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Effect of ascorbyl palmitate on the preservation of α-tocopherol in sunflower oil, alone and with herbs and spices

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Abstract

Ascorbyl palmitate (AP) (200 ppm) preserved α-tocopherol in sunflower oil at 95°C and delayed the onset of rancidity. Both effects increased with AP concentration ($r^2 = 0.96$ and 0.97 respectively) but levelled off near ~700 ppm. The improved anti-rancidity effect was due to the increased preservation ($P < 0.01$). Synergism was observed for both effects for AP combined with sage, turmeric, oregano and marjoram. Clove and thyme gave a smaller synergistic effect whereas basil inhibited. Neither bay nor cumin had any effect. Both the preservative (PF_p) and anti-rancidity effects (PF_r) were directly related to the thyme concentration (0–2000 ppm). Again, the decreased rancidity was due to the increased preservation ($P < 0.01$). The optimum AP concentration (0–1000 ppm) was around 250 ppm ($P < 0.01$) with thyme present (at 500 ppm) ($r^2 = 0.99$). The increased delay in rancidity was due to the improved preservation of α-tocopherol ($r = 1$, $r^2 = 0.99$, $P < 0.01$). Both the logarithm of the induction time and the preservative effect for the mixture of thyme and AP was directly related to the temperature (80–105°C). The mode of action of AP is also discussed.

Keywords: Anti-oxidant; Oregano; α-Tocopherol; Ascorbyl palmitate; AP; Sunflower oil; Rancimat®; Antioxidative mechanisms; Herb; Thyme; Sage; Turmeric; Marjoram; Clove; Basil; Bay

1. Introduction

α-Tocopherol is reported to have a number of health benefits (Azzi & Stocker, 2000), including the ability to help in controlling the excess of free-radical produced by the defence mechanisms of the body (Gardner & Fridovich, 1991) and some clinical conditions that have been related to the continuing excess of these free-radicals (Halliwell, Gutteridge & Cross, 1992; Morrissey, Buckley & Sheehy, 1994). Halliwell (1991) proposed that dietary antioxidants help to control this excess and vitamin E (α-tocopherol) may have great significance as it integrates into the vulnerable cellular membranes (Bjoneboe, Gunn & Drevon, 1990; Niki, Noguchi, Tsuchihashi & Gotoh, 1995).

In foods, free radicals cause oxidative rancidity, whereby unsaturated fats degrade to form volatile compounds. α-Tocopherol is lost during this process (Wong, Hashimoto & Shibamoto, 1995).

Ascorbyl palmitate (AP), a lipid-soluble ester of vitamin C, is an approved synthetic antioxidant that delays the onset of rancidity (Gwo, Flick & Dupuy, 1985; McMullen, Hawrysh, Lin & Tokarska, 1991).

In earlier work, some commercially-available herb extracts were shown to preserve α-tocopherol in sunflower oil but fairly high concentrations of these herbs (~500 ppm) were needed to produce a marked effect (Beddows, Jagait & Kelly, 2000). This work suggested that the mechanism of action was possibly through components of the herbs being more reactive with free-radicals than α-tocopherol, thus sparing the vitamin. Good linear relationships were found, for instance, with the concentrations of thyme and oregano.

Hras, Hadolin, Knez and Bauman (2000) evaluated the use of AP and α-tocopherol, in combination with rosemary extract, to delay rancidity in sunflower oil at 60°C. However, AP has not been assessed alone or in combination with herbs, for preserving α-tocopherol.

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Gordon and Kourimska (1995) reported that rosemary with AP, preserved α-tocopherol in rapeseed oil with repeated frying and Banias, Oreopoulou and Thomopoulos (1992) studied the effect of combinations of herbs with one concentration of AP for delaying rancidity in lard at 75°C.

Normally with polyunsaturated oils, initiation of rancidity begins slowly, with the polyenoic ester (LH) giving the free-radical (Reaction 1). This reacts with oxygen (Reaction 2) to give a peroxy species immediately, which in turn, reacts with a fresh alkyl-proton to give a new free-radical (Reaction 3). The mechanism of action of AP is not known (Coppen, 1994), although Lee, Jung and Kim (1997) reported that it quenched singlet oxygen in photosensitized oil mixtures. Coppen (1994) suggested that AP achieved its effect, by its ability to remove, or sequester, trace metals that catalyse peroxide formation.

However, ascorbic acid (AA) itself has a number of actions, including reaction with free-radical species such as $L^{\bullet}$ (Reaction 4) and $LOO^{\bullet}$ (Reaction 6), removing the single electron and donating a hydrogen, to form a more-stable free-radical. Also, AA scavenges oxygen (Reaction 5) and can react with peroxy species using a redox mechanism (Reaction 7), thus converting the peroxy species back to the alkyl group.

$$LH \rightarrow L^{\bullet} \quad (1)$$

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet} \quad (2)$$

$$LOO^{\bullet} + L^{\bullet}H \rightarrow LOOH + L^{\bullet} \quad (3)$$

$$AA\ (AP?) + L^{\bullet} \rightarrow LH + AP^{\bullet} \quad (4)$$

$$AA\ (AP?) + O_2 \rightarrow O_2\text{-}AP \quad (5)$$

$$AA\ (AP?) + LOO^{\bullet} \rightarrow LOOH + AP^{\bullet} \quad (6)$$

$$AP + LOO^{\bullet} \rightarrow LH + O_2\text{-}AP^{\bullet} \quad (7)$$

On the other hand, α-tocopherol (TOH) is affected by free-radicals by interacting with either the initial product ($L^{\bullet}$; Reaction 8) or the peroxy species. The method of heating used, can affect the mode of action (Chao-Liang and Schwarzer, 1998). In this work, the Rancimat® was used; thus the great excess of oxygen might be expected to produce a greater concentration of the peroxy species and should remove the free-radical directly (Reaction 9) or by oxidation (Reaction 10).

$$TOH + L^{\bullet} \rightarrow LH + TO^{\bullet} \quad (8)$$

$$TOH + LOO^{\bullet} \rightarrow LOOH + TO^{\bullet} \quad (9)$$

$$TOH + LOO^{\bullet} \rightarrow LH + O_2\text{-}TO^{\bullet} \quad (10)$$

Niki et al. (1995) suggested that AP regenerated oxidised α-tocopherol as with AA (Reaction 11).

$$AP + TO^{\bullet} \rightarrow AP^{\bullet} + TOH \quad (11)$$

The current work evaluates the ability of AP, alone and in combination with commercially available herb extracts, to preserve α-tocopherol, and to delay the onset of rancidity of sunflower oil at 95°C.

As in earlier work (Beddows et al., 2000), a protection factor for rancidity, PF_r was defined as the induction time for sample, compared to the control and a protection factor for preservation (PF_p) was the time for sample to lose 50% of α-tocopherol compared to that of a control. These are used here.

2. Materials and methods

2.1. Materials

α-Tocopherol, (AP) and all other materials were obtained from Sigma Chemical Co. Missouri, USA. Pure sunflower oil was purchased locally. Commercially available extracts of herbs were donated by Kalsec Inc, Kalamazoo, Michigan, USA. Sage extracts were prepared from fresh sage (*Salvia officinalis L.*) with methanol (Beddows et al., 2000). To add AP to oils, it was initially dissolved in a minimum volume of ethanol (HPLC grade; ~0.1 cm^{-3}).

2.2. Methods

Analysis of α-tocopherol was undertaken using HPLC (Beddows et al., 2000). AP, chlorophyll and β-carotene were assayed spectrophotometrically at 255, 663 and 453 nm, respectively, with calibration against standard solutions (Morgan, Shaw, Sidebottom, Soon & Taylor, 1985). Trace metals were measured by oxidising the oil with perchloric/nitric acids and analysing by atomic absorption spectrometry.

2.3. General method

Various materials were added, alone or in combination, to sunflower oil (10 g) and heated in a Rancimat® 679 (Metrohm, Herisau, Switzerland) at 95°C with an airflow rate of 20 $cm^3\ min^{-1}$. Portions were removed at intervals, and analysed for α-tocopherol as above:

induction time was measured directly. The materials assessed were:-

1. AP at concentrations 0–1000 ppm.
2. AP (200 ppm) and herb extracts (2000 ppm; see Table 2 below).
3. AP (200 ppm) with thyme or oregano concentrations at 0–2000 ppm.
4. AP at concentrations of 0–600 ppm, in the presence of thyme (500 ppm).
5. With sequestrant, citrate (500 ppm) alone and with AP (200 ppm).

2.3.1. Statistical analysis

All experiments were carried out in duplicate, and analyses were carried out in triplicate: thus the graphical results were based on $n=6$. Where appropriate, the results were analysed using the SPSS package. This includes linear regression analysis and the analysis of variance. The significances of *linear* relationships were determined using the Pearson correlation coefficient (r): (in all cases $P<0.01\%$). The coefficient of determination (r^2) was calculated from the results for all relationships and is included; the significance was assessed (at $P<0.01\%$).

Table 1
Effect of ascorbyl palmitate (AP) concentration on the preservation of α-tocopherol and rancidity of sunflower oil heated at 95°C

Initial AP concentration (ppm)	Induction time (hours)	PF_r	$t_{0.5}$ (hours)	PF_p
0	21.0	1.00	15.5	1.00
100	27.1	1.29	21.5	1.39
200	27.5	1.31	22.5	1.45
400	30.4	1.45	24.5	1.58
600	31.4	1.50	25.9	1.67
800	30.9	1.47	26.2	1.69

3. Results and discussion

3.1. AP concentration

AP preserved α-tocopherol (Table 1). This, as shown by the PFp, increased with the initial AP concentration ($r^2=0.97$ $P<0.01$) reaching a near maximum at ~700 ppm. An asymptotic curve fitted this relationship ($P<0.01$). AP delayed the onset of rancidity and the relationship of PF_r to the AP concentration was asymptotic ($r^2=0.96$ $P<0.01$). However, a possible explanation for these observations may be AP solubility. Coppen (1994) reported that solubility of AP is 300–1000 ppm in some vegetable oils at room temperature. In this work, AP was dissolved initially in ethanol, then added to the oil before heating at 95°C, when the solubility would be much greater. Ethanol alone, gave the same effects as the control. UV measurement of the oil at 255 nm, showed the AP to be fully soluble. However, the spectrum is complex, due to interference of other oil constituents. Under test conditions, some AP may come out of solution but not be observable and may thus not be easy to detect.

Table 2
Effect of ascorbyl palmitate (AP; 200 ppm) on the preservation of α-tocopherol and onset of rancidity by selected herbs and spices in sunflower oil heated at 95°C

Additive	Concentration ppm	Herb				Herb + AP				Effect of AP
		$t_{0.5}$ (hours)	PFp	T_i (hours)	PFr	$t_{0.5}$ (hours)	PFp[a]	T_I (hours)	PFr[b]	
Series A										
None	0	14.8	(1.00)	20.4	(1.00)	21.3	1.44 (1.00)	26.6	1.30 (1.00)	Preservation
Clove	2000	14.9	1.01	21.6	1.06	21.8	1.02	27.2	1.02	Slight synergism
Marjoram	2000	15.5	1.05	24.6	1.21	23.7	1.11	29.6	1.11	Synergism
Turmeric	2000	16.8	1.14	22.4	1.10	24.0	1.12	29.6	1.11.	Synergism
Cumin	2000	14.8	1.00	20.1	0.98	21.5	1.01	26.0	0.97	Little effect
Basil	2000	11.8	0.80	21.0	1.03	20.8	0.97	24.2	0.91	Inhibition
Oregano	2000	16.7	1.13	23.4	1.15	25.6	1.20	30.4	1.14	Synergism
Thyme	500	17.1	1.16	22.2	1.09	24.4	1.15	29.3	1.10	Slight synergism
Thyme	1000	19.9	1.34	24.8	1.22	25.9	1.22	31.7	1.17	Some synergism
Thyme	2000	23.3	1.57	29.2	1.47	29.0	1.36	36.8	1.38	Some synergism
Series B										
None	0	4.3	(1.00)	7.8	(1.00)	16.1	1.41 (1.00)	9.9	1.27 (1.00)	Preservation
Sage	2000	9.5	2.21	9.9	1.72	12.2	2.01	15.7	1.58	Synergism
Bay	2000	4.4	1.02	8.5	1.09	6.2	1.02	9.8	1.25	Slight inhibition
Thyme	2000	6.9	1.56	11.6	1.49	8.3	1.37	13.1	1.32	Some synergism
Bay + thyme	2000	7.1	1.54	12.0	1.53	7.1	1.16	12.9	1.29	Slight inhibition

[a] Calculated with the equivalent AP-containing sample as the control with value 1.00.
[b] Series A and B used different batches of sunflower oil.

Another explanation would be that different mechanisms are operating between AP and the various products at the different concentrations of AP.

The breakdown of lipids might be due to singlet oxygen rather than through free radical activity (Donnelly & Robinson, 1995). Chlorophyll, which assists the photochemical generation of singlet oxygen, was found to be quite low at 1.54 ppm and β-carotene, which quenches (Lee & Min, 1988), was 0.97 ppm. In addition, the non-conjugated hydroperoxide, that results from the action of singlet oxygen, (Scott, 1993; Terao and Matsushita, 1977), did not appear at 970 cm^{-1}. These observations agree with those of Cort (1974) and Terao and Matsushita (1977) that singlet oxygen was not involved to any great extent. Cort (1974) also showed that no differences were observed when singlet oxygen quenchers were included.

The PF_r was directly proportional to the PF_p (where $PF_p = 0.7226\ PF_r + 0.2793$ ($r^2 = 0.987$ $r = 0.99$, $P < 0.01$), thus the improved resistance to rancidity must to be due to the increased preservation of α-tocopherol.

Although it was not the intent to investigate the mechanism of action of AP, these results provide some evidence as to the possible mode of action:-

1. AP was proposed to be an oxygen-scavenger (Cort, 1974; Reaction 5). However, a rapid depletion of AP did not occur, even though oxygen was in great excess and any primary free radicals formed (Reaction 1) should have been converted quickly to the peroxy species (Reaction 2). So this mode of action seems less likely.
2. Another mechanism was that AP could act as a sequestrant (Coppen, 1994) as for AA. Citrate will combine with trace elements. Thus when it was added to the oil and heated, the rate of breakdown of α-tocopherol was unaffected, with both the AP and AP-free samples. However, such trace metals were found to be very low in concentration in the oil used (Beddows et al., 2000). This does not rule out the possibility that AP may act as a sequestrant if these trace elements were to be in higher concentration. Similar observations were made by McMullen et al. (1991) who noted that the delay of the onset of rancidity with AP, was not affected by the presence of monoglyceride citrate (MGC) in canola oil.
3. In earlier work, the preservation of α-tocopherol was proportional to its concentration (Beddows et al., 2000). Thus, if AP reformed α-tocopherol from the free-radical form (Reaction 11), the relationship of AP concentration to preservation, would be linear, as the tocopherol was in great excess.
4. Perhaps the most likely action is that AP reacts with free-radical species as they are formed (Reactions 4, 6 and 7), in preference to α-tocopherol (Reactions 8, 9 and 10) which, in effect, can be identified as a displacement. In this, the reactions are more likely to be reduction-oxidation of the single electron species, as identified by Büettner (1993).

3.2. Effect of AP on the ability of some herbs to preserve α-tocopherol

The aim was to identify whether synergism occurred between AP and commercially available herb extracts, as this would reduce the individual levels needed to achieve the same effect. The herb extracts were selected because they were commercially-available. Synergism, for both the anti-rancidity and preservative effects, was observed when AP was combined with sage, oregano or marjoram and to a lesser extent, clove (Table 2). Thyme was slightly synergistic and turmeric was additive whereas basil, bay and cumin reduced the preservative effect shown by AP alone and might be considered to be pro-oxidative.

3.3. The effect of herb concentration on the preservation effect by AP

Thyme was selected at an initial AP concentration of 200 ppm. The improved preservation of tocopherol (PF_p) and the delay in rancidity (PF_r), were directly proportional to the concentration of thyme ($P < 0.01$), suggesting that AP enhanced the activity (Fig. 1). The results with oregano were more complex, with a slight decrease in preservation at higher concentrations. However, the PF_r was directly proportional to the PF_p for both thyme and oregano ($P < 0.02$). Thus, for thyme, $PF_r = 0.941$. PF_p ($r^2 = 0.995$ $r = 0.999$, $P < 0.01$), indicating that both AP and thyme delayed the onset of rancidity.

3.4. Effect of AP concentration on the preservative effect of thyme

When the AP concentration was increased, in oil containing thyme extract, the relationship was not linear and a near maximum was reached at ~250 ppm for both the preservation (PF_p 1.62) and rancidity effects ($PF_r = 1.54$) ($r^2 = 0.99$; Fig. 2). Again, an asymptotic curve fitted for both ($r^2 = 0.99$ $P < 0.01$). Nevertheless, the PF_r was linearly related to the PF_p ($r = 0.999$ $P < 0.01$), indicating again that, even in the presence of AP, the increased delay in the rancidity was due to the improved preservation of α-tocopherol.

3.5. Multiple combinations

Initially bay was selected, as it had been shown to be ineffective on its own but was synergistic with thyme (Beddows et al., 2000). This was confirmed but no synergism was observed when AP was present. When

AP, thyme and bay were used in combination, slight inhibition was observed (Table 2).

Further brief studies, with turmeric, showed that, when used in combination with thyme and AP, a strong synergistic effect was obtained for the prevention of rancidity and preservation of α-tocopherol (Table 3).

3.6. Temperature

The logarithm of the induction time, and also the preservative effect, (log $t_{0.5}$) for the combination of thyme and AP, was directly related to the temperature in the range 80–105°C, as expected (Reynhout, 1991).

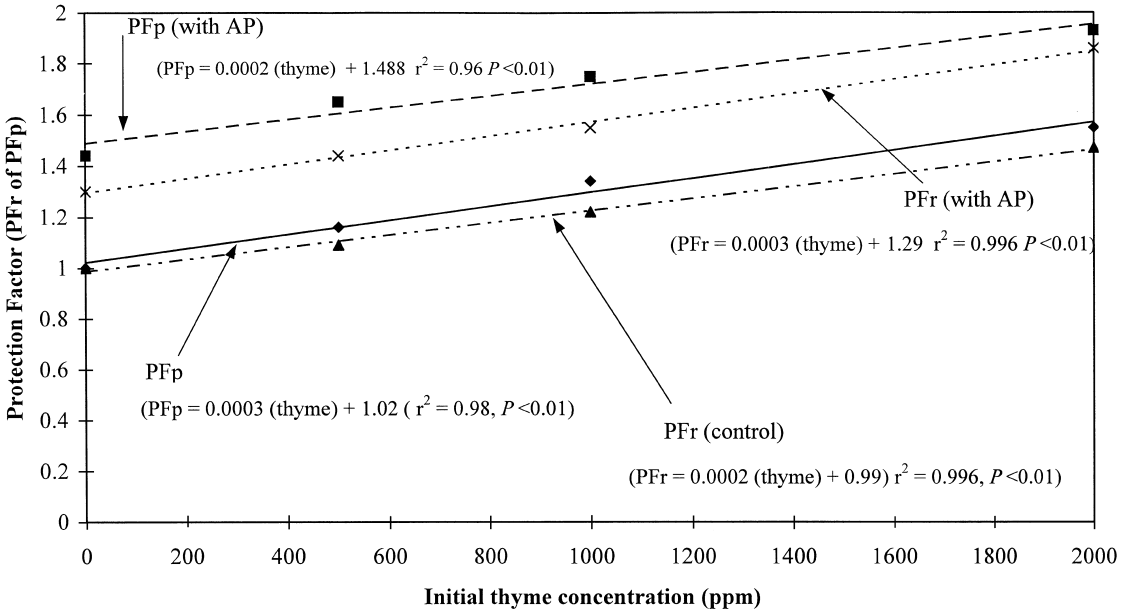

Fig. 1. The effect of thyme concentration on the preservation of α-tocopherol (PF_p) and the prevention of rancidity (PF_r) in sunflower oil heated at 95°C.

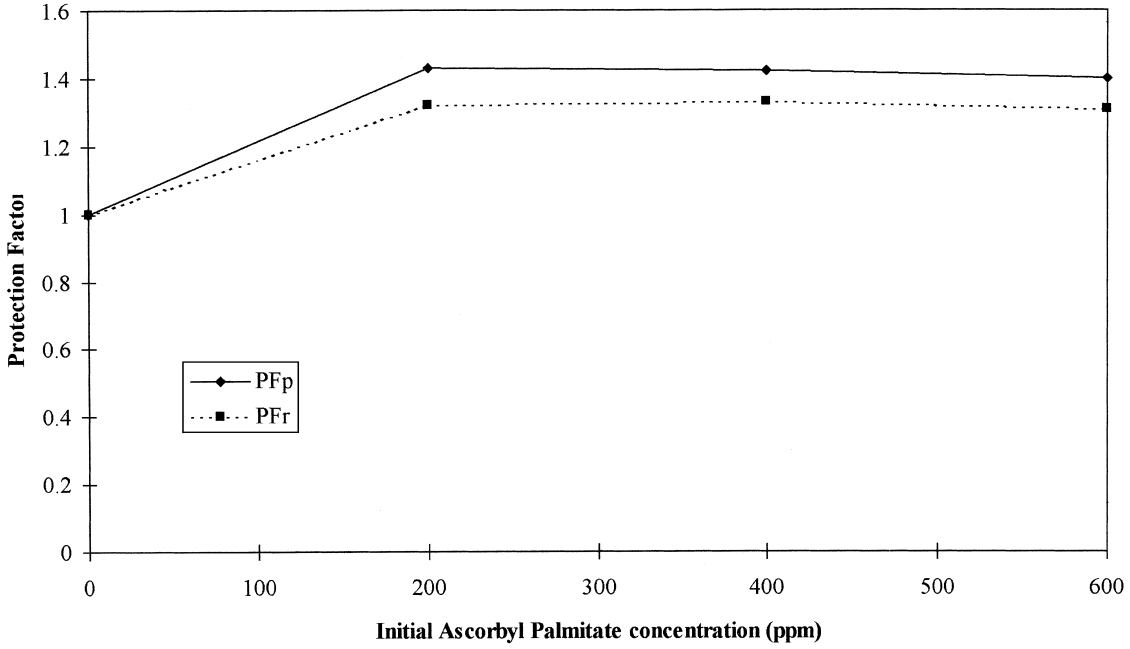

Fig. 2. The effect of ascorbyl palmitate (AP) concentration on the preservation of α-tocopherol (PF_p) and the prevention of rancidity (PF_r) in sunflower oil containing thyme (500 ppm) heated at 95°C.

Table 3
Effect of ascorbyl palmitate (AP) alone and in combination with of thyme and turmeric, on the rancidity and α-tocopherol concentration of sunflower oil heated at 95°C

Treatment	% α-Tocopherol left after heating for:		Induction time (hours)	PF_r
	4 h	7 h		
Control	59	0	8.1	1.00
Thyme	83	65	11.1	1.37
AP	85	40	10.3	1.27
Thyme + AP	92	84	13.6	1.68
Turmeric	58	28	9.7	1.20
Turmeric + AP	85	60	12.9	1.59
Turmeric + AP + Thyme	117	98	16.3	2.01

These results give some insight into the mode of action of AP as it did not appear to act as ascorbic acid is believed to do, by chelating with trace metals, quenching of singlet oxygen or reacting with oxygen and removing it. The regeneration of α-tocopherol could occur, but this appeared less likely from the current evidence.

Thus, AP appeared to act by being more sensitive to free-radical attack than herbs and spices or α-tocopherol. Thus, AP is subject to reduction-oxidation of the single electron species (Büettner 1993).

Whether this would be the same mode of action in the body is questionable, as such systems are much more complex and heterogeneous (Lambelet, Saucy & Löliger, 1994). However, AP was more protective than thyme or other herbs and greatly reduced the amount of extract needed to preserve α-tocopherol. Thus, with the normal limited dose of 200 ppm AP, 250 ppm thyme was as effective as 2000 ppm of thyme extract alone. The latter observation is in agreement with the work of Hras, Hadolin, Knez and Bauman (2000) who found that the optimum effect against rancidity for sunflower oil at 60°C, was with a mixture of AP (100 ppm) and rosemary extract (250 ppm). This type of combination is likely to be more usable in food oils than the herb extracts alone. Other synthetic antioxidants could also play a role and they could increase the effectiveness of the herb components against rancidity, as AP has done. This will lead to more effective mixtures being identified.

Work is progressing on the nature of the components, present in thyme, that may be responsible for the major α-tocopherol-sparing activity. Other permitted synthetic anti-oxidants are being evaluated, as well as other dietary components. This will help to identify structural characteristics that may be needed to protect α-tocopherol.

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