Antioxidant Activity of Tocopherols, Ascorbyl Palmitate, and Ascorbic Acid and Their Mode of Action

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ABSTRACT

In the quest to use antioxidant compounds occurring in nature or related compounds, extensive studies have been made on vegetable oils, animal fats, apocarotenal, and vitamin A as substrates with ascorbyl palmitate, tocopherols, and ascorbic acid as antioxidants. Antioxidant efficiency varies with the substrate. Ascorbyl palmitate at a level of 0.01% provides a useful increase in the shelf-life of vegetable oils. Alone it is better than butylated hydroxytoluene and butylated hydroxyanisole and in combinations with other known antioxidants improves the shelf-life of all vegetable oils, as well as potato chips. Solubility problems with ascorbyl palmitate and other esters of ascorbic acid are discussed. The tocopherols have their greatest effect in protection of animal fats, carotenoids, and vitamin A. Experiments utilizing tocopherols and tocopherol combinations are presented. The activity of ascorbic acid, an excellent scavenger of oxygen, is reviewed. Quenchers of singlet oxygen do not inhibit the direct oxidation of fats and oils under the conditions used.

INTRODUCTION

Tocopherols have been proposed, used, and studied as antioxidants for many years. They are classified among the natural antioxidants which function as electron donors (1). One of the purposes of this study was to determine the comparative activity of tocopherols as antioxidants.

Ascorbic acid and ascorbyl palmitate, which function by oxygen scavenging, an entirely different mechanism, also are used as antioxidants, particularly in closed systems, to remove oxygen in the head space and in solution. Ascorbyl palmitate is considered physiologically acceptable, although it is not found in nature; both the ascorbic acid and palmitic acid moieties produced on hydrolysis are natural compounds. Ascorbyl palmitate has been listed under Section 121.101 in the Federal Code for a number of years. It has not found widespread use because of the ready availability and low price of butylated hydroxytoluene (BHT). Because of the quantity of BHT now consumed, there has been some fear of restricted usage; however, BHT appears to be in a favorable position at the present time since the Shamberger (2) data became available. Ascorbic acid, on the other hand, has been used in many food applications to remove oxygen from solution.

The second purpose of this study was to determine efficacy of ascorbyl palmitate as an antioxidant and to delve into the mechanism of the action of ascorbic acid as an oxygen scavenger.

The third phase of this study sought to gain insight into the mode of action of air oxidations. For many years, autoxidation of substrates (fats, oils, and oxygen sensitive compounds) has been classified as a free radical chain reaction. The phenolic antioxidants have been said to act as electron or hydrogen donors and quench electron mobility with the subsequent interruption of free radical chain reactions. This has been aptly reviewed by Johnson (3). It has been stated that ultimately the antioxidants were swamped, and, thus, oxidation proceeded.

In the meantime, singlet oxygen has come into recognition since the molecular orbital description of the oxygen molecule was revealed (4). The lowest and most active configurations of oxygen are the singlets. $^1\Delta g$ Singlet is short lived (less than 10^{-6} sec), but it will withstand 10^8 collisions in methanol.

Rawls and Van Santen (5) presented what appeared to be a logical explanation for the mechanism of oil oxidations. They studied the oxidation of methyl linoleate by singlet oxygen, produced by radio frequency gas discharge or by photosensitization of photodynamic compounds, and obtained hydroperoxides at least 1400 times faster than with air oxidation. They identified the conjugated peroxides as the primary reaction products in all oxidations, but, in singlet reactions, *trans*-nonconjugated peroxides also were formed. They postulate that the nonconjugated peroxides are formed by singlet oxygen and these then

TABLE I

Comparative Antioxidant Activity Schaal Oven, Thin Layer, 45 C

Antioxidant	Concentration (%)	Days to reach meq/kg PV		
		Chicken Fat	Pork fat	Beef fat
None		8	3	10
dl-α-Tocopherol	0.02	13	15	24
dl-α-Tocopherol	0.05	13	15	---
dl-α-Tocopherol	0.2	10	15	---
d-α-Tocopherol	0.02	13	15	---
d-α-Tocopherol	0.05	13	15	---
d-α-Tocopherol	0.2	11	15	---
dl-γ-Tocopherol	0.02	29	37	40
dl-γ-Tocopherol	0.05	40	58	---
dl-γ-Tocopherol	0.2	46	61	---
Butylated hydroxyanisole	0.02	20	28	36
Butylated hydroxytoluene	0.02	15	18	24
dl-α-Tocopherol Ascorbyl palmitate	0.02 each	28	28	38
dl-γ-Tocopherol Ascorbyl palmitate	0.02 each	53	67	70
Ascorbyl palmitate	0.02	10	9	12

TABLE II

Stripped Oils meq/kg–Peroxides Comparative Antioxidant Activity in Days[a]

Oil	Days 1	4	5	6	7	8	9	12	13	14	16	19	Days to Reach 70 meq/kg PV
Soybean oil													
None	0	NR[b]	24	24	24	NR	33	54	56	75	76	79	14
γ-Tocopherol	0	NR	25	25	28	NR	38	38	36	48	54	68	19
α-Tocopherol	0	NR	38	38	49	NR	43	49	49	73	73	---	14
Safflower oil													
None	0	52	900	1120	NR	---	---	---	---	---	---	---	5
γ-Tocopherol	NR	58	54	58	63	73	---	---	---	---	---	---	8
α-Tocopherol	NR	64	59	70	75	89	---	---	---	---	---	---	6

[a]Oils contain no antioxidants–stripped on molecular still to reduce tocopherol. STripped soybean oil has 5 mg % α-tocopherol, 8 mg % total tocopherols. Stripped safflower oil has 0.32 mg % α-tocopherol, 1.6 mg % total tocopherols. Levels of tocopherols added-0.02%.

[b]NR = not run.

TABLE III

Oleic Acid vs Linoleic Acid

Antioxidant	Days to form 20 meq/kg PV in oleic acid	Days to form 70 meq/kg PV in linoleic acid
None	3	2
0.02% dl-α-Tocopherol	9	3
0.02% dl-γ-Tocopherol	19	5
0.02% Butylated hydroxytoluene	9	8
0.02% Butylated hydroxyanisole	16	9

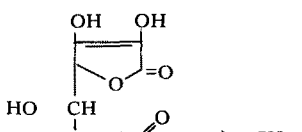
initiate autoxidation. One then wonders why the initiator is not found in the autoxidized product, but, nevertheless, the energy requirement for initiation of air oxidation is explained logically if singlet oxygen is involved.

Labuzza (6) also presents arguments for the involvement of singlet oxygen in air oxidation of fats and oils. However, it is difficult to rationalize the initiation of air oxidation by the concept of a singlet oxygen in systems which are not biological and do not have exposure to photodynamic compounds and light. Air oxidation of synthetic vitamin A, carotenoids, and 2-methyl-2-pentene, purified oleic acid, and, in fact, the highly purified methyl linoleate (5) did not involve biological exposure. Experiments on $^1\Delta g$ singlet oxygen and its quenchers will be reported.

TABLE IV

Physicochemical Data Ascorbyl Palmitate

ASCORBYL PALMITATE



Mol Wt 414.54
melting point 113 C
Listed in code of Federal Register 121.101 as chemical preservative. No limit given. Other Antioxidants are limited to 0.02% fat

Solubility	Temperature	G/100 ml
100% Ethanol	RT[a]	12.5
95% Ethanol	RT	10.8
50% Ethanol	RT	0.04
Methanol	RT	18.3
Acetone	RT	6.9
2-Propanol	RT	5.0
Ether	RT	0.76
Chloroform	RT	0.03
H_2O	RT	0.00018
H_2O	70 C	0.2
H_2O pH 8.1	RT	0.001
H_2O pH 8.1	60 C	10.1
Drewmulse GMC8	RT	5.5
Neobee M5	RT	0.1
Coconut oil	RT	0.12
Peanut, sunflower, and olive oils	RT	0.03
Soybean and safflower oil	RT	0.02
Propylene glycol[b]	80 C	10.0
Glycerine[b]	80 C	10.0
Decaglycerol octaoleate	80 C	11.0

[a]RT = room temperature.

[b]Forms semisolid at RT.

METHODS

The substrates have been studied in thin layers of 0.2 g

TABLE V

Oxidation of Soybean Oil, 45 C

Antioxidant[a]	Days to reach 70 meq/kg PV
None	7
0.01% AP	16
0.02% AP	19
0.05% AP	21
0.2% AP	25
0.02% BHA	9
0.02% BHT	10
0.02% TDPA	15
0.01% PG	20
0.02% PG	20
0.02% NDGA	21
0.02% TBHQ	26
0.02% Ascorbic acid	12
0.2% Ascorbic acid	17
0.01% AP + 0.01% PG	27
0.01% AP + 0.01% TDPA	21
0.01% AP + 0.01% BHA	18
0.01% AP + 0.01% BHT	17
0.01% AP + 0.01% NDGA	28
0.01% AP + 0.01% Tocopherol	16
AP at 0.05%, PG, TDPA at 0.01%	42
AP at 0.05%, BHA, TDPA at 0.01%	30
AP at 0.05%, BHA, PG at 0.01%	31
AP at 0.05%, BHT, TDPA at 0.01%	31

[a]AP = ascorbyl palmitate, BHA = butylated hydroxyanisole, BHT = butylated hydroxytoluene, TDPA = thiodipropionic acid PG = propyl gallate, NDGA = nordihydroguaiaretic acid, TBHQ = 2-tertiarybutylhydroquinone.

TABLE VI

Antioxidant Efficiency 45 C, Days To Reach 70 meq/kg PV

Antioxidant[a]	Concentration (%)	Substrate oil			
		Safflower	Sunflower	Peanut	Corn
None	---	7	6	15	12
AP	0.01	11	10	26	21
AP, PG, TDPA	0.01 each	25	22	46	31
BHA	0.02	8	8	15	15
BHT	0.02	10	9	15	13
PG	0.02	16	19	26	21

[a]AP = ascorbyl palmitate, PG = propyl gallate, TDPA = thiodipropionic acid, BHA = butylated hydroyanisole, and BHT = butylated hydroxytoluene.

in 50 ml beakers (1-1/2 in. diameter) at 45 C. Fats and oils were titrated daily using KI and $Na_2S_2O_3$ titration procedure of AOAC (7).

The animal fats were rendered in an autoclave, and only preparations containing no measureable tocopherol were used. Tocopherol assays were done by the gas liquid chromatographic (GLC) procedure of Slover, et al. (8). UV measurements were made in a single beam Hitachi Perkin-Elmer 139 and oxygen measurements on a Beckman oxygen analyzer 777.

RESULTS AND DISCUSSION

Table I shows the comparative activity of the various antioxidants in chicken, pork and beef fats. The peroxides were measured and calculated as the number of days required to reach 20 meq/kg. Where checked, the activity of d- and the dl-α-tocopherol were equivalent on a wt basis, and neither one had increasing antioxidant activity above the level of 0.02%. dl-γ-Tocopherol has more activity than α-, and activity increases as the concentration increases. This latter fact corroborates the findings in many previous publications, such as Lea and Ward (9), Griewahn and Daubert (10), and Parkhurst, et al. (11).

Antioxidant activity of tocopherol added to vegetable oils always has been low. The explanation usually given has been that tocopherols naturally present mask additional activity. Therefore, soybean and safflower oils were heated in a molecular still under high vacuum at 270 C to distill off the tocopherols. Table II contains the results with these stripped oils. Although the stripped safflower oil contained only 0.32 mg % α-tocopherol, fairly poor antioxidant activity was obtained after addition of tocopherol.

To find a reason for the low activity in vegetable oils, experiments were performed in 99% oleic and linoleic acids (NU-chek) which had no demonstrable tocopherol (below

TABLE VII

Stability of Potato Chips Cooked in Cottonseed Oil with Various Antioxidants, 45 C

Antioxidant[a] (0.02%)	Days to reach rancidity
Control (none)	10
BHA	10
AP	15
BHT	14
PG	24
Dilaurylthiodipropionate	14

[a]BHA = butylated hydroxyanisole, AP = ascorbyl palmitate, BHT = butylated hydroxytoluene, and PG = propyl gallate.

TABLE VIII

Peroxides Formed with Linoleic Acid, Oleic Acid, and 2-Methyl-2-Pentene Substrates[a] in Thin Layer Oxidation, 45 C

Antioxidant	Singlet quencher	Days									
		1	2	3	4	5	7	8	11	13	15
		Linoleic substrate PV meq/kg									
None	None	214	NR								
TOCOL	None	70	106	364							
None	APO	212	NR								
TOCOL	APO	86	104	384							
None	TEM	214	NR								
TOCOL	TEM	64	192	436							
		Oleic substrate									
None	None			0	0	0	20	50	70	NR	
TOCOL	None			0	0	0	0	0	0	20	52
None	APO			0	22	30	38	120	190	NR	
TOCOL	APO			0	0	0	0	0	0	22	70
None	TEM			0	26	26	34	66	130	NR	
TOCOL	TEM			0	0	0	0	0	0	21	63
		2-Methyl-2-pentene substrate									
None	None	70	270	422							
TOCOL	None	0	110	358							
None	APO	75	250	430							
TOCOL	APO	0	128	324							
None	TEM	80	344	410							
TOCOL	TEM	0	88	332							

[a]TEM = 10^{-3}M Triethylamine, APO = 10^{-4}M β-apocarotenal, TOCOL = dl-α-Tocopherol 0.02%, and NR = not run.

TABLE IX

Peroxides[a] Formed in Thin Layers at 45 C in Solvents and with Chemically Produced Singlet in the Same Solvents

Thin layer autoxidations						Chemical oxygenations[c]
(A) Substrate soybean oil[b] in benzene:methanol (4:1, v:v)						
	Days					
Singlet quenchers	6	8	9	10	13	---
None	48	66	70	74	156	20
Apo-8'-carotenal 10^{-4}M	54	72	80	92	160	0
Triethylamine 10^{-3}M	50	69	76	86	157	0
(B) 0.1 M Linoleic acid[d] in methanol						
	Days					
Singlet quenchers	2	3	4	5		---
None	30	70	110	210		95
Apo-8'-carotenal 10^{-4}M	30	75	120	215		0
Triethylamine 10^{-3}M	35	80	140	230		0
β-Carotene	32	77	125	250		0

[a]Peroxides meq/kg of substrate, soybean oil and linoleic acid.

[b]18 g made up to 300 ml. Benzene:methanol (4:1) 3 ml in 50 ml closed bottles 36 mm diameter.

[c]Singlet experiments run on 300 ml solvent solutions made up to 0.18 M H_2O_2 and 0.15 M NaOCl added dropwise at -20 C for 30 min; 60 ml water added and extracted three times with diethyl ether containing 0.1% BHT (in case of linoleic acid water adjusted to pH 2.5 HCl). The ether extracts were rewashed with water three times changing separatory funnels each time and then blown down with nitrogen. Recovery was 89-90% of the starting material on a wt basis. (Substrates without H_2O_2 NaOCl and reagents without substrates put through extraction procedures did not have peroxides.)

[d]28 g/liter methanol (0.1M): direct oxidations run on 7 ml in closed 250 ml bottles 62 mm diameter.

0.1 mg %) or peroxides (below 0.02 meq/kg). Table III presents the results from this experiment. The tocopherols were active in oleic acid; dl-α-tocopherol is as active as BHT and dl-γ-tocopherol was more active than BHT or butylated hydroxyanisole (BHA). In linoleic acid, BHT and BHA are more active than the tocopherols.

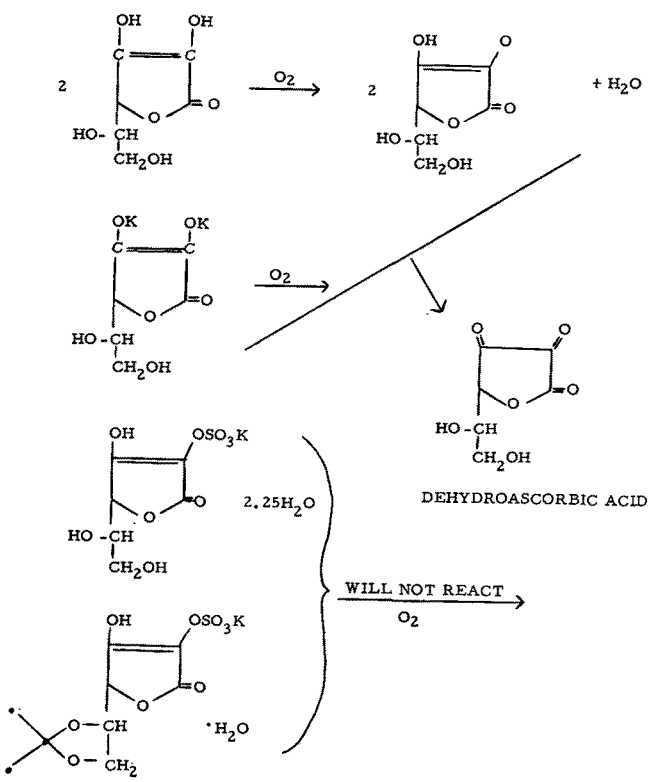


FIG. 1. Proposed ascorbic acid mechanism.

Also in Table I, the results show the activity in chicken, pork, and beef fats; and, similar to the oleic acid results, dl-α-tocopherol is equal to BHT and γ-tocopherol is better than BHT and BHA. Both α- and γ-tocopherol are synergized by ascorbyl palmitate. Thus, α and γ-tocopherols should be useful as antioxidants in animal fats and in countries where BHT and BHA are illegal.

Additionally, in a thorough piece of work, Kläui (12) has reported the effect of tocopherol, ascorbyl palmitate and various amines upon the stability of vitamin A palmitate. It is interesting to see from his publication that a small amount of tocopherol, 1 mg million international units of vitamin A (MIU), is indeed effective, provided ascorbyl palmitate and an amine are added. In the same publication, β-carotene in paraffin oil was protected from cooper oleate induced oxidation by a mixture of tocopherol and ascorbyl palmitate. Here again, we have an example of efficacy based upon substrate and the use of combinations.

Proceeding to the second phase of this study, Table IV shows the structure and solubility of ascorbyl palmitate. Ascorbyl palmitate can be weighed directly into oils, dissolved in ethanol, and added to the oils or dissolved in a special oil, such as decaglycerol octaoleate. With the last method, solubility of 0.05% in oils can be achieved. Antioxidant activity has been demonstrated by levels from 0.003-5.0% with increasing activity in spite of the fact that the solubility limit is exceeded.

Table V shows the activity in soybean oil; ascorbyl palmitate at 0.01% is more active than BHT or BHA at 0.02%. Since the present food laws place no restriction on the levels of use of ascorbyl palmitate, it may be used in higher concentrations than the legal limits of the conventional antioxidants. Ascorbyl palmitate, therefore, offers a means of obtaining extra stability when it may be required. For example in dual combinations, it increases the total protection time; at 0.05% in the combinations shown in Table V, ascorbyl palmitate prolongs shelf-life for a considerable period.

Comparative activity in four vegetable oils is shown in Table VI. Here again, ascorbyl palmitate is more active than BHT or BHA. Ascorbyl palmitate in combination with propyl gallate and thiodipropionic acid gave extended protection to all four oils.

To see if ascorbyl palmitate could protect deep fried foods, potato chips were made in cottonseed oil with several antioxidants (Table VII). The chips then were placed in open beakers at 45 C, and the number of days required to reach 70 meq of peroxide were determined. Ascorbyl palmitate did function better than BHT in potato chips, as well as in the oils.

Since ascorbic acid, as previously stated, functions as an oxygen scavenger, it offers a distinct advantage for products contained in bottles or cans with a head space of air. Based upon theoretical calculations of the amount of air required to convert 1 mole ascorbic acid to 1 mole dehydroascorbic acid, 3 mg ascorbic acid would be required for each cc of head space. When oxygen is measured in solution with the oxygen analyzer, 7 mg ascorbic acid actually is required for each cc of head space.

Bielski and Allen (13) have shown that two free radicals are formed from ascorbic acid after pulse radiolysis, one at the 3 position which absorbs at 360 nm and the other on the 2 position which absorbs at 290 nm. When direct oxygen scavenging was studied in constantly mixed, closed containers with a head space of 5 cc air and filled with a solution of 50 mg ascorbic acid in 50 ml water, no absorption at 360 nm was found when examined at hourly intervals, but absorption at 290 nm appears. In this sytem, no oxygen is left in solution or in the head space after 8 hr.

1-Ascorbic acid-2-sulfate and 5,6-isopropylidine-1-ascorbic acid-2-sulfate have no activity as oxygen scavengers (Fig. 1). Thus, to initiate oxygen scavenging, the 2 position must be unsubstituted to form the free radical as shown.

Levandoski, et al., (14) identified the monodehydro ascorbic acid-ascorbic acid (MDHAA-AA) complex from ascorbic acid oxidation. The proposed structure of MDHAA-AA has been through the 3 and 1 positions. In pulse radiolysis, however, Bielski, et al., (15) found no other intermediates other than the free radical prior to formation of dehydroascorbic acid. Laroff, et al., (16) has shown a number of free radicals by electron spin resonance spectrophotometry, but the present investigation demonstrates that the free radical in the 2 position is essential.

Bauernfeind (17) also has surveyed ascorbic acid reactions in food systems and potential reaction pathways.

MECHANISM OF AUTOXIDATION

Foote, et al., (18,29) produced $^1\Delta g$ singlet oxygen both chemically and photodynamically and have oxidized a number of substrates including 2-methyl-2-pentene. They measured the peroxides formed by reduction to the alcohol and by GLC determination. These oxidations could be quenched with β-apo-8′-carotenal, β-carotene, and trimethylamine. We have repeated this work on 2-methyl-2-pentene. Because of its greater solubility in methanol, we prefer β-apo-8′-carotenal as a quenching agent.

Therefore, it occurred to us that a quenching experiment be performed in our thin layer air autoxidation tests. Apo-8′-carotenal was added by direct weighing or from a methanol solution to safflower oil, oleic acid, and linoleic acid. These substrates contained less than 0.02 meq/kg peroxides initially. In all three substrates, the time required to reach 70 PV was the same with and without apocarotenal. Similar experiments were performed in the same substrates, with and without conventional antioxidants (BHT, propyl gallate); both in the presence of apocarotenal and without. In none of these experiments did apocarotenal extend the time to reach 70 PV.

Table VIII presents additional data with singlet quenchers, triethyamine and apocarotenal, on oleic and linoleic acids and 2-methyl-2-pentene with and without dl-α-tocopherol. The singlet quenchers showed no effect upon peroxide formation. It is interesting to see that autoxidation of substrates used for singlet oxidation are not quenched by singlet oxygen quenchers.

Because of the possibility of solvent involvement, quenching experiments were performed with substrates in methanol and methanol:benzene (1:4) combinations in thin layer autoxidations. Results of these experiments appear in Table IX and quenching of thin layer autoxidations did not occur in solvents used for chemically produced $^1\Delta g$ singlet oxygen and for quenching singlet oxygen.

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