THE JOURNAL OF THE American Oil Chemists' Society

Volume 31 AUGUST, 1954 No. 8

The Relation of Synergist to Antioxidant in Fats[1]

O. S. PRIVETT[2] and F. W. QUACKENBUSH, Department of Biochemistry, Purdue University, Lafayette, Indiana

WHILE much progress has recently been made toward an understanding of the mechanism of autoxidation of fats and the probable means by which antioxidants retard the process (1, 2, 5, 13), relatively little has been established concerning the reactions through which synergists perform their spectacular role in prolonging antioxidant action.

Calkins and Mattill (4) studied reactions between ascorbic acid and quinone and postulated that ascorbic acid was oxidized, as fat peroxides were reduced, through the catalytic action of quinone. Golumbic extended this view in his studies with tocopherols. He believed (7) that synergists, generally, provided a reservoir of hydrogen to regenerate the antioxidant after its partial oxidation by activated fat peroxides while phosphoric acid (6) performed the additional function of recycling tocohydroquinone to tocopherol. Calkins (3) subsequently rejected this view after further study of the behavior of quinone with phosphoric acid in an autoxidizing medium. He postulated that phosphoric acid reacted with activated fatty-ester molecules and that the excess energy became dissipated in the complex; through subsequent dissociation both phosphoric acid and fatty-ester molecules were regenerated in the inactivated state. The function of quinone (and phenolic antioxidants in vegetable oils) was to increase the solubility of phosphoric acid or other synergist in the fatty medium.

Studies on autoxidation of fats in this laboratory (9, 10, 11) have occasionally yielded data which were inconsistent with any of the above postulates. Experiments were then initiated to obtain additional information on the antioxidant-synergist relationship, and the results are presented in this paper.

Experimental

The effect of synergists on "pro-oxidant" levels of antioxidants. Samples of lard (3 g.) in 50-ml. Erlenmeyer flasks containing synergistic combinations of ascorbic, citric, NDGA (nordihydroguaiaretic acid), and α-tocopherol were placed in an oven at 75°C., and peroxide determinations were made at frequent intervals (Figures 1 and 2). The initial rapid rate of peroxide accumulation during the induction period with .05% of NDGA or 2.0% α-tocopherol was suppressed sharply by the presence of .10 to .15% of either citric acid or ascorbic acid. The suppression was not complete however since peroxide accumulation in these samples was greater than in samples containing low concentrations of antioxidants, *e.g.*, .01% NDGA (Figure 1). In a similar series with 2-g. samples of lard, tocopherol analyses (11) were made at or near the end of the induction period (Table I).

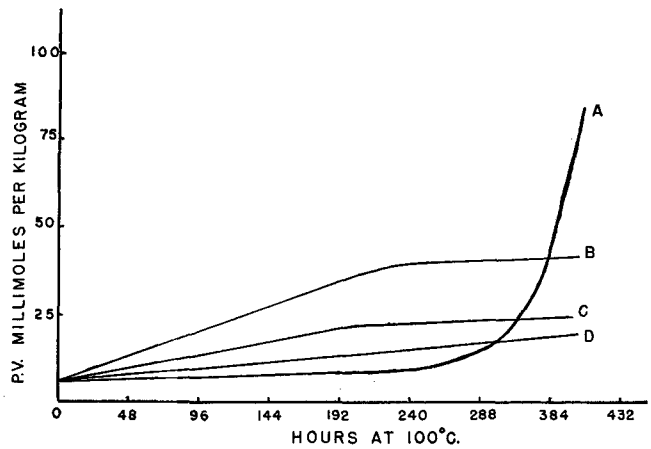

FIG. 1. Effect of synergists on the pro-oxidant action of NDGA.

A. Lard containing .01% NDGA.
B. Lard containing .05% NDGA.
C. Lard containing .05% NDGA + 0.1% citric acid.
D. Lard containing .05% NDGA + 0.1% ascorbic acid.

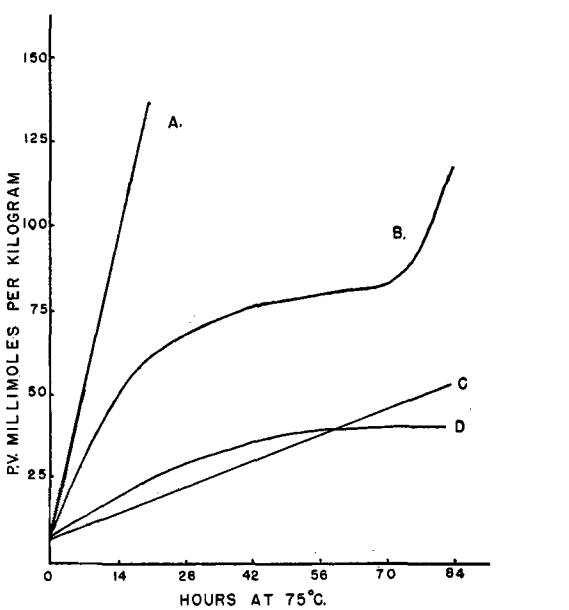

FIG. 2. Effect of synergists on the "pro-oxidant" action of α-tocopherol.

A. Lard.
B. Lard + 2.0% α-tocopherol.
C. Lard + 2.0% α-tocopherol + 0.15% ascorbic acid.
D. Lard + 2.0% α-tocopherol + 0.14% ascorbic acid.

[1] Journal Paper No. 742 of the Purdue University Agricultural Experiment Station, Lafayette, Ind.
[2] Present address: Hormel Institute, University of Minnesota, Austin, Minn.

TABLE I

Effect of Ascorbic Acid on Tocopherol Loss and Length of Induction Period of Lard at 75°C.

Sample	Initial			Induction period	Final		
	Ascorbic acid	α-Tocopherol			Time	α-Tocopherol remaining	
	(%)	(%)	(μg)	(hrs.)	(hrs.)	(%)	(μg)
1	....	0.15	3100	25	24	9.0	280
2	....	0.3	6000	33	24	10.8	650
3	....	0.45	9000	30	24	12.2	1100
4	0.025	0.15	3150	158	120	10.8	340
5	0.05	0.15	3150	142	120	7.6	240
6	0.1	0.15	3150	119	120	6.3	200
7	0.3	0.15	3150	119	120	5.6	175
8	0.1	0.3	6000	165-178	165	5.0	300
9	0.1	0.45	9000	178-200	165	5.3	475
10	Control lard			1½	.....	.....	.....

While the ascorbic acid had served to "spare" or delay destruction of tocopherol and thereby greatly prolong the induction period, more than 90% of the initial tocopherol had been lost in every case at the end of the induction period. The lowest level (.025%) of ascorbic acid seemed to be more effective than higher levels in prolonging the effect of tocopherol.

Similar experiments with increments of citric acid and tocopherol in lard samples which contained both additives also revealed an optimal level for citric acid as a synergist for α-tocopherol (Table II). Neither citric acid nor ascorbic acid showed any substantial effect on peroxide development when added to lard alone.

TABLE II

Effects of Varying the Amounts of Citric Acid and Tocopherol in Lard Samples Containing Both (75°C.)

Sample	Level of citric acid	Level of tocopherol	Induction period
	%	(%)	(hrs.)
1	0.1	0.025	70
2	0.1	0.05	110
3	0.1	0.1	118
4	0.1	0.3	139
5	0.025	0.1	136[a]
6	0.05	0.1	125
7	0.1	0.1	118
8	0.3	0.1	105

[a] Induction period not ended.

Rate of destruction of ascorbic acid in fat containing α-tocopherol. Five series of 25-ml. Erlenmeyer flasks, containing 1-g. samples of lard and different amounts of ascorbic acid with and without α-tocopherol, were placed in the oven at 75°C. The total amount of unreacted ascorbic acid was determined at intervals. For this determination the entire content of one flask in each series was transferred to a separatory funnel by rinsing with benzene and then with 0.5% aqueous oxalic acid. The separatory funnel was shaken vigorously for about a minute to extract the unreacted ascorbic acid into the aqueous phase. An aliquot of the aqueous phase was analyzed for ascorbic acid by a colorimetric method (8).

The ascorbic acid was oxidized much less rapidly in the presence than in the absence of α-tocopherol (Table III). Similar tests at 100°C. with additions of ascorbic acid and α-tocopherol to lard which had been previously oxidized to a peroxide number of 90 showed the same protective effect of tocopherol upon ascorbic acid in the presence of peroxides (Figure 3). It was evident therefore that these compounds exerted a mutual protective action on each other.

TABLE III

Rate of Oxidation of Ascorbic Acid as Affected by α-Tocopherol in Lard (1-g. samples at 75°C.)

Time of heating[a]	Additives				
	5.5 mg. A.A.[b]	2.75 mg. A.A.	1.5 mg. α-toc.[b] 5.5 mg. A.A.	.75 mg. α-toc. 5.5 mg. A.A.	1.5 mg. α-toc. 2.75 mg. A.A.
(hrs.)	Ascorbic acid found (mg.)				
1½	5.0	2.0	5.0	5.0	2.2
5	4.7	1.8	4.9	5.1	2.2
11	4.4	1.5	4.9	4.9	2.0
23	3.2	....	4.3	4.2	2.0
50	1.7	.7	4.1	4.1	1.5
69	1.0	.3	3.6	3.5	1.4
144	nil	nil	3.3	3.2	1.1

[a] Induction period of lard containing ascorbic acid was approximately 4 hours.
[b] A.A. = Ascorbic acid; α-Toc. = d,l-α-tocopherol.

Effect of α-tocopherol on phosphoric acid in autoxidizing lard. In similar oven tests at 75°C. phosphoric acid reacted less rapidly in autoxidizing fat in the presence of α-tocopherol than in its absence (Table IV). The phosphoric acid recoverable by aqueous extraction of the fat was considered to be unreacted. In a previous study (10) it was shown that phosphoric acid reacted with fatty peroxides at 75°C. It

TABLE IV

Recovery of H_3PO_4 From Lard Containing Various Levels of α-Tocopherol (3-g. samples at 75°C.)

Time of heating	Additives				
	.05% P.A.[a]	.1% P.A.	.1% α-Toc.[a] .1% P.A.	.05% α-Toc. .1% P.A.	.1% α-Toc. .05% P.A.
(hrs.)	Phosphoric acid recovered (mg.)				
0	1.57	3.15	3.15	3.15	1.57
½	1.47	3.11	3.00	3.00	1.52
2	1.45	2.80	2.95	2.85	1.35
5	....	2.50	2.65	2.65	1.20
12	.89	1.75	2.65	2.55	1.10
25	.26	1.17	2.55	2.50	1.07
48	....	.95	2.40	2.50	1.07

[a] α-Toc. = d,l-α-tocopherol; P.A. = phosphoric acid.

was evident that α-tocopherol exerted a sparing action on the phosphoric acid, perhaps indirectly by inhibiting the rate of autoxidation and consequent peroxide accumulation.

Quinone-ascorbic acid synergism. In accordance

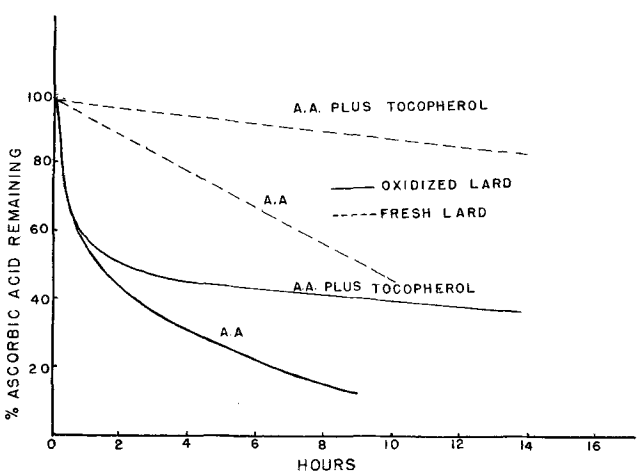

FIG. 3. Sparing action of tocopherol on ascorbic acid in lard in oven tests at 100°C.

with the observations of Calkins and Mattill (4) ascorbic acid was found to be oxidized very quickly in the presence of quinone. Lard containing 0.1% each of ascorbic acid and quinone showed an induction period of 380 hours in the oven tests at 75°C. Only 23% of the ascorbic acid was recovered after the first hour. In another experiment 3.0 milligrams of quinone were dissolved in 2 ml. of absolute methanol containing 3.15 milligrams of ascorbic acid, and the solution was warmed on a steam bath until most of the alcohol evaporated. Only 20% of the ascorbic acid was recovered. In an identical experiment in which α-tocopherol was used in place of quinone all of the ascorbic acid was recovered. These observations suggest that synergism is not involved between ascorbic acid and quinone as such but that intermediates and/or end products of their reaction are involved in a complex mechanism, perhaps involving synergism, which accounts for the inhibitory action of this combination of compounds.

Discussion

The Calkins theory of synergism (3) based on quinone, which is neither antioxidant nor synergist, and phosphoric acid which also presents an unusual case, seems to be limited to a special system and does not lend itself to interpretation of the systems commonly encountered in fats. It fails to explain the activity of phenolic antioxidants, the lack of activity of the fat-soluble organic acids alone, or the potentiating effect of these compounds upon each other.

The concept that a synergist, such as ascorbic acid, functions as a reservoir of hydrogen to regenerate the antioxidant is also untenable in view of the data presented. One of its premises (7) was the rapid disappearance of ascorbic acid in oxidizing fat, its even more rapid disappearance when quinone was added, and the assumption that tocopherol would act in a manner similar to quinone. This assumption was not found to be valid since loss of ascorbic acid was markedly slowed by the addition of tocopherol (Figure 3) in both fresh and partially oxidized lard.

Increments of either citric or ascorbic acid with a constant level of tocopherol progressively shortened the induction period (Tables I and II), instead of lengthening it as a larger reservoir should be expected to do.

With a given level of tocopherol in autoxidizing fat, both ascorbic and citric acids showed their greatest stabilizing effect at the lowest level (.025%). Evidently these synergists, like the phenolic antioxidants, are capable of performing a dual role of retarding autoxidation at low levels and accelerating autoxidation at higher levels. This suggests a greater similarity of function between antioxidant and synergist than has been hitherto envisaged.

In this connection let us consider the behavior of tocopherol in its well known pro-oxidant role at the higher levels. α-Tocopherol was shown to catalyze the decomposition of peroxides *in vacuo* (11), presumably in the same manner as in the presence of air. Yet the tocopherol was not destroyed by these products of decomposition. And both citric and ascorbic acids inhibited the catalytic action of tocopherol as well as NDGA and hydroquinone (13) on peroxide decomposition. Perhaps this provides a clue to the real function of the synergist—that of an inhibitor in the decomposition of peroxides induced by the antioxidant. A reduced form of free radical formed by, but not destructive of, tocopherol probably would change rapidly in contact with air to forms destructive of the antioxidant and synergist alike. Only an inhibitor for the catalysis of peroxide decomposition would save the antioxidant from indirect self-destruction in a system involving chain reactions.

Phosphoric acid and its derivatives appear to warrant special consideration. While it is possible that they may perform the same function as other synergists, they are also capable of reacting directly with peroxides to form products which do not impair the effectiveness of tocopherol (9, 10). When these compounds are present in sufficient concentration, fats can absorb large amounts of oxygen without accumulating substantial titratable peroxides. One of the products is an insoluble phosphorus-containing substance, presumably polymeric in nature.

One of the revelations of this study which invites further attention is the diminishing effect of increments of ascorbic and citric acids at high levels. A complete investigation as to the general occurrence of this effect may be hampered by low solubilities of many compounds which act as synergists with antioxidants. Nevertheless its further study may provide substantial aid to an eventual understanding of the complex process of antioxygenesis in fats.

Summary

Citric and ascorbic acids delayed substantially the rate of peroxide accumulation, during the induction period, of lard which contained pro-oxidant levels of α-tocopherol or NDGA. While low levels (.025%) of both acids showed effective synergistic action with α-tocopherol, higher levels were proportionately less effective.

Tocopherol had a marked protective effect on ascorbic and phosphoric acids in autoxidizing lard.

Evidence indicates that ascorbic and citric acid function as synergists in natural fats and oils by inhibiting the antioxidant catalysis of peroxide decomposition. This concept is discussed in relation to current theories of the mechanism of synergist action.

REFERENCES

1. Bolland, J. L., and Gee, G., Trans. Faraday Soc., *42*, 236 (1946).
2. Bolland, J. L., and ten Have, P., Trans. Faraday Soc., *43*, 201 (1947)
3. Calkins, V. P., J. Am. Chem. Soc., *69*, 384 (1947).
4. Calkins, V. P., and Mattill, H. A., J. Am. Chem. Soc., *66*, 239 (1944).
5. Farmer, E. H., Bloomfield, Sundralingam, A., and Sutton, D. A., Trans. Faraday Soc., *38*, 348 (1942).
6. Golumbic, C., Oil and Soap, *19*, 181 (1942).
7. Golumbic, C., Oil and Soap, *23*, 184 (1946).
8. Morell, S. A., Ind. Eng. Chem., *13*, 793 (1941).
9. Privett, O. S., and Quackenbush, F. W., "Studies on the Antioxygenic Properties of Wheat Germ Phosphatides," manuscript in preparation.
10. Privett, O. S., and Quackenbush, F. W., "Functions of Phosphoric Acid in the Autoxidation of Fat," manuscript in preparation.
11. Privett, O. S., and Quackenbush, F. W., "Effects of Antioxidants on the Thermal Decomposition of Fat Peroxides *in Vacuo*," manuscript in preparation.
12. Biological Antioxidants, Trans. First Conference, p. 16 (1946). Publisher: Josiah Macy Jr. Foundation.
13. Robertson, A., and Waters, W. A., Trans. Faraday Soc., *42*, 201 (1946).

[Received September 17, 1953]