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Effects of Antioxidants on the Thermal Decomposition of Fat Peroxides in Vacuo[1]

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THE significance of peroxide decomposition in the processes of autoxidation and antioxygenesis has received considerable attention in the last few years. Robertson and Waters (10) observed that the presence of tetralin hydroperoxides increased the susceptibility of tetralin to autoxidation. They suggested that the tetralin hydroperoxides decomposed with the formation of free radicals. Bolland and his associates (1) also favored the view that hydroperoxides decomposed into radical fragments. Swift (11), in his studies on the effect of the addition of methyl hydroperoxide-oleate on the autoxidation of methyl oleate, concluded that hydroperoxides either promoted the formation of radical centers in oxidizing methyl oleate or decomposed with the formation of free radicals. He also observed that the thermal decomposition of methyl hydroperoxide-oleate was accelerated when mixed with 42% of α-tocopherol.

In this laboratory we have previously shown that phosphoric acid and its derivatives can accelerate decomposition of fat peroxides (9). In the present paper the effects of some well-known antioxidants and synergists on thermal decomposition of fat peroxides are presented.

Experimental

The role of α-tocopherol in the decomposition of lard peroxides heated in vacuo at 100°C. A portion (0.84 g.) of oxidized lard was introduced by means of a calibrated glass tube (1 mm. i.d.) into a series of 25-ml. Erlenmeyer flasks, the necks of which previously had been constricted and sealed to adapters fitted with 10/30 ₸ ground glass joints. A known amount of α-tocopherol dissolved in petroleum ether (b.p. 40-60°C.) was introduced in the same manner. As much as possible of the solvent was removed with the aid of a water aspirator. After the walls of the adaptor were constricted to capillary size, for subsequent sealing, the flasks were placed on a high vacuum line (less than 10 microns pressure). In each experiment five to 10 samples were degassed for at least four hours before the flasks were sealed at the point of constriction.

It was found that the Emmerie-Engel reaction (3) could be used for the estimation of α-tocopherol when the Parker-McFarlane sulfuric acid modification (8) for removal of interfering substances was employed.

Peroxides were determined by the Wheeler method (12) modified in this laboratory for use with a small sample (0.1 g.).

Tocopherol analyses and the rate of peroxide decomposition at various levels of added α-tocopherol are shown in Table I. As the concentration of α-tocopherol was increased to pro-oxidant levels, the rate of peroxide decomposition was accelerated without any appreciable loss of the tocopherol, once the tests were started. Most of the destruction of the α-tocopherol occurred immediately on the addition of the petroleum ether solution to the oxidized lard before the vacuum was established. There was no concurrent change in the peroxide number. A greater loss occurred at the higher concentrations of tocopherol in accordance with pro-oxidant action in actively oxidizing conditions (5). Thus it was evident that α-tocopherol a) was quickly destroyed at room temperature by substances formed in the course of active oxidation, b) was not oxidized by the accumulated

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TABLE I

The Catalytic Effect of α-Tocopherol on Peroxide Decomposition in Oxidized Lard *in vacuo* at 100°C.

Time of heating	None	Level of d,l-α-Tocopherol added							
		0.013%		0.051%		0.12%		0.98%	
		P.V.[a]	α-Toc.[a] found	P.V.	α-Toc. found	P.V.	α-Toc. found	P.V.	α-Toc. found
(hrs.)			%		%		%		%
0	94	94	.002	100	.027	86	.058	90	.725
2	....	....	....	....	....	....	....	50	.676
3	....	....	....	....	....	62	.063	....	....
4	89	88	.003	78	.026	....	....	....	....
6	....	....	....	....	....	....	....	15	.640
8	....	....	....	....	....	33	.062	....	....
10	59	56	.002	38	.026	....	....	0	.684
24	25	19	....	15	.026	9.5	.066	....	....
36	7	5	.003	2	.027	0	.063	0	.667

[a] α-Toc. = α-tocopherol; P.V. = Peroxide value, expressed as millimoles of oxygen per kilogram of fat.

peroxides *in vacuo* even at 100°C., and c) catalytically accelerated the rate of peroxide destruction. The catalytic action was not an "all or none" reaction depending on concentration but became more pronounced with each successive increase in tocopherol concentration.

The effect of synergists on the in vacuo, α-tocopherol-catalyzed decomposition of peroxides. Preliminary tests carried out under the same conditions as described above showed that ascorbic acid and citric acid exerted a well-defined inhibitory action on the catalytic effect of α-tocopherol (Tables I and II). These acids had little or no effect on the rate of peroxide decomposition when added alone.

TABLE II

Effect of Ascorbic and Citric Acids on α-Tocopherol-catalyzed Decomposition of Peroxides in Oxidized Lard *in vacuo* at 100°C.

Time of heating	0.92% α-Toc.[a] 0.1% A.A.[a]		0.92% α-Toc. 0.1% C.A.[a]		0.1% A.A.	0.1% C.A.
	P.V.[a]	α-Toc. found	P.V.	α-Toc. found	P.V.	P.V.
(hrs.)		%		%		
0	98	0.78	102	0.78	108	105
5½	71	0.68	89—	0.66	....	....
10	55	0.66	65	0.66	73	70
24	22	0.68	28	0.66	43	50
36	5	0.66	8	0.66	18	21

[a]A.A. = Ascorbic acid; C.A. = Citric acid; α-Toc. = d,l-α-tocopherol; P.V. = Peroxide value (millimols/kg. of fat).

In another series of tests conducted in a similar manner, but under slightly different conditions of temperature (96°C.) and pressure (0.2-0.3 mm. Hg.), the relative effectiveness of various concentrations of ascorbic acid to inhibit the α-tocopherol-catalyzed decomposition of peroxides was studied. Ascorbic acid was most effective at 0.025%, which was the lowest level used (Table III). When the level of ascorbic acid was kept constant and the amount of α-tocopherol was varied, the effectiveness of ascorbic acid to inhibit catalyzed peroxide decomposition diminished as the concentration of α-tocopherol was increased (Table IV).

TABLE III

Effect of Various Concentrations of Ascorbic Acid on the α-Tocopherol-catalyzed Decomposition of Peroxides in Oxidized Lard *in vacuo* at 96°C.

Time of heating	None	Additives			
		No A.A.[a] 0.75% α-Toc.[a]	0.025% A.A. 0.75% α-Toc.	0.12% A.A. 0.75% α-Toc.	0.35% A.A. 0.75% α-Toc.
		[a] P.V. (mo. mols./kg.)			
(hrs.)					
0	120	120	120	120	120
4-5	102	60	98	88	81
10-11	77	30	62	50	44
16	....	20	....	....	....
20-22	57	....	38	30	15

[a]A.A. = Ascorbic acid; α-Toc. = d,l-α-tocopherol; P.V. = Peroxide value.

Investigation of other phenolic antioxidants. It was found that NDGA (nordihydroguaiaretic acid) and hydroquinone performed in essentially the same manner as α-tocopherol in the experiments described above. The rate of thermal decomposition of peroxides was accelerated in the *in vacuo* experiments as the concentration of NDGA and hydroquinone were increased to pro-oxidant levels (Table V).

TABLE V

Effect of Various Concentrations of NDGA and Hydroquinone on the Rate of Peroxide Decomposition in Oxidized Lard Heated *in vacuo* at 100°C.

Time of heating	N.D.G.A.			Hydroquinone		
	0.01%	0.1%	0.5%	0.01%	0.1%	0.45%
(hrs.)	P.V.(m. mols./kg.)					
0	109	109	109	119	120	118
4	90	79	7		75	15
10	58	29	0	53	36	0
24	22	10.5		30	15	
36	14	4		6.5	3.5	

To determine whether NDGA and hydroquinone functioned as catalysts in peroxide decomposition (as was found in the case of α-tocopherol) an attempt was made to employ the Emmerie-Engel iron-bipyridyl color reaction (3) for their estimation as devised by Lundberg *et al.* (7). Although hydroquinone and NDGA ordinarily can be extracted from either fresh or oxidized lard by successive extractions with 80% ethanol, only a small fraction of the original amount added was recoverable by this method in the *in vacuo* heating experiments. However it was found that the petroleum ether solution (epiphase) in a separatory funnel gave a strong positive test for their presence while the hypophase representing a second extraction with 80% ethanol gave a negative test. Attempts to apply the iron-bipyridyl reaction directly to a petroleum ether and a chloroform solution on a quantitative basis were unsuccessful due to interfering substances, but strong qualitative reactions indicated that the antioxidants were still present. The hydroquinone color reaction, normally complete in 10-15 minutes, was still increasing in intensity after 20 minutes in these tests, indicating nearly 100% recovery of the hydroquinone at this time. Thus it appeared that these antioxidants also were acting as catalysts in the reaction. The presence of either ascorbic or citric acid also greatly diminished the effect of these antioxidants (Table VI) as in the α-tocopherol experiments. Both ascorbic and citric acids

TABLE IV

Effect of Ascorbic Acid With Increments of α-Tocopherol on the Decomposition of Peroxides in Oxidized Lard *in vacuo* at 96°C.

Time of heating	None	Additives					
		.15% α-Toc.[a]	.12% A.A.[a] .15% α-Toc.	.30% α-Toc.	.12% A.A. .30% α-Toc.	.75% α-Toc.	.12% A.A. .75% α-Toc.
		P.V.[a](m. mols./kg.)					
(hrs.)							
0	120	120	120	120	120	120	120
4-5	103	86	100	75	....	60	88
10-11	77	58	74	48	65	30	50
16	....	....	....	....	....	20	....
20-22	57	38	49	30	40	....	30

[a]A.A. = Ascorbic acid; α-Toc. = d,l-α-tocopherol; P.V. = Peroxide value.

TABLE VI

Effects of Ascorbic Acid and Citric Acid on NDGA and Hydroquinone Catalysis of Peroxide Decomposition in Oxidized Lard *in vacuo* at 100°C.

Time of heating	None	Additives							
		.5% NDGA[a]	.1% A.A.[a] .5% NDGA	.5% A.A. .5% NDGA	.1% C.A.[a] .5% NDGA	.5% C.A. .5% NDGA	.5% Hq.[a]	.12% A.A. .5% Hq.	.077% C.A. .5% Hq.
(hrs.)		P.V.[a] (m. mols./kg.)							
0	200	168	200	200	200	200	118	145	145
2-2.5	145	14	138	130	100	77	47	....	....
4-5	97	0	95	62	91	40	15	90	80
12	56	....	40	30	38	10	0	57	40
24	14	....	6	2	9	....	....	8	9

[a]A.A. = Ascorbic acid; C.A. = Citric acid; NDGA = Nordihydroguaiaretic acid; Hq. = Hydroquinone; P. V. = Peroxide value.

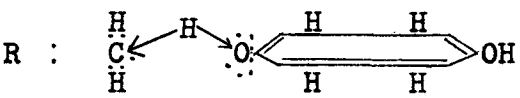
appeared to show their greatest effectiveness at low levels.

Discussion

Although Swift was using a 42% concentration of α-tocopherol when he observed that this compound accelerated the rate of peroxide decomposition, the evidence presented here has shown that even the low levels of α-tocopherol as well as NDGA and hydroquinone used for antioxidants exerted a marked increase in the rate of *in vacuo,* thermal decomposition of fat peroxides. It was further shown, in this investigation, that these antioxidants function as catalysts since they persisted substantially throughout the reaction and became progressively more effective as their concentration was increased.

Since the antioxidants studied here are relatively stable in oxidized lard heated *in vacuo,* it appears that under actively oxidizing conditions, they must be oxidized by a reactant formed directly in the reaction between fat and oxygen. It is also evident that, because of its great reactivity, this reactant does not accumulate to any extent in oxidizing fat. Bolland and ten Have (2) in their studies on the action of hydroquinone in the stabilization of ethyl linoleate concluded that peroxide radicals react with the antioxidant to effect its oxidation. Perhaps an oxidizing fat contains two or more forms of peroxide radicals which differ widely in their ability to react with an antioxidant. Polarographic studies (6) have shown evidence for three different reducible substances in autoxidizing lard. Whether any of these may bear a relation to the rapid initial oxidation of added antioxidant is not established.

Evidently the same peroxide radicals do not form during decomposition of peroxides to their more stable end-products, otherwise the antioxidants would be oxidized in the *in vacuo* heating of oxidized lard. On the other hand, the formation of hydrocarbon radicals, similar to those believed to be formed under actively oxidizing conditions (4), during decomposition of peroxides is plausible and has been visualized by other investigators as mentioned previously. The oxidative state of these alkyl-type of radicals is too low to effect an oxidative attack on a phenolic antioxidant, but a loose chemical combination may occur through a hydrogen bond in the absence of oxygen. Example of such a combination with hydroquinone is represented below:



The inextractability of hydroquinone and NDGA from petroleum ether by 80% ethanol in the *in vacuo* experiments is convincing evidence that the antioxidant has bonded with a product of autoxidation, perhaps a hydrocarbon radical as shown above or more strongly with an OH or peroxide group.

Summary

1. The antioxidants, α-tocopherol, NDGA and hydroquinone, effected a marked acceleration on the *in vacuo,* thermal decomposition of fat peroxides. Their action, which appeared to be purely catalytic, increased progressively as their concentration was increased.

2. The synergists, citric and ascorbic acids, exerted no influence on the rate of decomposition of the fat peroxides. However these agents suppressed the catalytic effect of the antioxidants to varying degrees. Both citric and ascorbic acids exerted their greatest influence at relatively low levels.

3. The evidence lends support to the concept that the peroxide radical is the point of intervention by the antioxidant in the chain reaction of autoxidation.

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