An Antagonistic Effect with Antioxidants for Unsaturated Fats[1]

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DURING A STUDY of the effect of various antioxidants on marine oils it was observed that a mixture of α-tocopherol (α-T)[2] and EMQ[2] provided much less protection than did EMQ alone. Furthermore an oil which had been stabilized with EMQ could be induced to begin to oxidize during the induction period by the addition of α-T. These effects were observed at lower concentrations of α-T than are needed to demonstrate its known pro-oxidant effect when used in excessive amounts. The term "antagonism" is used in discussing this phenomenon, in contrast to "synergism" which describes a more than additive effect with mixtures of antioxidants (1).

Previous observations of an antagonistic effect have been limited or poorly documented. Fisher *et al.* (2) noted that α-T depressed the antioxidant activity of NDGA and norconidendrin, and Kraybill and Dugan (3) reported that mixtures of α-T with EMQ, BHA, NDGA, and DPPD exhibited "negative synergism" in lard under the conditions of the AOM test.

Experimental

Oxidation of oils was followed by the weighing technique described recently (4). Briefly 200 mg. or 1-g. samples in 10- or 50-ml. beakers containing the antioxidants were held in constant-temperature ovens. At daily intervals the beakers were removed, allowed to cool, and weighed. The end of the induction period was detected by an easily identified, accelerating increase in weight. The data are shown in graphical form in Figures 1 and 2, or they are presented as the length of the induction period, arbitrarily chosen as the elapsed time from the start of the experiment until the samples have gained 0.4% in weight. At this point most mixtures had begun to gain weight rapidly and were rancid in odor.

EMQ was a redistilled sample of a commercial product (Monsanto Chemical Company) made available through the courtesy of C. R. Thompson, Western Regional Research Laboratory, U.S.D.A., Albany, Calif. DPPD was a commercial product (Goodrich Chemical Company), which was crystallized twice from alcohol. The other antioxidants were used as obtained.

The tocopherols were Eastman Chemical Company products. Marine oils were furnished through the courtesy of the U. S. Fish and Wildlife Service. The menhaden oil sample was especially prepared from fresh fish and obtained through the courtesy of M. Bender, College Park, Md. Purified esters, acids, and trilinolein were obtained from the Hormel Institute, Austin, Minn.

Results

The effect of adding α-T to a menhaden oil containing 0.05% EMQ is illustrated in Figure 1. With

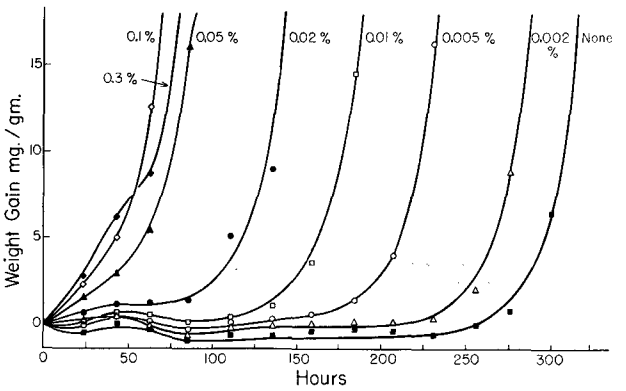

FIG. 1. Effect of added α-tocopherol on the induction period of menhaden oil containing 0.05% EMQ (50°C.). The numbers refer to the concentration of α-T.

increasing concentration the induction period decreased until the protection afforded approximated that afforded by α-T alone (*cf.* Figure 2). Both series of runs also illustrate the pro-oxygenic effect of the larger amounts of α-T. Similar data qualitatively were obtained with anchovy, sardine, and tuna oils.

A separate series was run in which γ-T was substituted for α-T. The γ-T was a more effective antioxidant than α-T when used alone, and the differences between the induction periods of the samples with EMQ and those containing EMQ plus γ-T are therefore not as striking as in the EMQ-α-T series. The data for menhaden oil are recorded in Table I.

An attempt was made to determine whether other phenolic inhibitors would antagonize EMQ in the same manner as did α-T and γ-T. The substrate was menhaden oil at 50° and the antioxidants included EMQ plus PG, NDGA, BHA, BHT, 2246, and THBP. In no case was antagonism demonstrable to the ex-

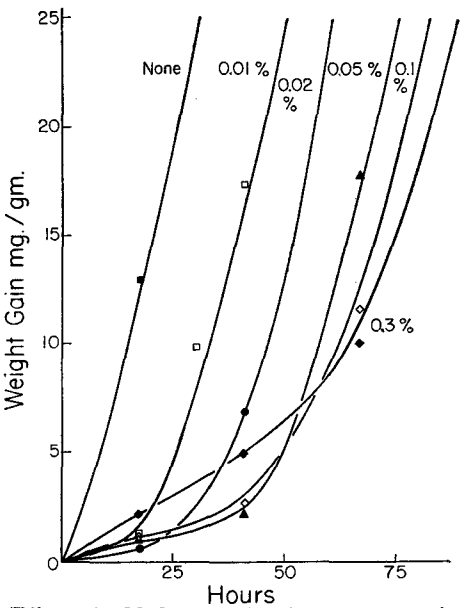

FIG. 2. Effect of added α-tocopherol on the rate of oxidation of menhaden oil. The numbers refer to the concentration of α-T.

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[2] Abbreviations used are as follows: EMQ, 6-ethoxy-2,2,4-trimethyl-1, 2-dihydroquinoline (Santoquin); α-T, d-α-tocopherol; γ-T, d-γ-tocopherol; DPPD, diphenyl-p-phenylenediamine; NDGA, nordihydroguaiaretic acid; BHA, 2(or 3)-tertiarylbutyl-p-cresol; BHT, 2,6-di-tertiarybutyl-p-cresol; PG, propyl gallate; 2246, 2,2methylene-bis (4-methyl-6-tertiary butylphenol); THBP, 2,4,5-trishydroxy-butyrophenone; ADPA, 4-amino-p-diphenylamine; AOM, active oxygen method.

TABLE I

Effect of Mixtures of EMQ and γ-T on Induction Period of Menhaden Oil at 50°C.

Gamma-tocopherol added %	Induction period—days	
	With 0.05% EMQ	Without EMQ
0	7.5	0.5
0.01	5.5	1
0.02	4.5	1.5
0.05	4	3
0.10	4	4

tent seen with the tocopherols. A mixture of 0.02% BHT and 0.02% EMQ had an induction period of 4.5 days compared to 5 with 0.02% EMQ alone, but the other mixtures all had induction periods of more than 5 days. Thus the tocopherols appear to be somewhat unique in their antagonistic effect to EMQ.

It was desirable to determine whether the tocopherols would antagonize antioxidants other than EMQ. A limited survey indicated the most active antagonism was limited to the nitrogen-containing inhibitors, EMQ, DPPD, and ADPA. Representative data are shown in Table II.

TABLE II

Effect of Mixtures of α-T with Other Antioxidants on the Induction Period of Menhaden Oil at 50°

Additive[a]	Induction period—days	
	With 0.1% α-T	Without α-T
0	2	0.5
0.1% EMQ	2.5	24
0.1% DPPD	2	23
0.1% ADPA	2	10
0.1% BHA	3.5	4.5
0.1% BHT	3	2
0.1% NDGA	10	16
0.1% PG	11	7

[a] See footnote 2 for explanation of abbreviations.

TABLE III

Effect of EMQ, α-T, and EMQ Plus α-T on the Induction Period of Purified Substrates

Substrate	Temp. °C.	Conc. of additives %	Induction period—days			
			Additives			
			None	EMQ	α-T	EMQ+ α-T
Ethyl oleate	60	0.01	6	14	9	11
Methyl linoleate	40	0.05	0.5	17.5	1.4	10
Methyl linolenate (95%)	40	0.05	0.5	10.5	2.5	5.5
Methyl arachidonate	40	0.10	0.5	11	3.5	4
Linoleic acid	40	0.05	0.5	6.5	1	5.5
Linolenic acid	40	0.05	0.5	8	1.5	6
Trilinolein	50	0.02	1	23	10.5	12

The effect of mixtures of EMQ and α-T on the induction period of purified substrates is compared in Table III. These data show that the antagonistic phenomenon can be demonstrated with unsaturated fatty esters, acids, and a triglyceride, and therefore that it is not likely to be mediated through other substances which generally occur in natural oils, such as traces of heavy metals, phospholipides, etc., although these would undoubtedly influence the quantitative aspects.

Discussion

The mechanism by which the tocopherols act as pro-oxidants in the presence of the nitrogenous antioxidants is obscure. Privett and Quackenbush (5) suggest that tocopherols, in addition to their antioxidants or chain-breaking action, may act as catalysts for the decomposition of peroxides to form new chain-promoting radicals. No exidence is as yet available to determine whether this mechanism is operative in the experiments described.

Recognition of the ability of tocopherols both to promote and antagonize the effects of other antioxidants will help to explain further the markedly different effects of antioxidants in animal and vegetable oils since the latter often contain substantial amounts of natural tocopherols. Preliminary results show that EMQ at a level of 0.01% had no antioxidant effect on samples of refined olive, peanut, cottonseed, or corn oil at 60°, suggesting that the antioxidant effect of EMQ is neutralized by the tocopherols in these oils.

Further work on factors which influence the effects of antioxidants on natural oils is in progress.

Summary

α- and γ-tocopherol act as pro-oxidants with marine oils containing the antioxidant EMQ. The term "antagonism" is suggested for such phenomena. Antagonism was also demonstrable in purified unsaturated fatty ester acids and triglycerides. Nitrogenous inhibitors (EMQ, DPPD, ADA) are more affected by added tocopherols than are the phenolic antioxidants examined (BHT, BHA, PG, NDGA). Phenolic antioxidants other than tocopherols do not show marked antagonism with EMQ.

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