The Autoxidative Behavior of Vegetable and Animal Fats*

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Vegetable fats in general possess certain autoxidative characteristics which differ markedly from those of animal fats. In contrast to the latter, vegetable fats have no sharply defined induction period in which slow absorption of oxygen with gradual accumulation of peroxides changes swiftly to a period of rapid oxygen absorption with concomitant development of oxidative rancidity. Instead, they exhibit organoleptic rancidity before any marked increase in oxygen uptake and peroxide oxygen appears (1). Also unlike animal fats, vegetable oils are not as readily stabilized by tocopherols which are themselves obtained from vegetable fat sources (1, 2).

In seeking an explanation for these differences, attention has been focused on the occurrence of tocopherols in natural fats; they have been found in a wide variety of vegetable fats but are much less commonly found in fats of animal origin (1, 3). If the tocopherols are responsible for the characteristic behavior of vegetable fats during autoxidation, their functional activity as inhibitors would be expected to reside in the oxidative changes they undergo during the process. Little is known of these changes except that they lead to total loss of vitamin E activity (4, 5) and disappearance of tocopherol (6).

The experiments to be reported here have demonstrated that during the autoxidation of vegetable fats both tocoquinones and chroman-5,6-quinones are formed, whereas in autoxidizing animal fats containing added tocopherol, only tocoquinones are detectable. The chroman-5,6-quinones, unlike the tocoquinones, are fat antioxidants (7); as has been described in detail elsewhere (8) they arise from some source other than the oxidation of tocopherols. The kinetics of this oxidation have now been studied by the current methods available for the determination of tocopherol (9) and its quinone oxidation products (10, 11).

Experimental

The animal and vegetable fat substrates were the ethyl esters of the fatty acids of lard and of hydrogenated cottonseed oil prepared as previously described (1). Their induction periods were measured by the oxygen absorption method at 75° (12). The cottonseed oil esters already contained adequate amounts of tocopherol; to the lard esters, α-tocopherol was added in the form of a synthetic product.[1]

Samples were removed frequently during the induction period and analyzed for tocopherol by the Emmerie and Engel method (9) and for total tocopherol plus quinones by the Furter and Meyer method (10, 11). The difference represents the quinone forms, both tocoquinone and chroman-5,6-quinone. When both these forms were present, the chroman-5,6-quinone was determined by omitting the nitric acid oxidation in the Furter and Meyer method.

The oxidation of α-tocopherol in the animal fat substrate is illustrated in Figure 1. The tocopherol (curve A) is rapidly oxidized during the progress of the induction period, which ends at X. During the early part of the induction period, it is converted almost quantitatively into steadily increasing amounts of tocoquinone (curve B). As the induction period proceeds, this relation no longer holds. Instead of showing a progressive rise the quinone concentration

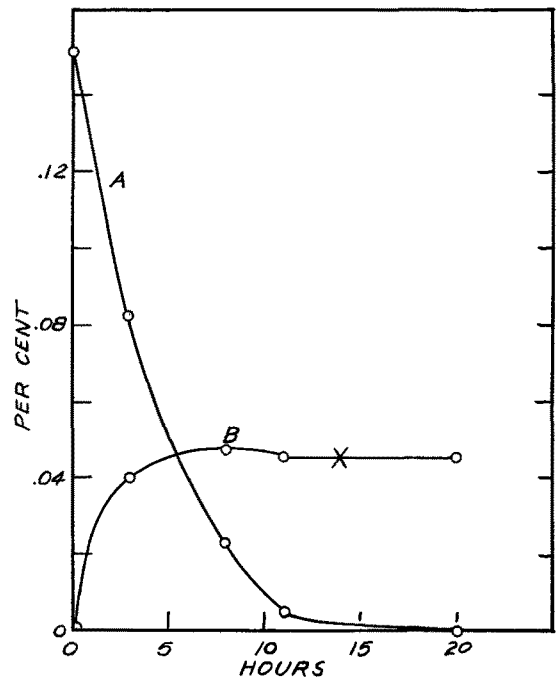

FIG. 1. The oxidation of α-tocopherol in the ethyl esters of lard fatty acids at 75°.

A, α-tocopherol.
B, tocoquinone.
X, end of induction period (oxygen absorption method).

attains a maximum and thereafter appears not to change even beyond the end of the induction period. On the other hand, the tocopherol continues to disappear rapidly and when its oxidation is complete, the induction period comes to an end.[2] At this point

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[1] From α-tocopherol acetate generously furnished by Hoffman-La-Roche, Inc.

[2] In this connection it should be emphasized that the action of this type of antioxidant (tocopherols and inhibitols) is not merely that of a reducing agent. The autoxidation of unsaturated fatty acids is generally held to be a chain reaction in which activated molecules of fat peroxides pass their energy on to unactivated fat molecules, thus initiating reaction chains. When tocopherol acts as an antioxidant it is not merely being oxidized to tocoquinone (and to further but unknown products) but at the same time, it reduces the "active" peroxides, and thereby breaks the reaction chains that would otherwise have been started. As long as tocopherol is present, peroxides do not accumulate in amount sufficient to cause oxidative formation of the secondary products that characterize rancidity.

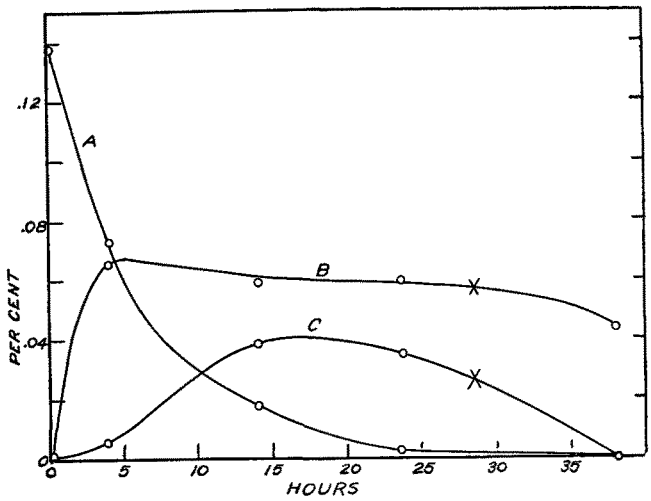

FIG. 2. The oxidation of tocopherol in the crude ethyl esters of hydrogenated cottonseed oil at 75°.

A, tocopherol.
B, tocoquinone.
C, chromane-5,6-quinone.
X, end of induction period (organoleptic).

about 70% of the initial tocopherol has been oxidized to substances other than tocoquinone. No attempt was made to identify these products but additional evidence for the presence of tocoquinone was obtained by subjecting the rancid fat mixture to the combined action of a reducing and a cyclizing agent. Thereafter the unsaponifiable fraction of the fat possessed antioxidant properties for lard, indicating the regeneration of tocopherol from tocoquinone.

Spectrophotometric measurements revealed the absence of the red chroman-5,6-quinone in autoxidizing animal fats but appreciable quantities of it always appeared during the induction period of cottonseed oil esters (Figure 2). It is formed rather slowly at first (curve C) and soon reaches a maximum concentration. Later, it gradually disappears but it is not completely destroyed until some time after the termination of the induction period (designated by X). The behavior of tocopherol and tocoquinone (curves A and B) is similar to that observed in the lard esters.

The relatively slow oxidation rate of the chroman-5,6-quinone in comparison to tocopherol is even more pronounced in a hydrogenated vegetable fat. As discussed in detail below, this variance in oxidation rates between the two inhibitors offers an adequate explanation for the absence of sharply defined induction periods in vegetable fats. In the specific case studied (Figure 3) a hydrogenated vegetable fat was exposed to air in an oven maintained at 60° and tocopherol, chroman-5,6-quinone and peroxide contents were determined at weekly intervals. The peroxide curve (A) follows the S-shaped pattern typical of an autocatalytic reaction; the initially slow peroxide development is succeeded by a phase of rapidly increased peroxide production. Organoleptic rancidity (designated by X on curve A) was detected after the twenty-fifth day, yet the beginning of rapid peroxide formation did not occur until after the fiftieth day. In the intervening period of twenty-five days peroxide formation continued at the same slow rate as in the early stages of the oxidation. The tocopherols alone (curve B) cannot account for this interval of retarded peroxide development because they had disappeared before the thirty-fifth day. The only other known antioxidants present, the chroman-5,6-quinones (curve C), must therefore be responsible for the continued slow rate of peroxide formation. When their concentration began to diminish after about the forty-fifth day there was an abrupt rise in peroxide values.

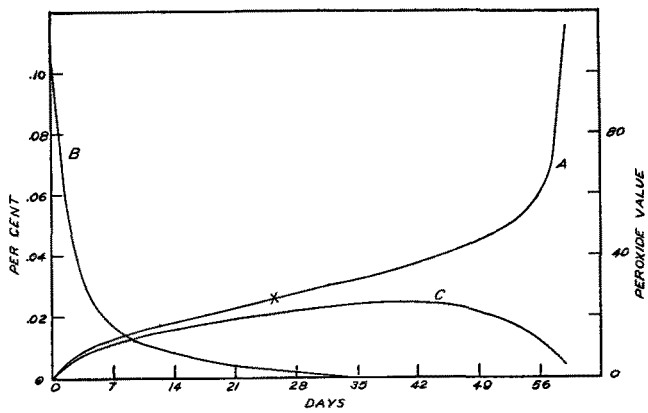

FIG. 3. The autoxidation of a hydrogenated cottonseed oil at 60°.

A, peroxide oxygen.
B, tocopherol.
C, chromane-5,6-quinone.
X, end of induction period (organoleptic).

Effect of Concentration

To study the influence of the concentration of tocopherol on its rate of oxidation, samples of fresh lard containing 0.05%, 0.10%, and 0.20% α-tocopherol respectively were placed in an oven maintained at 60 degrees and the tocopherol and peroxide contents of the fat were determined at frequent intervals. The results plotted in Figure 4 demonstrated that the rate of oxidation of α-tocopherol increased with progressive increase in its initial concentration, but that the time necessary for the oxidation of any given fraction of tocopherol was independent of the initial concentration of tocopherol. This relation is indicative of a first order reaction.

In agreement with the recent observations of Swift, Rose, and Jamieson on cottonseed oil (14) the rate of peroxide formation also became more rapid with progressive increase in the amounts of tocopherol present at the beginning of the induction period (Table I). With the highest concentration of tocopherol (0.20%), and the enhanced development of peroxide oxygen, the stability of the lard began to decrease and the onset of organoleptic rancidity was hastened. However, stability still remained higher than that of the lard without any addition of tocopherol.

Similarly, addition of large amounts of tocopherol to vegetable fats which already contained substantial quantities of tocopherol as a natural constituent increased the peroxide accumulation and shortened the induction period. On the other hand when the naturally occurring tocopherols were removed from vegetable fats by chromatographic adsorption on activated alumina (13), tocopherols added to fats so treated had an antioxygenic action. Above a certain concentration however (0.10% for cottonseed oil esters) further additions of tocopherol were decreasingly effective, as was the case when lard was the substrate.

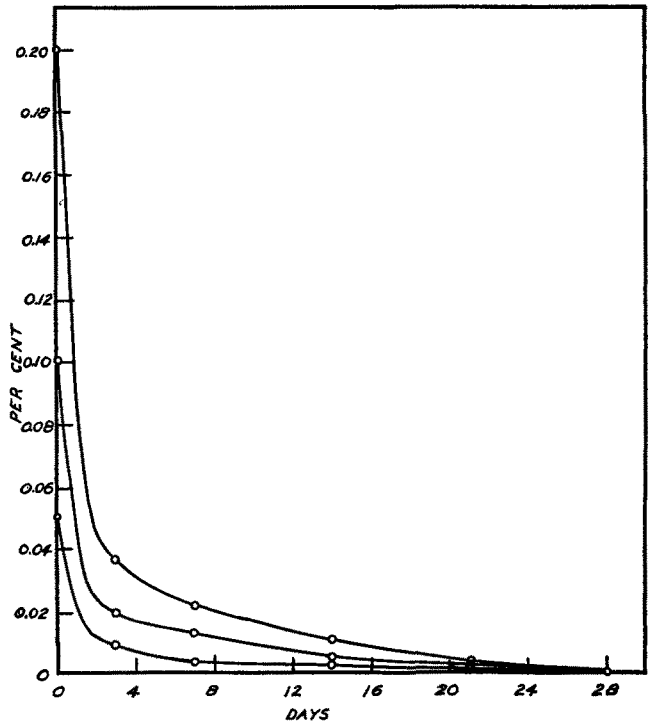

FIG. 4. The oxidation of α-tocopherol in lard exposed to air at 60°.

The optimum concentration of tocopherol for the stabilizing of any given fat can thus not be stated categorically because of the possible presence of other natural inhibitors, and other variables. The presence of more than optimum amounts of tocopherol accomplishes better stabilization than obtains when none is present but the optimum amount may vary from one fat to another.

TABLE I

The Effect of Tocopherol Concentration on the Formation of Peroxides in Lard at 60°.

Time, days	Peroxide Value			
	(No α-tocopherol)	(0.05% α-tocopherol)	(0.10% α-tocopherol)	(0.20% α-tocopherol)
0	1.3			
3	2.5	3.2	6.9	8.8
7	13.7	10.3	17.5	17.7
14	68.3	19.0	25.9	34.4
21		27.7	40.0	54.3
28		61.5	51.8	67.7
Length of induction period, days [1]	11	28	24	19

[1] Determined organoleptically. The shortening of the induction period by high concentrations of α-tocopherol was also observed when the induction period was measured by the oxygen absorption method at 75°.

These observations offer an explanation for the paradoxical results of Olcott and Mattill (1) who found that tocopherol concentrates were ineffective as antioxidants for the vegetable fats from which they were obtained. It is apparent now, that in such fats, the tocopherols are already exerting their maximum antioxygenic activity and that further additions of them yield diminishing returns in stabilization.

The demonstration that the maximum antioxygenic activity of tocopherols is related to their concentration raises an interesting question. Is the tocopherol content of plant fats merely fortuitous or is it regulated by the metabolic processes of the plant so as to provide for the maximum stability of the fat? No answer can yet be given but if the former circumstance is true, some vegetable fats may contain more than enough tocopherol to insure maximum protection against autoxidation.

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Summary

The kinetics of the oxidation of tocopherol (vitamin E) during the induction period of animal and vegetable fats were studied by photometric methods. The concentration of tocopherol and the nature and origin of the substrate fat markedly influence the course of the oxidation.

Tocoquinones were recognized as the immediate oxidation products of tocopherols in animal and vegetable fats. Chroman-5,6-quinones also appeared during the course of the induction period of the vegetable fats but never during the autoxidation of animal fats even when tocopherol had been added to them. These antioxygenic o-quinones retarded the accumulation of fat peroxides in vegetable fats after the complete disappearance of the tocopherols. The successive action of these two antioxidants explains the absence of sharp induction periods in vegetable fats.

When employed at higher levels, tocopherols are decreasingly effective as antioxidants. This accounts for the previously recognized ineffectiveness of tocopherols and inhibitol concentrates when added to vegetable fats.

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