Antioxidants and The Autoxidation of Fats

V. Mode of Action of Anti and Pro-Oxidants

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THE prevention of rancidity in edible fats and oils is a problem of tremendous practical importance, yet except for empirical studies of the antioxygenic activity of numerous compounds, the subject has scarcely been touched. Many helpful facts are, however, available by analogy from related and more intensely investigated fields, such as those concerning the drying oils (17), cracked gasoline (9), and unsaturated hydrocarbons (18), etc.

In a previous communication (5), it was pointed out that fundamental data on the oxidation mechanism in fats must be obtained from purified fatty acids and esters rather than from natural fats of complex make-up. Some phases of such investigations (6) have now been completed and will be reported in detail elsewhere. The present paper is concerned particularly with the mode of action of inhibitors and accelerators as revealed by some of the data obtained in that study.

Experimental

The methyl oleate used in these studies was prepared from olive oil by a combination of the methods of Skellon (15) and Raymond (13), and involved (I) removal of saturated acids by a lead salt precipitation, (II) crystallization of the lithium soaps of the unsaturated acids, (III) fractional distillation of the methyl esters of the acids recovered from the lithium soaps, (IV) crystallization, from acetone at —40° C., of the oleic acid obtained from the methyl esters, and (V) reesterification, and refractionation of the methyl esters. The final product contained methyl oleate with a very small amount of methyl palmitate.

An especially devised apparatus (6) was used to measure the rate of oxygen absorption at 80° C. and atmospheric pressure. As the purification described above proceeded, the induction period declined from 14 hours or more to as little as 1 hour. Even this short induction period may have been due to traces of impurities.

Hydroquinone, *a*-naphthol, and an inhibitol concentrate from wheat germ oil[1], were used as antioxidants. The oxygen absorption curves for methyl oleate with and without these inhibitors are shown in Fig. 1. With the exception of D, each curve represents several separate runs, the data of which are superimposable. The crosses mark the places at which runs were discontinued.

In the runs represented by C, D, and E, the carbon dioxide and water formed during the oxidation were not removed from the reaction flask, whereas in those represented by A and B, these products were removed as formed; a fact which probably accounts for the differences in the character of the two groups of curves. The actual amount of oxygen absorbed may have been the same in the two cases. The curves show that the inhibitors had no effect on the rate of absorption of oxygen after the end of the induction period.

The rates of formation of various combined forms of oxygen: peroxide, water, carbon dioxide, aldehyde, free hydroxyl, free and total carboxyl, together with the rate of decrease in unsaturation, were measured during the oxidation of methyl oleate with and without an inhibitor. These data, which are being presented elsewhere, demonstrate that the inhibitors used also had no effect on the course of the oxidation after the end of the induction period.

Our results confirm those of Yamaguchi (21) (22) who studied the oxidation of oleic acid and olive oil with and without inhibitors. His data indicate that the phenolic inhibitors, such as hydroquinone, lengthen the induction period but do not change the rate of destruc-

[1]The natural antioxidant which occurs in the unsaponifiable fraction of wheat germ oil (3). The unsaponifiable fractions of many vegetable fats and oils contain compounds which are effective antioxidants. It is proposed to call these compounds as a class "inhibitols," a name which indicates their function as inhibitors and also the invariable occurrence of hydroxyl groups upon which their inhibiting action depends.

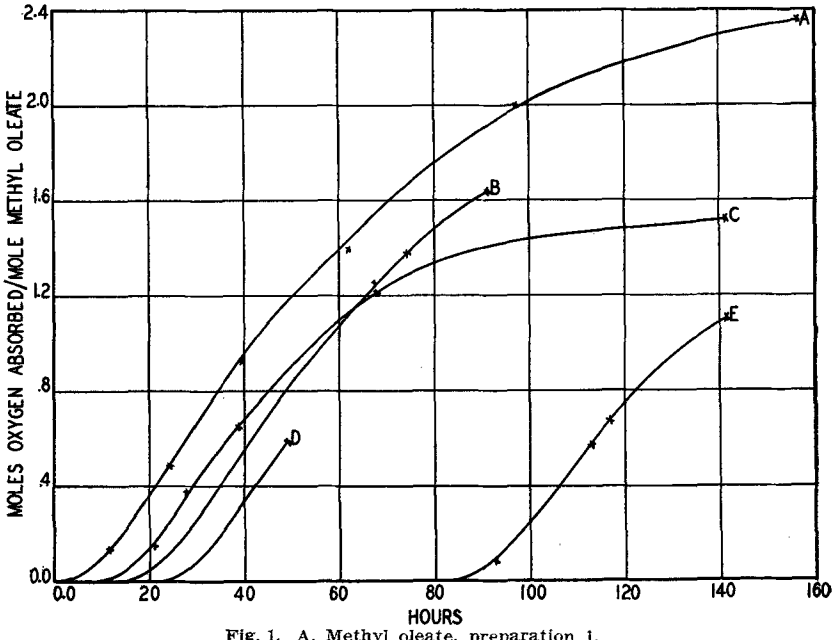

Fig. 1. A. Methyl oleate, preparation 1.
B. Same + 0.0002% W5-10 (inhibitol).
C. Methyl oleate, preparation 2.
D. Same + 0.0001% hydroquinone.
E. Same + 0.00005% *a* naphthol.

tion of the double bond as measured by iodine number[2].

Previous experiments (5) from this laboratory showed that the activity of inhibitol concentrates was completely destroyed at the end of the induction period. Samples of fat protected by such concentrates were allowed to oxidize until the absorption of oxygen was appreciable or a rancid odor could be detected. They were then saponified and extracted. The unsaponifiable lipid fraction was invariably ineffective as an anti-oxidant in fresh samples of lard.

Similar experiments with hydroquinone and *a*-naphthol indicate that these compounds also are destroyed during the induction period.

The activity of inhibitols is also destroyed by peracids. If a small amount of inhibitol concentrate is dissolved in a chloroform solution of perbenzoic acid and allowed to stand cold for 24 hours, the recovered concentrate has no antioxygenic activity.

Discussion

The formation of a reactive moloxide is considered by most authors (16) (12) (4) (20) to be the initial step in the oxidation of unsaturated compounds. The existence of two kinds of peroxides in rancid fat, one more stable than the other, has been suggested by several workers (19) (16). Data from this laboratory (6) indicate that the initial reactive moloxide of oleic acid changes rapidly to a more stable peroxide.

Aldehydes, which are known to be present in rancid fats, occur in small amounts even in the very early stages of oxidation (7) (14). Since aldehydes autoxidize to form acids by a mechanism involving intermediate reactive peracids (2) (1), it seems apparent that at least three types of peroxides are present in oxidizing oleic acid, methyl oleate, or fats containing oleic acid; these are, the original moloxides, the less active peroxides formed from the moloxides, and the reactive peracids.

The mechanism of autoxidation is generally conceived to be a chain reaction initiated by the active peroxide (moloxide). Inhibitol concentrates and phenolic inhibitors apparently prevent the formation of chains of appreciable length.

It is now possible to advance an explanation for the phenomena previously observed (5) concerning the effect of adding inhibitol concentrates to rapidly oxidizing lard-cod liver oil mixtures. Under these conditions the absorption of oxygen ceases temporarily and the peroxide level is slightly depressed. The further the oxidation has advanced, the more antioxidant is required to give the same inhibition.

Apparently the inhibitol destroys the transitory original moloxide and the peracids but not the less reactive secondary peroxides into which the moloxides are normally transformed; therefore only a slight drop in the peroxide level results. Oxygen absorption also stops, for the excess inhibitor over that required to destroy the moloxides and peracids now breaks any new chains before they reach appreciable length.

Some of the inhibitol is destroyed in the reaction with moloxide and peracids. The remainder exerts an antioxygenic effect until the combined destructive reactions of newly forming moloxides and peracids remove it completely, after which the oxidation proceeds as before the addition of inhibitor. Since the concentration of aldehydes is higher in the later stages of oxidation, the formation of peracids and hence the destruction of the inhibitol proceeds with greater rapidity.

Perbenzoic acid is a pro-oxidant in lard (10). However, it has been demonstrated (11) that perbenzoic acid reacts with oleic acid to form an oleic acid oxide. This compound, we have shown, is not readily autoxidizable (6). It would thus appear that the prooxygenic effect of perbenzoic acid must be attributed to the oxidative destruction of inhibitors which are normally responsible for the induction period. In confirmation of this hypothesis, perbenzoic acid exerted only a very slight pro-oxygenic effect on a highly purified sample of oleic acid, presumably almost entirely free from inhibitors.

The carotenoid pigments, carotene, xanthophyll and lycopin, are also pro-oxygenic in lard and other fats and oils (10). In this instance, the pro-oxygenic effect is probably due to the easily formed and highly reactive peroxide derivatives of the carotenoids which are able to initiate reaction chains in the fat. In rapidly oxidizing linolic acid, according to Monaghan and Schmidt, carotene behaves as an antioxidant (8). Under such circumstances, it seems likely that the number of chains initiated by the formation of reactive carotenoid peroxides might be less in number than those broken by reaction of "hot" moloxides of linolic acid with the highly reactive unsaturated bonds of carotene; resulting in a decrease in the rate of oxygen absorption.

Yamaguchi (21) (22) has studied the properties of still another type of pro-oxidant, copper oleate. In oleic acid, this substance (I) greatly decreases the effect of added phenolic inhibitors, (II) inhibits slightly the rate of destruction of unsaturated linkages, (III) increases the amount of carbon dioxide produced, and (IV) increases the amount of oxygen absorbed (by calculation from his data).

Yamaguchi (21) also showed that copper oleate in ether solution greatly accelerates the oxidation of hydroquinone by gaseous oxygen, and suggests that the pro-oxygenic effect of copper oleate in fats may be due to its property of catalyzing the autoxidation of the inhibitor.

In addition, his data indicate that the copper soap catalyzes a more vigorous oxidation of the first addition products of oleic acid and oxygen, resulting in an increase in rate of oxygen absorption and CO_2 liberation, although the rate of destruction of double bonds is slightly decreased.

Thus, copper oleate is a powerful pro-oxidant to the inhibiting substances and to the secondary products of oxidation but has a weak inhibiting action on the initial oxidative reaction; i.e., the formation of the moloxide.

Such studies demonstrate that natural and phenolic antioxidants in fats may be subject to destruction not only by reaction with moloxide chains and peracids, but also by catalyzed autoxidation of the antioxidant molecule itself.

In general, the statements which have been made above are in agreement with the conclusions and deductions of Stephens concerning oxidation of drying oils (17) and cyclohexene (18).

The chemical configurations required for an effective antioxidant have been discussed elsewhere (10). It is obvious that an easily oxidized compound need not be an effective antioxidant. Hydroquinone and carotene are both easily oxidized, but one is a powerful antioxidant and the other a pro-oxidant. It may be pertinent to emphasize the important role played by the free hydroxyl group in conjunction with unsaturation in the antioxidant molecule. What part these struc-

[2]We have verified his statement that the peroxides in rancid fats do not interfere with the determination of iodine number.

tures play in the complex reactions in an oxidizing fat is still obscure.

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Summary

Experiments on the oxidation of purified methyl oleate support the view that its induction period, and probably that of natural oils, is due to the presence of inhibitors and that purified unsaturated compounds have no induction period, other than the time required for gaseous oxygen to diffuse into the liquid.

Experiments with antioxidants indicate that phenolic inhibitors and inhibitols cause no change subsequent to the end of the induction period, that they exert their effect solely by inhibiting the formation of the initial active moloxide, and that they are entirely destroyed before the start of rapid oxidation which characterizes the end of the induction period.

The mode of action of several different pro-oxidants is analyzed. Perbenzoic acid, and presumably other peracids, and copper oleate decrease the induction period by virtue of their destruction of natural inhibitors.

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ABSTRACTS

Oils and Fats

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The problems of chemistry in the new Germany. XIII. Auto-oxidation and ketonic decomposition of fats as problems in the fat industry. K. Täufel. *Angew. Chem.* **49**, 48-53 (1936); cf. *C. A.* **30**, 775.—The following subjects are discussed: (1) synthesis of fats, (2) refining of fats, (3) research for fat substitutes, (4) steps to reduce fat losses by spoilage, (*a*) the chem. spoiling of fats and (*b*) the biol. spoiling of fats. Conclusions: Fats can be stabilized by keeping bacteria away in the mfg. process, by retardation of bacterial growth by cooling means, and by the application of bacteria-killing substances. Thirty-four references. (*C. A.* **30**, 1597.)

The determination of the oil content (of seeds) by the refractometer. A. Rasteryaev. *Maslobo˘ino-Zhirovoe Delo* **1934**, No. 3, 10-11.—Cover 2 g. of the ground seeds with 15 cc. $CHCl_3$ and allow to stand for 12 hrs. at room temp. Calc. the percentage oil content (*P*) from the difference (*D*) of the *ns* of $CHCl_3$ and the $CHCl_3$ soln. by the formula: $10 \times D = 55P \times 10 \times 15/2$. The method is exact within 0.5%. (*C. A.* **30**, 1597.)

What is the most economical process for extracting oil from decorticated cotton seed? J. de Raedt. *Mat. grasses* **27**, 10671-3 (1935).—From a comparison of the relative costs of (1) discontinuous hydraulic pressing, (2) continuous mech. pressing and (3) preliminary continuous pressing followed by solvent extn., it is concluded that (3) is the most economical, particularly under the conditions prevailing in the Argentine Chaco. (*C. A.* **30**, 2030.)

Characteristics of halibut-liver oils. R. T. M. Haines and J. C. Drummond. *Analyst* **61**, 2-7 (1936). Norman Evers, A. G. Jones and Wilfred Smith. *Ibid.* 7-11 (1936); cf. *C. A.* **29**, 4960.—The increasing use of this oil in medicine makes it important that the analyst should know the characteristics of a pure sample of oil to detect adulteration. In the first of these 2 independent papers, the values obtained in the analysis of 18 samples of West Greenland oils, 9 samples of Labrador oils and 3 samples of Iceland oils are given. In the second paper, the values obtained from 41 samples of Iceland, Faröes and West Greenland oils and of 5 samples of Norwegian oils are tabulated. The "blue values" of West Greenland oils, indicating the vitamin A content, varied from 625 to 12,930. The lowest blue value in any sample of pure oil was 495. The sp. gr. of all the oils was 0.928 or a little less. The I values ranged from 114.0 to 161.0. Apparently there is some relation between a high I value and a high content of vitamin A but it is not quite clear what this is. The refractive indices varied from 1.47 to 1.48, the unsaponifiable matter from 6.34 to 17.6%. (*C. A.* **30**, 2030.)

Chemical studies of cottonseed and its products. W. D. Gallup. Okla. Agr. Expt. Sta., *Rept.* **1932-4**, 177-80 (1934).—Cottonseed having a high oil content also had a high content of *gossypol*. For seeds of low oil content the oil: gossypol ratio was 55:1, and for oil-rich seeds the ratio was only 35:1. The presence of gossypol in the crude oil reduces the alkali refining loss of the oil. The nutritive values of cottonseed meal are discussed. (*C. A.* **30**, 2030.)

Composition of rape-seed oil. Riichiro Yamasaki and Kentaro Ichihara. *J. Chem. Soc. Japan* **56**, 1332-4 (1935).—Fat acids of the oil consisted of behenic 0.8, erucic 55, oleic 14, linolic 24, linolenic 2 and palmitic 3.5%; it contained also myristic, palmitoleic and stearic acids but the amt. was small. The presence of rape-seed oil in the other oils can be identified by the detection of erucic acid, and the presence of the other oils in rape-seed oil by estg. stearic acid. (*C. A.* **30**, 1598.)

Detection of rape oil in edible fat. J. Grossfeld. *Chem.-Ztg.* **59**, 935-6 (1935).—The method for detg. the amt. of rape oil in other oils depends on the estn. of the content of erucic acid by detg. the I absorption of the insol. fat acid Pb salts obtained from the sample. The method is similar to the Grossfeld and Peter procedure for estg. isoöleic acid in oils. The sensitivity is increased by adding palmitic acid to the test sample so that the insol. Pb salt of the erucic acid is absorbed by the Pb palmitate. As little as a 2%