The Kinetics of the Oxidation of Several Antioxidants in Oxidizing Fats*

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THERE are very few reports in the literature concerning the kinetics of the destruction of antioxidants in oxidizing fats. The paucity of information on this subject may be attributed in part to the fact that there has been a lack of analytical methods for the determination of various antioxidants in fat.

Golumbic (1), using the Emmerie and Engel and the Furter-Meyer methods of analysis, has followed the decrease in concentration of tocopherols in animal and vegetable fat substrates during oxidation. He reported that in lard containing added alpha-tocopherol, oxidized in an oven at 60° C., the rate of oxidation of alpha-tocopherol increased with progressive increase in its initial concentration. Moreover, the time necessary for the oxidation of any given fraction of the tocopherol was independent of the initial concentration. In this respect therefore the oxidation of the tocopherol exhibited the characteristics of a first order reaction.

Filer and coworkers (2) studied the oxidative deterioration of gallic acid and ascorbic acid, individually and in combination, in a commercially refined cottonseed oil aerated at 110° C. They used an analytical method for gallic acid in fats developed in their laboratory (3), based on a color reaction between polyphenolic compounds and a ferrous tartrate reagent. They found that the rate of loss of gallic acid was approximately constant with respect to time and was virtually independent of the initial concentration, thus having characteristics similar to those of a zero order reaction.

In the present study the oxidative deterioration of four antioxidants—hydroquinone, catechol, NDGA (nordihydroguaiaretic acid), and gallic acid—at initial concentrations of 0.02%, 0.1%, and 0.5% in lard was followed at 100°C. In addition, studies were conducted involving combinations of 0.02% NDGA with 0.02% citric acid, and 0.02% NDGA with 0.02% methionine in lard.

About 200 gms. of lard containing the added antioxidant in a 500-ml. Erlenmeyer flask was immersed in a constant temperature bath maintained at 100° C. A continuous stream of oxygen was passed through the sample at an approximately constant rate.

In the cases of hydroquinone and catechol it was found that under these conditions there appeared a condensate of these substances in the outlet tube of the flask. This condensate was washed back into the main portion of the fat twice during each 24-hr. period. Volatilization of these antioxidants would presumably occur likewise in applying the commonly used active-oxygen method to measurement of the stability of fats containing them. NDGA, gallic acid, and tocopherols did not volatilize appreciably under these conditions.

From time to time small portions of the fat were withdrawn from the flask and analyzed for peroxides by King, Roschen, and Irwin's modification of the Wheeler method (4). Analyses of the residual antioxidant concentrations were also made, using a method developed in our laboratories (5). This method consisted in an extraction of the antioxidant from the fat, followed by an analysis of the extract, essentially by a modification of the Emmerie and Engel method for tocopherols. It was so adapted that it could be applied to a variety of phenolic antioxidants, including all those used in this study, with no variations in procedure except in the length of the reaction time.

Briefly, the extraction and analytical procedures were as follows: The fat (1 to 10 grams) was introduced into a separatory funnel together with 40 to 60 ml. of Skellysolve F. The solution of fat was extracted with three successive portions of 80% ethyl alcohol. The combined extracts were shaken in a separatory funnel with 100 ml. of Skellysolve F, and the alcohol layer was withdrawn together with a small amount of alcohol washings. After making the necessary adjustments in volume, aliquots of the extract were taken for analysis. Two reagent solutions were used, one consisting of 1,000 mgm. of 2,2'bipyridine in 1 liter of 95% ethyl alcohol, and the other of 832 mgm. of reagent ferric chloride hexahydrate in 1 liter of 95% alcohol. To one part of the antioxidant extract were added two parts of bipyridine solution followed by 2 parts of ferric chloride solution in the dark. The mixture was allowed to stand in the dark for the necessary reaction time: 1 minute for catechol and NDGA, 15 minutes for hydroquinone, and 30 minutes for gallic acid. The optical density of the solution was then determined in a Coleman spectrophotometer at 510 millimicrons wave length.

There may be a question whether this nonspecific method accurately determined the residual amount of the original antioxidant, or whether there were interfering substances present which could also reduce ferric chloride. Direct evidence was obtained to show that no substances present in the original lard interfered with the determination. Similarly it was found through experiment that the lard peroxides did not interfere with the determinations. The possibility that oxidation products of the antioxidant might have reducing properties that would interfere was contraindicated by the observation that in every case the time at which the antioxidant was found by this method to disappear completely from the fat coincided approximately with the time at which a marked acceleration in the peroxide accumulation occurred. This coincidence could not have occurred if oxidation products of the antioxidant interfered unless the oxidative products of the antioxidant were themselves antioxygenic.

Direct evidence was also obtained which showed that the presence of any of these antioxidants did not

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lessen the accuracy of the method used in determining the peroxides present at any given time although some of the antioxidants may slowly reduce the peroxides in lard at 100° C.

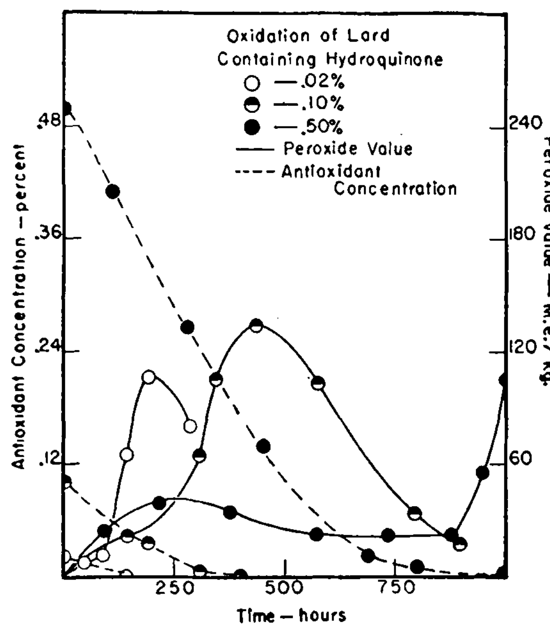

FIG. 1.

Figure 1 illustrates the deterioration of hydroquinone and peroxide accumulation in the fat at several initial concentrations of this antioxidant. The broken lines indicate the progress of the destruction of the antioxidant, and the corresponding solid lines represent peroxide accumulation. A study of these curves makes it evident that under these conditions the oxidation of hydroquinone does not have the kinetic characteristics, even approximately, of either a first order or a zero order reaction, as was the case with results obtained previously with alpha-tocopherol and gallic acid, respectively, under quite different conditions (1, 2). It appears very likely that in

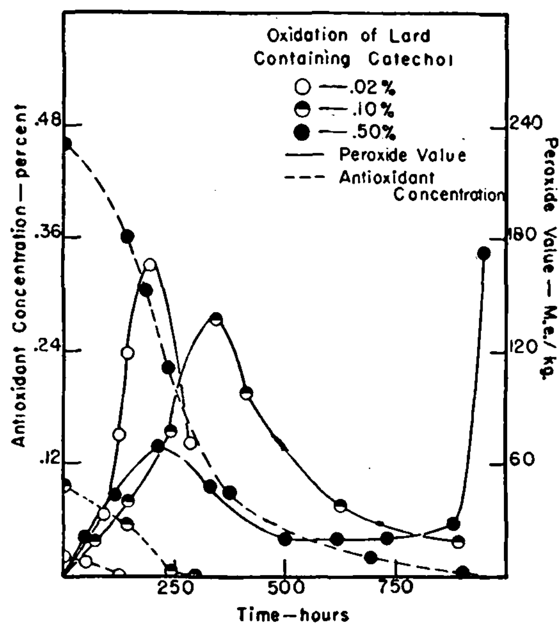

FIG. 2.

addition to direct oxidation of the antioxidant by molecular oxygen, complicated side reactions are involved, including possibly oxidation of the antioxidant by fat peroxides and also catalytic effects of some of the oxidative products of the fat and the antioxidant.

Figure 2 illustrates similar data obtained with catechol under the same conditions. The progress of the antioxidant destruction in this case is quite similar to that of hydroquinone. In both cases 800 to 900 hrs. were required for the destruction of the antioxidant at an initial concentration of 0.5%, slightly more than 250 hrs. at 0.1%, and slightly more than 100 hrs. at 0.02%.

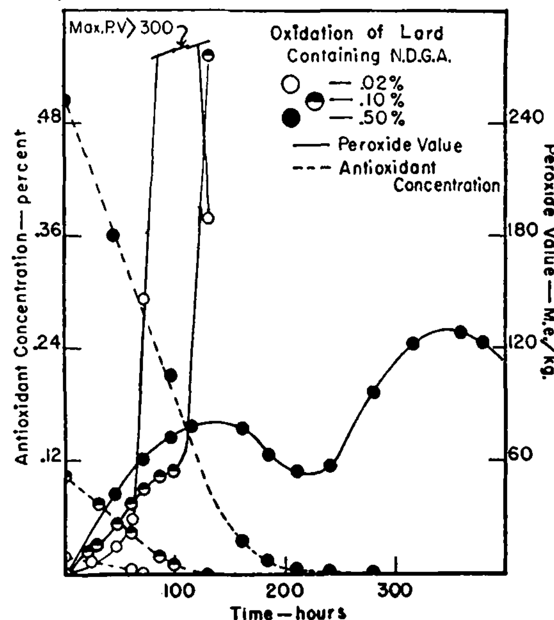

FIG. 3.

Figure 3 illustrates the data that were obtained with NDGA. Here again the antioxidant destruction curves are similar to those of hydroquinone and catechol, except that the antioxidant disappeared much more rapidly. Gallic acid also disappeared more rapidly than hydroquinone or catechol. However, this must not be interpreted to mean that hydroquinone and catechol are better agents for inhibiting rancidity than are NDGA and gallic acid. Actually the reverse is often found to be true, depending on the conditions used. With all four of these antioxidants at initial concentrations of 0.5 and 0.1%, and with hydroquinone and catechol also at an initial concentration of 0.02%, rancidity was detected before the antioxidant had disappeared. From a practical standpoint the effectiveness of an antioxidant in retarding peroxide development and particularly in retarding the formation of rancid products in the fat is more directly important than durability of the antioxidant.

There are several other observations common to all four of these antioxidants that are well illustrated in Figure 3. The higher concentrations of the antioxidant have a positive catalytic effect on the formation of peroxides during the initial stages of the reaction. This has previously been reported to be true of tocopherols (6) and appears to be characteristic of phenolic antioxidants in general. Because of this phenomenon it is found that each such phenolic antioxidant possesses an optimal concentration insofar as

the prevention of organoleptically detectable rancidity is concerned. At antioxidant concentrations above and below this optimum rancidity develops more rapidly.

The positive catalytic effect of higher concentrations of the antioxidant on early peroxide development is probably responsible for the peculiar character of the peroxide curves that are obtained with the higher concentrations of the antioxidant. Thus at 0.5% concentration of NDGA the peroxide accumulation curve rises quite rapidly initially. Then, as the concentration of NDGA decreases, the positive catalytic effect decreases, and the rate of peroxide decomposition becomes equal to the rate of peroxide formation, thus producing a leveling of the curve. As the antioxidant concentration continues to decrease, peroxide decomposition exceeds peroxide formation and the curve falls to a minimum. This point represents the time at which the antioxidant and its antioxygenic effect completely disappear. The peroxide then increases again until a second maximum is reached. In this region of the curve the available oxidizable fat has been so depleted that peroxide decomposition again equals, and later exceeds, peroxide formation.

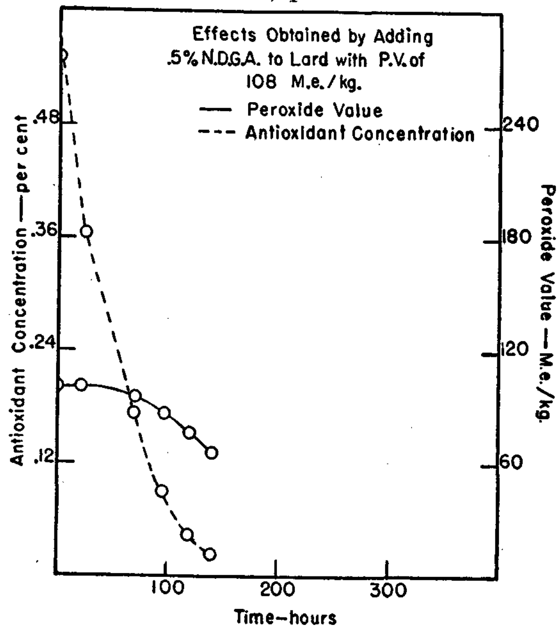

FIG. 4.

Figure 4 illustrates the destruction of NDGA having an initial concentration of 0.5% in a lard with an initial peroxide content of 108 m.e./kg. The deterioration curve in this case follows much the same course as with the same concentration in the previous figure but the deterioration occurs more rapidly. This again indicates that either the peroxides of other oxidation products of the fat or antioxidant materially hasten the destruction of the antioxidant. In general, therefore, it appears that one may not expect the deterioration of the phenolic antioxidants in fats to follow an uncomplicated curve representing a single low order reaction.

The peroxide curve in this case remains approximately constant initially, representing a state of balance between peroxide formation and decomposition. As the concentration of the antioxidant decreases, its catalytic effect on peroxide formation decreases and the peroxide value falls, partly because of thermal

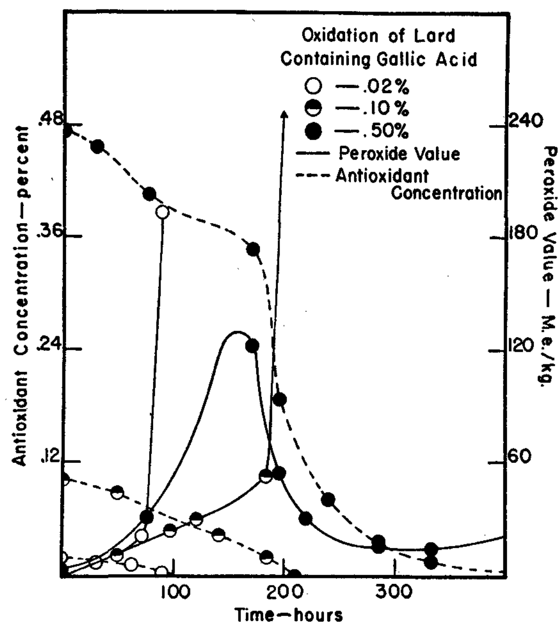

FIG. 5.

decomposition and possibly also due to reduction and polymerization reactions.

The deterioration data for gallic acid are illustrated in Figure 5. Results in this case are somewhat different from those obtained with the other three antioxidants particularly at 0.5% concentration, where the curve is sigmoidal. This may be explained tentatively on the basis of the synergistic action of gallic acid upon itself if one may assume that the synergistic action does not involve gallic acid destruction. On this basis gallic acid may be presumed to exert its greatest synergistic effect at the higher concentrations, but as its concentration gradually decreases, the synergistic effect also decreases, and the rate of gallic acid destruction is accelerated. Finally, as the concentration approaches zero, the curve becomes like those obtained with the other antioxidants.

This explanation is also suggested to some extent by data not included here concerning the destruction of NDGA at 0.02% concentration of NDGA in combination with 0.02% citric acid, and also 0.02% NDGA in combination with 0.02% methionine. In both of these cases the deterioration of the NDGA followed a sigmoidal type of curve with deviations similar to but less pronounced than in the case of gallic acid. Although the destruction of the synergists in these two cases was not followed quantitatively, data obtained in the absence of NDGA showed that they undergo changes under these conditions, and it is reasonable that with the elimination of their synergistic effects, the destruction of the primary antioxidant would be accelerated.

The differences between the curves for gallic acid shown in Figure 5 and those obtained by Filer and coworkers (2) are largely attributable to differences in the composition of the substrate, in the concentrations of the added antioxidants, and in the conditions under which the experiments were conducted.

Summary

The deterioration of hydroquinone, catechol, NDGA (nordihydroguaiaretic acid), and gallic acid in lard oxidizing at 100° C. has been quantitatively studied.

Initial concentrations of 0.02, 0.1, and 0.5% were used.

The results indicate that, in general, the deterioration of phenolic antioxidants in oxidizing fats does not occur as a single low order reaction but is complicated by products formed in the oxidation of the fat and possibly also of the antioxidant.

The deterioration curves for gallic acid are quite different from those of the other three antioxidants. This is tentatively explained on the basis of the synergistic action of gallic acid upon itself.

There is an increasing catalytic effect with increasing initial concentrations of all of these antioxidants on the formation of peroxides during the early stages of the autoxidation of lard.

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Abstracts

Oils and Fats

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THE COMPONENT FATTY ACIDS OF BUFFALO COLOSTRUM FAT. C. P. Anantakrishnan, V. R. B. Rao, T. M. Paul, and M. C. Rangaswamy (Imperial Dairy Research Inst., Bangalore, India). *J. Biol. Chem. 166,* 31-3 (1946). The colostrum fat is found to differ in chemical composition from that of the normal butter fat of the buffalo. The chief changes to be found were the gradual increase in the amount of butyric, myristic, and palmitic acids and a decrease in the amount of stearic and oleic acids, the decrease in the latter being more pronounced.

A STUDY OF METHODS FOR ASSESSING RANCIDITY IN LARD. G. A. Grant (National Research Labs., Ottawa) and H. J. Lips. *Can. J. Research 24F,* 450-60 (1946). Lard from 26 sources was stored in glass jars at 26.7° until definitely rancid. Spoilage was evaluated at 2-week intervals by chemical tests and odor ratings. Correlation coefficients between odor scores and the logarithms of chemical test values were: iodometric peroxide—.90; α-dicarbonyl test—.85; Stamm test—.82; Kreis test—.81; ferrometric peroxide—.80; fluorescence—.79; free fatty acids—.10. Association between chemical measurements was greatest between α-dicarbonyl and iodometric peroxide values ($r = .97$). As peroxides are not thermostable, the measurement of the stable α-dicarbonyl compounds, although less precise, is considered the best available chemical method for assessing rancidity.

SUSCEPTIBILITY TO OXIDATION OF THE FAT OF THE BACON PIG. W. Tuck, P. B. D. De la Mare, F. B. Shorland, and R. N. Seelye (N. Z. Dept. Agr., Wellington). *New Zealand J. Sci. Tech. 27A,* 212-20 (1945). Lea's peroxide-value method was used to determine the changes in stored inner-back fat, outer-back fat, and flare fat of pigs fed solely on buttermilk during the fattening period. The results were variable and not in agreement with previous findings of Lea or Johns. Sampling errors and the effects of bacterial and metallic contaminations are suspected as causes of the discrepancy. (*Chem. Abs. 40,* 7435.)

THE DETERMINATION OF THE PEROXIDE VALUES OF EDIBLE FATS AND OILS. THE IODIMETRIC METHOD. C. H. Lea (Univ. Cambridge). *J. Soc. Chem. Ind. 65,* 286-90 (1946). The increase in peroxide value observed in earlier iodimetric procedures when the quantity of fat taken for determination was reduced has been shown to be due to an increasing error resulting from further oxidation of the fat by dissolved O_2 during the determination rather than to loss of I by reabsorption. The effect of other factors, such as moisture in the reagent and time and temperature of reaction, were measured. The data were the bases for development of 2 procedures in which O_2 exclusion was adequate.

X-RAY INVESTIGATION OF GLYCERIDES. V. DIFFRACTION ANALYSES OF SYNTHETIC DIACID DIGLYCERIDES. S. S. Sidhu and B. F. Daubert (Univ. Pittsburgh). *J. Am. Chem. Soc. 68,* 2603-5 (1946). X-ray diffraction and thermal data are reported for a series of 3 symmetrical diacid diglycerides, 2 of which, namely, 1-palmityl-3-laurin and 1-myristyl-3-caprin, are new compounds. The side-spacing diffraction data for 1-myristyl-3-caprin and 1-palmityl-3-laurin correspond to Malkin's type α pattern for the β form of monoacid diglycerides, while the data for 1-stearyl-3-myristin seemed to correspond more nearly to Malkin's type β pattern.

AMINOSTEROIDS. I. α- AND β-FORMS OF 7-AMINOCHOLESTEROL. J. Barnett, B. E. Ryman, and F. Smith (Univ. Edgbaston, Birmingham). *J. Chem. Soc. 1946,* 524-6. The α- and β-isomers of 7-aminocholesterol have been isolated by fractional crystallization of the mixture obtained by reduction of the oxime of 7-ketocholesteryl acetate. The isomeric acetyl derivatives have also been separated, but all attempts to hydrolyze them to the free bases have failed. α-7-Aminocholestanol has been obtained by catalytic reduction of α-7-aminocholesterol. Both the α- and β-7-aminocholesterol isomers show high anti-bacterial activity *in vitro* against Gram-positive organisms, but no appreciable difference has been observed between the activities of the 2 spatial isomers. II. PREPARATION OF 3:7-DIKETOCHOLESTENE. *Ibid.* 526-8. 3:7-Diketocholestene has been prepared from 7-ketocholesterol. An improved method for the preparation of the latter is also described. The diketone appears to exist only in the enolic form. III. SOME MONO- AND DI-AMINOSTEROIDS. *Ibid.* 528-30. Various mono- and di-aminosteroids have been prepared by reduction of the oximes of the corresponding mono- and di-ketosteroids. Examination of their properties as antibacterial agents *in vitro* against Gram positive organisms showed a marked and similar activity in all compounds studied. Only the diaminosteroids had