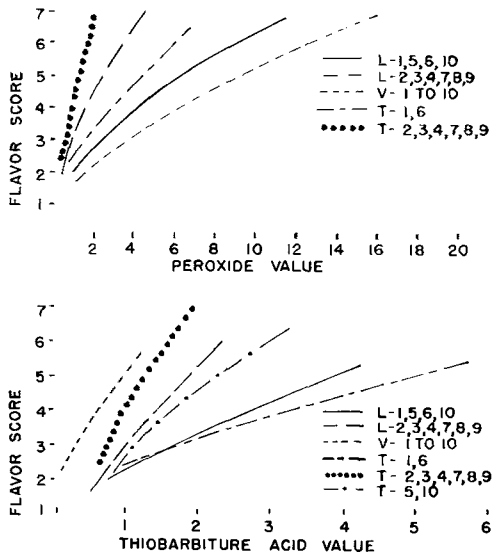

FIG. 2. Approx relationship between flavor score vs. PV and flavor score vs. TBA value after grouping formulations that had similar characteristics.

changes with time will be taken up in another paper.

A typical plot of flavor score vs. PV and TBA value is given in Figure 1. The plot is made without regard to whether the values were obtained from samples of lard and hydrogenated vegetable oil before and after selected intervals of storage at 140 and 85F. The distribution of the points shows clearly that a PV or a TBA value cannot be relied upon to indicate the flavor quality of a fat or shortening of unknown history.

Since the PV and TBA value have been used to follow the development of off flavor, the data was examined to see whether the samples could be placed in groups with respect to the relationship between PV and flavor, and TBA value and flavor. Figure 2 shows the approx relationships drawn by inspection from data for samples that showed similar characteristics.

It is evident that the change in PV or TBA value with change in flavor is different for different types of products and formulations and that a good correlation may be obtained for a series of tests on a given formulation. This limits the value of these tests except possibly for research, as the relationship between PV or TBA value and change in flavor would have to be established for a given formulation from a series of tests before PV or TBA value could be used as an index of flavor characteristics.

Conclusion

The data indicate that the PV or TBA value of a sample cannot be used as an index of flavor quality unless it is part of a series on which previous data is available.

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[Received October 7, 1963—Accepted June 17, 1964]

The Kinetics of Autoxidation of Methyl Linoleate. The Effect of Added Antioxidants and a New Method for the Evaluation of Antioxidants

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Abstract

A new method is described for the evaluation of antioxidants. Oxygen uptake by autoxidizing methyl linoleate both with and without antioxidants is measured in the Warburg apparatus and reaction orders, reaction rate constants, and length of induction periods determined. Tenox's BHT and BHA and Griffith's G-16 are evaluated and compared.

Introduction

MOST ANTIOXIDANTS are evaluated by their ability to increase the stability of fats. That no standard limits have been set for defining fat stability is appreciated by noting various definitions which have been used. Lundberg (4) defines induction period as that time during which no off-flavors can be detected. In the oxidation of tocopherol in the ethyl esters of hydrogenated cottonseed oil, Golumbic (3) also determined the end of the induction period organoleptically. Filer et al. (2) in their work on cottonseed oil with added antioxidants measured the induction period as the number of hr aeration necessary to raise the peroxide value of the sample to 120 meq sodium thiosulfate/kg fat. Riemenschneider et al. (5), working on the effect of deodorizing and antioxidants on the stability of lard, stated the end of the induction period to be that amt of time needed for 0.5 cc sample to absorb 300 cc oxygen. Stirton et al. (7), in measuring the effect of antioxidants on the oxygen absorption of methyl esters, used an oxygen absorption of 1 g/kg as the end of the induction period. Smith and Stolz (6), in their studies on the effects of copper and antioxidants on linoleic acid autoxidation, defined the incubation period as the time taken to reach 200 μm^3 oxygen uptake. Stability in the past, then, has been judged solely on the basis of prolonging the induction period. Stirton, et al. (7) further in their oxygen ab-

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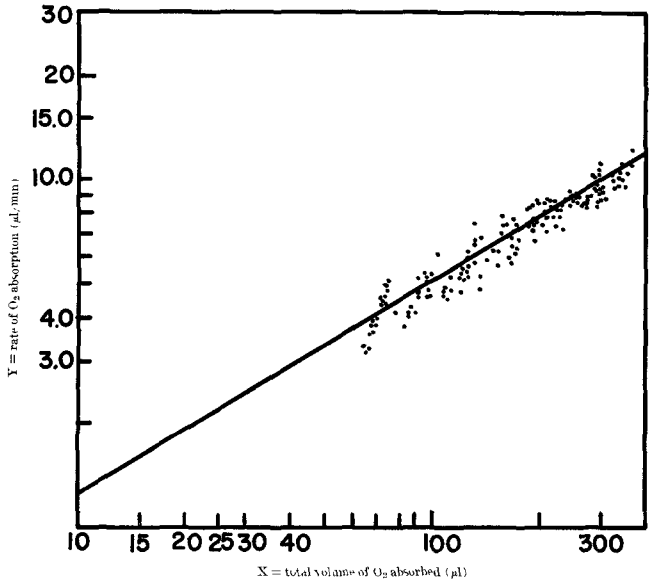

FIG. 1. Rate of oxidation vs. total volume of oxygen absorbed in 15% methyl linoleate at 90.5C. Log $Y = -0.509 + \log X$.

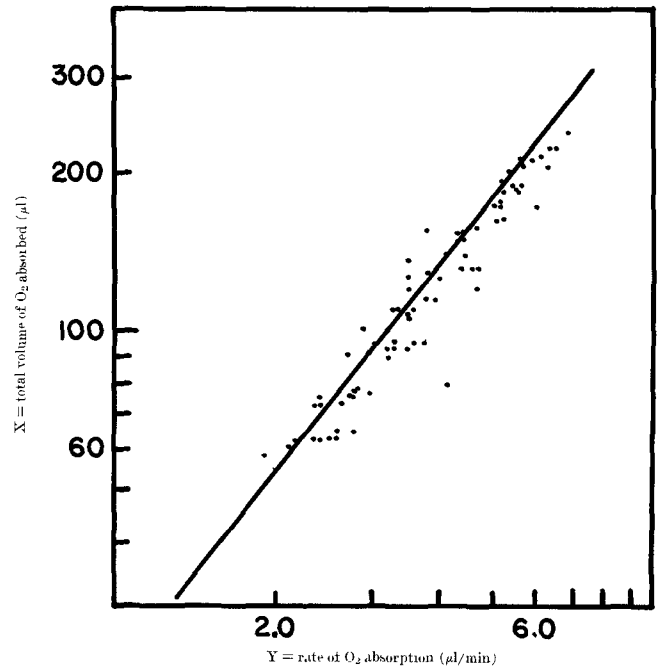

FIG. 2. Rate of oxidation vs. total volume of oxygen absorbed in 15% methyl linoleate with 0.001% antioxidant G-16 at 90.5C. Log $Y = -0.983 + 0.756 \log X$.

sorption experiments made judgments on the relative merits of added antioxidants by comparing induction periods only, although visual comparison of the graphs presented indicate that the curves behave differently when the induction period is passed. In the work by Budowski and Bondi (1) on the autoxidation of carotene and Vitamin A, an attempt was made, if not to quantify that period of oxidation which follows the induction period, at least to account for it. Octyl gallate (OG), butylated hydroxytoluene (BHT), and N,N diphenyl-*p*-phenylene diamine (DPPD) all lengthened the induction period of the autoxidation. The authors noted, however, after the induction period had passed the rate of volume of oxygen absorbed was decreased substantially from the same curves for OG and BHT. This was attributed to quinonedianil (QDN), the oxidation product of DPPD which retained some antioxidant properties. In spite of this significant observation, the antioxidants were judged solely on their ability to prolong the induction period. We decided that by comparing induction periods and kinetic data obtained in the rapidly oxidizing phase of autoxidation, a more meaningful evaluation of antioxidants would be possible.

The values obtained in oxygen absorption experiments can be fit to a general kinetic equation:

$$dv/dt = kV^a$$

where dv/dt represents the rate of gas absorption, V the total gas volume which has been absorbed, k the rate constant, and a the reaction order. The units of k are

$$(\mu \text{ liters})^{1-a}/\text{min} = k$$

If dv/dt is represented by Y and V by X, the rate equation becomes

$$Y = kX^a$$

Plotting the rates of oxygen absorption vs. total oxygen absorbed on log-log paper, the Y intercept will yield k, the rate constant, and the slope gives the value of a, the reaction order.

Experimental

Materials. Methyl linoleate, lot number 5-M, obtained from The Hormel Foundation, Minn., was used and had a stated purity greater than 99% by gas-liquid and paper chromatographic analysis. Paraffin oil, lot number V 249, Baker and Adamson Products, New York City, was used as the solvent for the oxidizable fats and for the antioxidants. Antioxidants Tenox Butylated Hydroxytoluene (BHT) and Tenox Butylated Hydroxyanisole (BHA) were donated by the Eastman Chemical Products, Inc., Rochester, N. Y. Antioxidant G-16, donated by the Griffith Laboratories, Chicago, is a mixture processed from vegetable oil containing monoglyceride citrate, BHT, BHA, propyl gallate, and propylene glycol.

The percentages of these various constituents are not stated.

Methods. One hundred mg material were weighed into standard Warburg flasks, using a melting point tube for transfer. To insure a proper oxygen atmosphere, oxygen was streamed into the flask for five min via the chimney on the side arm. The flow was maintained at five liters/min. After flushing for five min, the oxygen was trapped by closing the chimney and the three way stopcock at the top of the manometer. Temp of 90.5–91.0C were used in order to speed up the oxidation process and to obtain kinetic values within a reasonable span of time. Due to these high temp, oxygen in the flasks expanded quite rapidly when transferred to the constant temp bath. This gas expansion necessitated the bleeding off of the excess gas via the three way stopcock on the top of the manometer tube. Anti freeze, with a higher specific heat than water, was used in the constant temp bath, so that high temp levels could be maintained. The flask and its contents were allowed to equilibrate with the bath for five min before the first reading was taken from the manometer filled with Brodie's solution. Simultaneous with the first reading the shaker was turned on and the time noted. Oxidation ensued and the time at which 50 μl oxygen had been absorbed was noted. The elapsed time was recorded and using this figure, the induction period was later determined. After 50 μl oxygen had been absorbed, rates of pressure change and total pressure change were recorded for eight

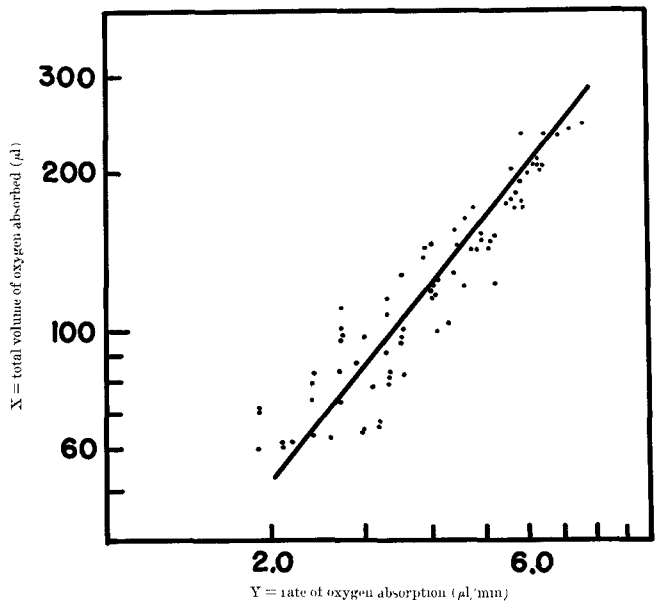

FIG. 3. Rate of oxidation vs. total volume of oxygen absorbed in 15% methyl linoleate with 0.001% Tenox BHT at 90.5C. Log Y = −1.075 + 0.804 log X.

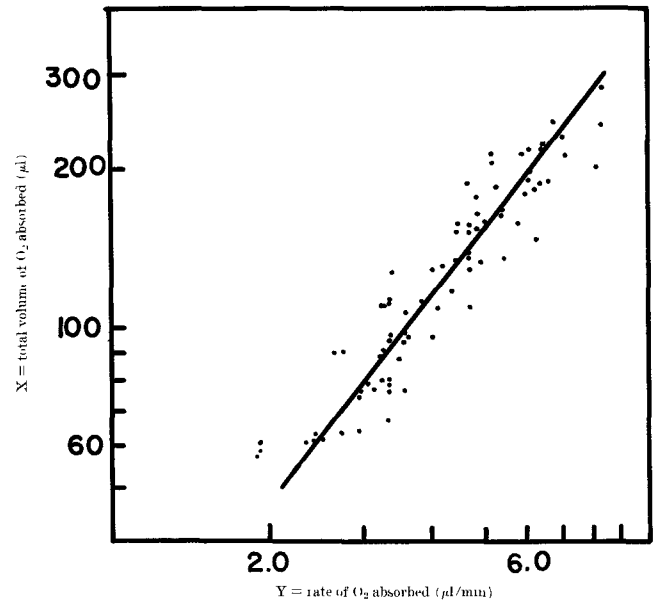

FIG. 4. Rate of oxidation vs. total volume of oxygen absorbed in 15% methyl linoleate with 0.001% Tenox BHA at 90.5C. Log Y = −9.984 + 0.766 log X.

5-min intervals. By using previously determined flask constants, the pressure readings were converted to volume measurements. A stopwatch was used to insure proper timing. Upon completion of the measurements, the flasks were rinsed in benzene, washed in a boiling detergent solution 20 min, rinsed thoroughly in distilled water, dried in an oven and stored in a desiccator until used again.

In determining the induction period the values of the average total volume of oxygen absorbed are plotted vs. time employing a graphical analysis especially designed for this work. Since the total volume of absorbed oxygen is not linearly related to time, the values are literally "forced" to fall on a straight line. The ordinate in Figure 5 shows average values of oxygen absorbed, and a straight line is drawn through the origin which represents zero volume absorbed. The abscissa as time in min corresponds to the volume of oxygen absorbed. Since the first reading of time vs. volume was taken at 50 μl, it is necessary to project values of time back to zero volume of absorbed oxygen. By measuring the distance on the abscissa between five-min intervals, the ratio of the distance on the abscissa can be determined. Applying this ratio to the time at which 50 μl were absorbed, the time during which no oxygen had been absorbed can be found. The plot of these points shows in Figure 5. The period of time during which no measurable volume of oxygen was observed to have been absorbed, under the conditions of the experiment, is used as the definition of induction period in these studies.

Procedure. Four separate experiments were performed following the generalized method described above. 1) The effect of different shaking speeds on the rate of oxygen absorption was determined. A 15% solution of methyl linoleate was oxidized using three different shaking speeds—378 oscillations/min, 289 oscillations/min and 230 oscillations/min. The rates of oxidation, from 35–40 min after the 50 μl had been absorbed, were measured and compared. 2) The kinetic constants—reaction order, reaction rate constant and induction period—of an autoxidizing 15% methyl linoleate solution were determined using a shaking speed of 289 oscillations/min. Results are plotted in Figure 1. 3) The kinetic constants—reaction order, reaction rate constants and induction period—of an autoxidizing 15% methyl linoleate with added antioxidants were determined using a shaking speed of 289 oscillations/min. Three antioxidants, BHT, BHA and G-16 were used in concn of 0.001%. Weighing out such a low antioxidant concn in the Warburg flask was accomplished as follows: 75 mg 20% methyl linoleate was combined with 25 mg 0.004% antioxidant in paraffin oil solution. The resulting sample was thoroughly mixed before the oxidizing procedure was started. Results are plotted in Figures 2,3 and 4. 4) The effect of various concn of antioxidant on the length of time necessary for the absorption of 50 μl oxygen in a 15% methyl linoleate solution was determined using a shaking speed of 289 oscillations/min. Concn of 0%,0.0005%,0.001%,0.005%,0.010% and 0.20% antioxidant G-16 were used and the results plotted in Figure 6.

The values for the rate of oxidation and total oxygen absorbed were analyzed by the Burroughs 220 Digital Computer at the Cornell Computing Center and regression coefficients for the straight lines were obtained. The plotted points along with the best fit straight lines show in Figures 1–4.

TABLE I

Tabulation of Kinetic Constants Determined at 90.5C and the Appropriate Rate Equations. Data Taken from Figures 1–5

	No antioxidant	G-16	BHT	BHA
Induction period min	6	19	30	55
Reaction order (a)	0.60	0.76	0.80	0.77
Rate constant (k) $(\mu l)^{1-a}$ min	0.310	0.104	0.084	0.104

	Rate equations
No antioxidant	$Y = 0.310\ X^{0.60}$
G-16	$Y = 0.104\ X^{0.76}$
BHT	$Y = 0.084\ X^{0.80}$
BHA	$Y = 0.104\ X^{0.77}$

Y = Rate of O_2 absorbed (μl/min).
X = Total volume O_2 absorbed (μl).

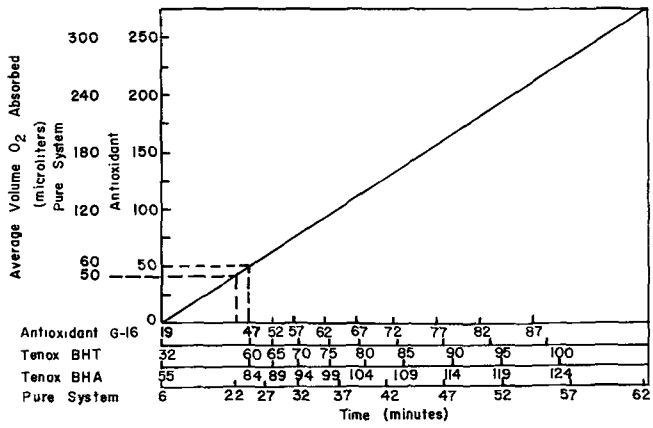

FIG. 5. Average volume of oxygen absorbed vs. time.

Discussion

The kinetic constants for all the oxidizing systems are summarized in Table I. The induction period varies significantly for each of the four systems, whereas there is a similarity in the rate equation for the added antioxidants. These differ greatly from the rate equation for the pure methyl linoleate with no added antioxidant. The order of the effectiveness of the antioxidants in terms of induction period is BHA, BHT and G-16, whereas in terms of the rate of reaction constant BHT appears superior to G-16 and BHA. Explanation of the fact that the systems with added antioxidants behave differently than the pure 15% methyl linoleate may be that the oxidation products of the antioxidants have a residual effect on the course of the autoxidation once the induction period has passed.

On this basis, a new method for rating antioxidants is proposed. First, measurements and calculations are made by which the induction period is determined. The advantage of defining the induction period as the time during which no oxygen is absorbed is that this definition is now a more meaningful concept. If the induction period of methyl linoleate is arbitrarily assigned a value of 1, then we could assign G-16 an antioxidant value of 3.1 since $19/6 = 3.1$. In a similar manner BHT and BHA could be assigned antioxidant values of 5.0 and 9.1, respectively. Secondly, the results of this particular rating must be tempered with the values obtained from the rate equation which in this case shows BHT to be more effective than either BHA or G-16. The differences in antioxidant effectiveness as demonstrated by induction period and by rate equations may be explained by the fact that during rapid oxidation, the oxidized species of the antioxidant may have a different effectiveness toward oxidation than the original reduced species of the antioxidant.

The above results, showing that one antioxidant may have a longer induction period but a greater rate of reaction constant than another once the induction period is completed, is not to be considered abnormal. Oxidation products of the antioxidants, as Budowski and Bondi (1) have shown in their work on Vitamin A and carotene autoxidation, may mediate this effect. This may in fact advocate the use of two different antioxidants in combination; one being effective in prolonging the induction period, the other in decreasing the rate of reaction.

The simplicity of this method would seem to lend itself to routine laboratory evaluations of antioxidants. The entire test can be run in relatively short periods of time depending on the concn and types of

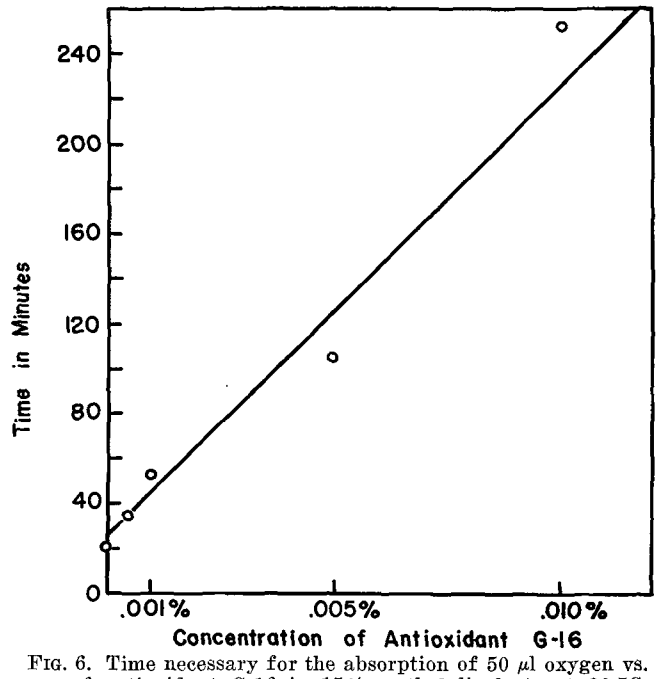

FIG. 6. Time necessary for the absorption of 50 μl oxygen vs. concn of antioxidant G-16 in 15% methyl linoleate at 90.5C.

antioxidants and fats used. Eliminated is the necessity to standardize a chemical reagent or to depend on the personal variations of a taste panel performing organoleptic analyses.

Varying the shaking speed has no appreciable effect on the rate of oxygen absorption of the 15% methyl linoleate system. This means that the measured rates of oxidation were factual and were not limited by the diffusion of oxygen into the system.

Figure 6 demonstrates the effect of increased antioxidant concn on the time necessary for the absorption of 50 μl oxygen. As would be expected, the relationship is linear and thus first order in nature. This observation makes this method of antioxidant evaluation valid over the concn of antioxidants studied.

A statistical analysis of the kinetic parameters for the oxidizing 15% methyl linoleate system with and without added antioxidants show that the experimental procedure followed permits the determination of accurate results. The 100 mg material transferred into the Warburg flasks was weighed to $\pm$ 0.5 mg; temp of 90.5C was kept constant to $\pm$ 0.5C, readings at 5-min intervals were taken to $\pm$ 1 second and the levels on the manometer tube were read to $\pm$ 1 mm. Computer calculations, showing how well the rate of oxygen absorption is related to the total volume of oxygen absorbed, gave the following correlation coefficients:

15% Methyl linoleate (no antioxidants) . .	0.960
15% Methyl linoleate (0.001% G-16) . . .	0.958
15% Methyl linoleate (0.001%BHT) . . .	0.931
15% Methyl linoleate (0.001%)	0.937

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[Received December 13, 1963—Accepted March 18, 1964]