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Reactions of Phosphoric Acid in the Autoxidation of Fats[1]

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ALTHOUGH phosphoric acid and its derivatives have received considerable attention as synergists in inhibiting the autoxidation of fat, their mode of action has not been established. Recently Calkin (1), rejecting the older view that phosphoric acid facilitated the regeneration of the antioxidant (3), advanced the theory that the phosphoric acid in a quinone-phosphoric acid system added across the olefinic linkages of activated fat molecules in a heterogeneous reaction, thereby dissipating the excess energy required for activation of the fat molecule. Subsequently the phosphoric acid was split off, and the fat molecule regenerated in its original inactive state. The quinone acted as a fat-solubilizing carrier for the phosphoric acid.

In this laboratory studies with wheat germ oil and with lard showed that wheat germ phosphatides and likewise phosphoric acid prevented the accumulation of peroxides, but the oils became highly viscous and highly oxidized as evidenced by oxygen absorption measurements (7). This evidence of polymerization cast some doubt on the validity of the reactions proposed by Calkin (1) and led us to re-investigate the action of phosphoric acid in autoxidizing fats.

Experimental

The effect of phosphoric acid on the autoxidation of lard. The course of autoxidation of a bleached and deodorized lard was followed by making peroxide determinations essentially by the same procedure as was employed by Lundberg (4) and concurrently measuring oxygen absorption in a Warburg respirometer, as described in a previous paper (7). The amount of oxygen absorbed by this lard showed relatively close agreement with the peroxide values, especially at 59°C. (Figures 1 and 2). The addition of phosphoric acid did not substantially diminish the rate of autoxidation of the lard as evidenced by oxygen absorption. However the absorbed oxygen accumulated only temporarily in the form of titratable peroxides. This behavior was analagous to that of phosphoric acid in degummed wheat germ oil (7).

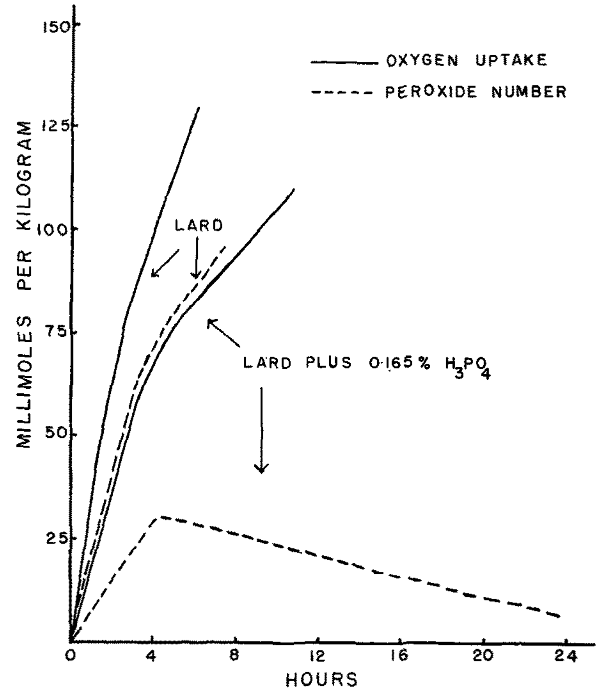

FIG. 1. Effect of H_3PO_4 on the autoxidation of lard at 99°C.

Simultaneously with the decrease in the peroxide values, a dark insoluble substance formed. This substance was observed to occur in all oxidation tests in which phosphoric acid was present. On separation the substance was observed to be insoluble in fat solvents as well as aqueous solvents. Phosphorus analysis of these precipitates showed them to contain up to 2% phosphorus, the amount depending on the duration of the heating. Part of the added phosphoric acid was readily extractable from the lard with distilled water, but some remained in the lard and was not extractable with water. Thus, after a few hours at 99° C., the phosphorus was found to be present in three different forms: a) free or unreacted, b) a fat-soluble complex, and c) a fat-insoluble complex.

To determine whether the observed changes represented reactions of phosphoric acid with unsaturated fatty acid esters or with some other constituent of the lard, further studies were made with ethyl linoleate as the substrate in oven tests at 100°C. In these tests a series of 25-ml. Erlenmeyer flasks containing linoleate and phosphoric acid was placed in the oven. At intervals a flask was removed, and the entire oil-soluble contents were transferred to a 200-ml. separatory funnel by washing with petroleum ether and distilled water. The water-extractable (unreacted) phosphoric acid was estimated by a colorimetric method (5). The amount of unreacted phosphoric acid was also determined at various intervals in a similar oven test in the presence of quinone. The results of these experiments (Figure 3) showed that a) the amount of un-

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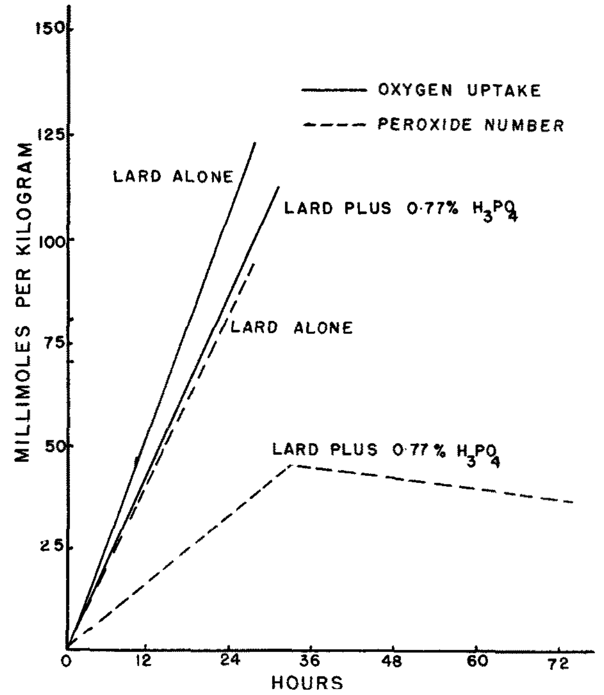

FIG. 2. Effect of H_3PO_4 on the autoxidation of lard at 59°C.

reacted phosphoric acid diminished as the heating was continued, and b) the presence of quinone retarded the rate of disappearance of the phosphoric acid. It was also observed in these experiments that as the heating was continued, more of the insoluble polymeric substance was formed.

In another experiment similar mixtures of ethyl linoleate and phosphoric acid were degassed at a pressure of less than 10 microns overnight, and the flasks were then sealed and placed in an oven at 100°C. All of the phosphoric acid was recoverable by aqueous extraction after 36 hours of heating (Table I). There also was no visible formation of insoluble residue. It was evident therefore that phosphoric acid did not form either fat-soluble or fat-insoluble compounds in the unsaturated fatty esters in the absence of air. This suggested that these substances were formed through reactions of phosphoric acid with fat peroxides, thereby destroying the latter and preventing their determination by the usual iodometric methods.

TABLE I

Effect of Heating Ethyl Linoleate With Phosphoric Acid at 100°C. *in vacuo*

Time of heating	Phosphorus recoverable by water extraction (micrograms)
0	1750
10	1750
24	1750
36	1750

The effect of temperature and phosphoric acid concentration on the phosphoric acid-peroxide reaction. A 100-g. portion of lard was allowed to oxidize to a peroxide value of *ca.* 60 by aspirating air through it at room temperature. Ten-gram samples of the oxidized lard with and without 0.2% of phosphoric acid were placed in covered petri dishes (9 cm. in diameter) and heated in ovens at various temperatures. Peroxide values were determined at intervals (Figure 4). At 40°C. the lard continued to oxidize slowly, and this rate was only slightly diminished by the presence of phosphoric acid. However, as the temperature was raised, the rate of peroxide formation increased rapidly; also the phosphoric acid affected a correspondingly rapid decrease in the peroxide number. The effect of temperature was also clearly marked by the formation of a progressively larger amount of dark precipitate as the temperature was raised.

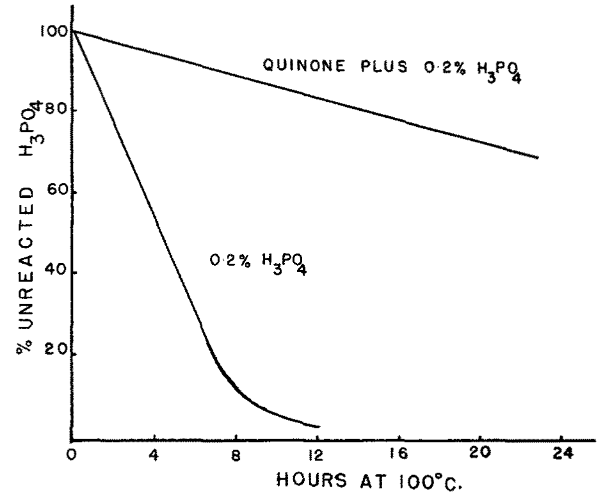

FIG. 3. Sparing action of quinone on phosphoric acid in ethyl linoleate at 100°C.

With fresh lard as the substrate the effect of phosphoric acid concentration was studied in similar oven tests at 100°C. (Figure 5). At low concentrations the phosphoric acid produced only a brief interruption in peroxide accumulation. However, at the higher phosphoric acid concentrations, the slight initial accumulation of peroxides was followed by almost complete destruction of the titratable peroxides. In all cases there was an initial accumulation of peroxides (to a value of 20-30) before the phosphoric acid began to show its effect.

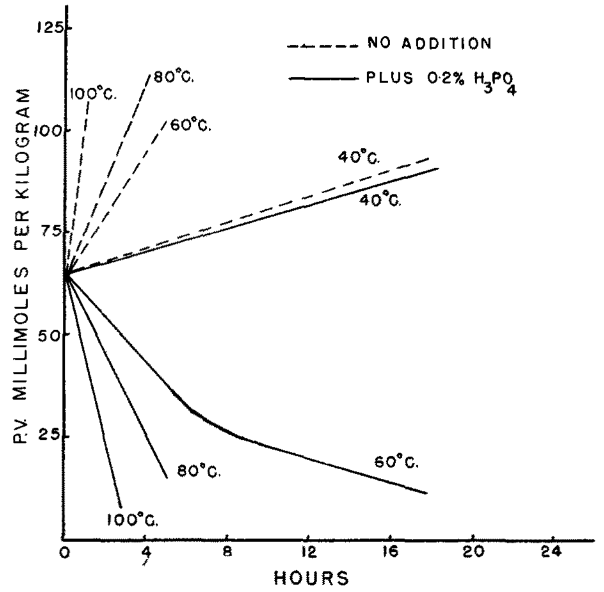

FIG. 4. Effect of temperature on the destruction of preformed peroxides in lard by phosphoric acid.

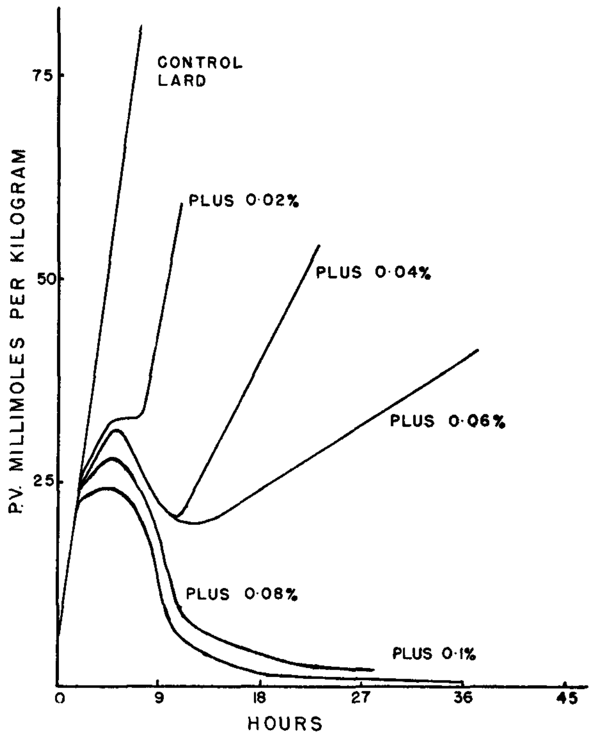

FIG. 5. Effects of increments of H_3PO_4 in lard in oven tests at 100°C. (H_3PO_4 as percent phosphorus).

Mutual sparing action of α-tocopherol and phosphoric acid in lard. The comparative rates of disappearance of phosphoric acid and d,l-α-tocopherol, each alone and in the presence of the other, was determined (Figure 6). The tocopherol, 0.1%, and phosphoric acid, 0.1%, were added to oxidized lard, and 2-g. samples were placed in a series of 25-ml. Erlenmeyer flasks and held at 40° C. At intervals a flask was removed, and the entire contents were transferred to a separatory funnel by washing with petroleum ether (b.p. 40°-60°C.) and distilled water. The aqueous phase was analyzed for phosphoric acid and the petroleum ether phase for α-tocopherol by the Emmerie-Engel color reaction (2), following the Parker-McFarlane sulfuric acid treatment for removal of interfering substances (6). The results of these experiments showed that, in the presence of each other, the α-tocopherol and the phosphoric acid exhibited a marked mutual sparing action.

Although the sparing action of phosphoric acid on α-tocopherol has long been recognized, the results presented here showed that the α-tocopherol also exerted a protective action on the phosphoric acid.

Discussion

The reaction of phosphoric acid with fatty peroxides does not seem to be clearly related to its function as a synergist. Below 40°C. there appears to be very little destruction of the peroxides by phosphoric acid. Phosphoric acid exerts synergistic activity at much lower temperatures. In these experiments the rate of disappearance of the phosphoric acid appeared to be associated with the amount of fat oxidation since it was expended less rapidly in combinations with α-tocopherol and quinone.

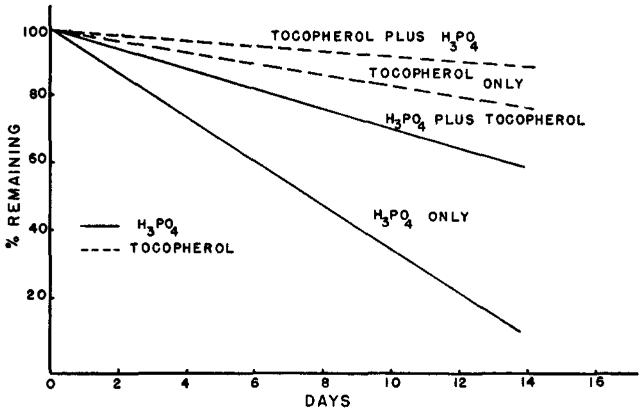

FIG. 6. Mutual sparing action of tocopherol and phosphoric acid in partially oxidized lard (peroxide number 90) in oven tests at 40°C.

Evidently the phosphoric acid underwent a sequence of reactions in which intermediate fat-soluble compounds were formed prior to the production of highly insoluble polymers. Although the mechanism of the reaction of phosphoric acid with peroxides in autoxidizing fat is not known, it is plausible that even at very low temperatures some of these transitory intermediates may form which do not interfere with the iodometric estimation of the peroxides. Evidence for such formation is indicated by the fact that regardless of the concentration of phosphoric acid (Figure 5) an initial accumulation of peroxides was observed prior to their destruction at 100°C. Calkin (1) postulated a transitory complex involving reaction of phosphoric acid with the olefinic linkage, but this is discounted in view of (1) the greater ease with which phosphoric acid reacts with oxygenated compounds present in autoxidizing fat during the induction period and (2) the resistance of olefinic linkages to attack by phosphoric acid under the mild conditions under which autoxidation occurs.

Summary

At the elevated temperatures used in accelerated stability tests phosphoric acid was shown to react with fatty peroxides in such a manner as to prevent their estimation by the usual iodometric methods. A dark, phosphorus-containing precipitate formed, which was apparently polymeric in nature.

Phosphoric acid also reacted with oxidized fat to form an undisassociated fat-soluble complex but did not react with fresh unsaturated fatty esters.

In addition to the well known sparing action of phosphoric acid on α-tocopherol, it was shown that α-tocopherol and quinone also exerted a marked sparing action on the phosphoric acid.

REFERENCES

1. Calkin, V. P., J. Am. Chemists' Soc., *69*, 384 (1947).
2. Emmerie, A., and Engel, C., Rec. Trav. Chem., *57*, 1351 (1938).
3. Golumbic, C., Oil and Soap, *23*, 184 (1946).
4. Lundberg, W. O., "Biological Antioxidants," Trans. of First Conference, p. 28 (1946), Josiah Macy Jr. Foundation.
5. Methods of Analyses, A.O.A.C., Seventh Edition, p. 104 (1950).
6. Parker, W. E., and McFarlane, W. D., Can. J. Res. B., *405* (1940).
7. Privett, O. S., and Quackenbush, F. W., "Studies on the Antioxygenic Properties of Wheat Germ Phosphatides," J. Am. Oil Chemists' Soc., *31*, 169 (1954).

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