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Studies on the Antioxygenic Properties of Wheat Germ Phosphatides[1]

O. S. PRIVETT[2] and F. W. QUACKENBUSH, Department of Biochemistry, Purdue University, Lafayette, Indiana

THE phosphatide fraction of wheat germ has been found to contain various phosphatidic acids, lecithin and cephalin (2). Olcott and Mattill (7) demonstrated that the antioxidant activity of a number of commercial phosphatide preparations was due to their cephalin content and that lecithin was inactive. They attributed the activity of cephalin to an ionizable phosphoric acid group and the inactivity of lecithin to the internal neutralization of this group with choline. This view has been widely accepted, and the effectiveness of the phosphatides as inhibitors of autoxidation has been regarded as being due to their synergistic action with phenolic antioxidants (8, 9). Calkins (1) proposed that the synergistic action of these compounds was limited by their ease of oxidation whereby phosphoric acid was liberated. However Dutton *et al.* (4) have attributed the function of metal scavenger to phosphatides which increase the stability of soybean oil.

Our experiments with wheat germ phosphatides indicated that the antioxygenic properties of these compounds cannot be ascribed simply to the phosphoric acid constituent. That other constituent groups may have a greater influence than heretofore realized is indicated by the recently reported pro-oxidant action of choline (3) and the discovery of serine instead of colamine in various phosphatide fractions (5).

In this investigation a study was made of the antioxygenic properties of wheat germ phosphatides in an attempt to define more clearly the action of these compounds during autoxidation.

Experimental

Separation of phosphatides from wheat germ oil. Crude wheat germ oil[3] (ethylene dichloride extract) containing 8% phosphatides was degummed either by shaking it vigorously in centrifuge tubes or by mixing it in a Waring Blendor with 6-8% by volume of distilled water. The aqueous sludge was centrifuged from the degummed oil. About 30% of the crude oil remained in the break material. This was extracted with petroleum ether (b.p. 40-60° C.), and the extract was concentrated and poured into 20 times its volume of acetone. The phosphatide fraction precipitated and collected on the walls of the flask. The acetone solution was decanted, and the phosphatides were redissolved in a minimum amount of petroleum ether. Generally, three or four successive precipitations, followed finally by two or three washings with fresh acetone, gave a product free from neutral oil.

Oxygen absorption studies. Oxygen absorption measurements were carried out in a Warburg respirometer, using 2-ml. samples in 20-ml. rectangular flasks under static conditions at 59 and 99°C. The course of the oxidation was also followed by withdrawing samples at appropriate intervals and determining peroxide values by essentially the same procedure as was employed by Lundberg (6), in which oxygen was excluded during the critical steps. A reaction time of 10 minutes at 35°C. was found sufficient for completion of the reaction.

Effect of wheat germ phosphatides on autoxidation of the oil. When crude wheat germ oil was allowed to oxidize in a Warburg flask at 99°C., the peroxide number was found to be a relatively poor index of the degree of oxidation which had taken place (Figure 1). Agreement with oxygen absorption was somewhat better at 59°C. With degummed oil however there was a relatively close relationship between oxygen absorbed and peroxide number, especially at 59°C. (Figure 2). When the purified phosphatide fraction was redissolved in the degummed oil at a

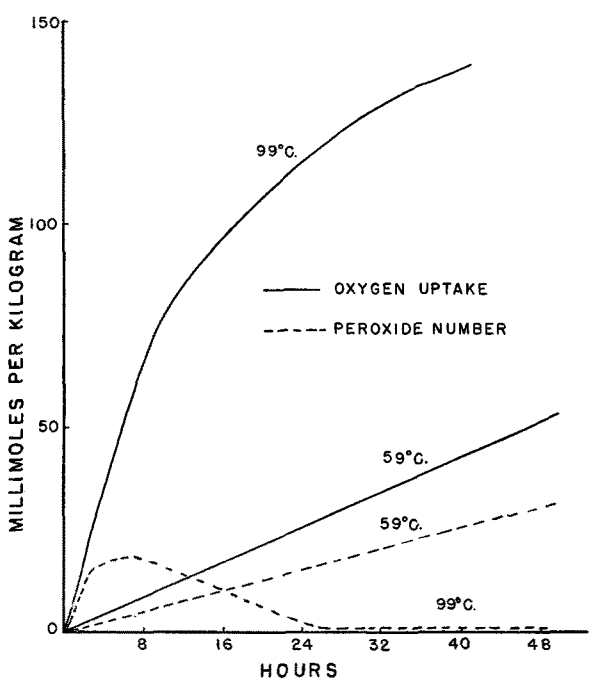

FIG. 1. Autoxidation of crude wheat germ oil in Warburg flasks.

[1] Journal Paper No. 682 of the Purdue University Agricultural Experiment Station, Lafayette, Ind.
[2] Present address: Hormel Institute, University of Minnesota, Austin, Minn.
[3] Supplied by the Viobin Corporation, Monticello, Ill.

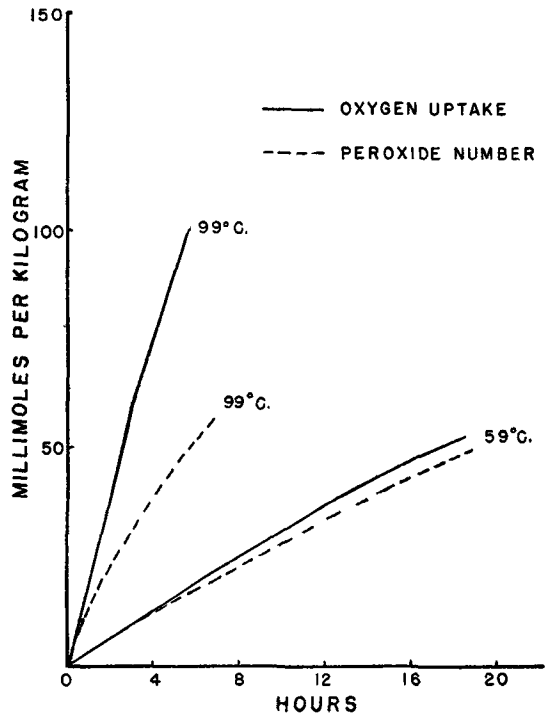

FIG. 2. Autoxidation of degummed wheat germ oil in Warburg flasks.

10% level, the stability of the mixture was similar to that of the crude oil (Figure 3). It was observed that in the presence of the phosphatides the oils darkened and became increasingly viscous during the oxidation period. This change was accompanied by the gradual formation of a tarry precipitate.

Since the action of phosphatides has been associated with their phosphoric acid component, the effect of phosphoric acid in degummed wheat germ oil was tried. The phosphoric acid (analytical reagent 85%) was diluted with distilled water to contain 6 milligrams of phosphorus per ml. Aliquots of this solution were added to the Warburg respirometer flasks, and the water was removed by evaporation at 100°C. before the fat was added. The slow rate of oxygen absorption in the presence of phosphoric acid showed that oxidation of the degummed oil was inhibited (Figure 4). There was no appreciable accumulation of peroxides even at 59°C., a fact which indicated that the action of phosphoric acid was qualitatively the same as that of the phosphatides in this respect.

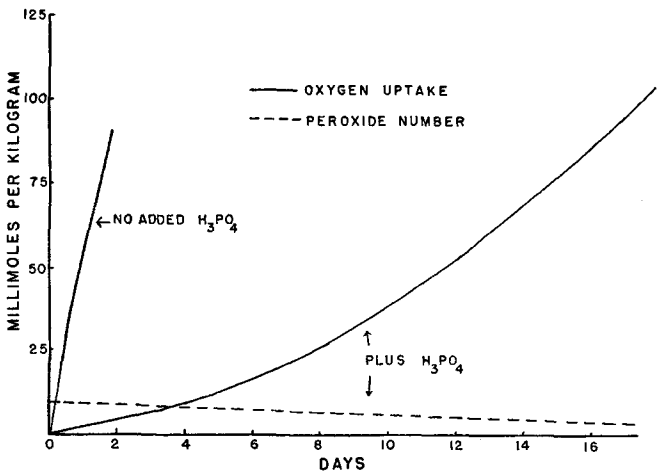

FIG. 4. Effect of 0.77% H_3PO_4 on the autoxidation of degummed wheat germ oil at 59°C.

Effect of wheat germ phosphatides on the autoxidation of lard. Bleached, deodorized lard was chosen as a relatively simple natural fat for comparison with the degummed wheat germ oil. The autoxidation of

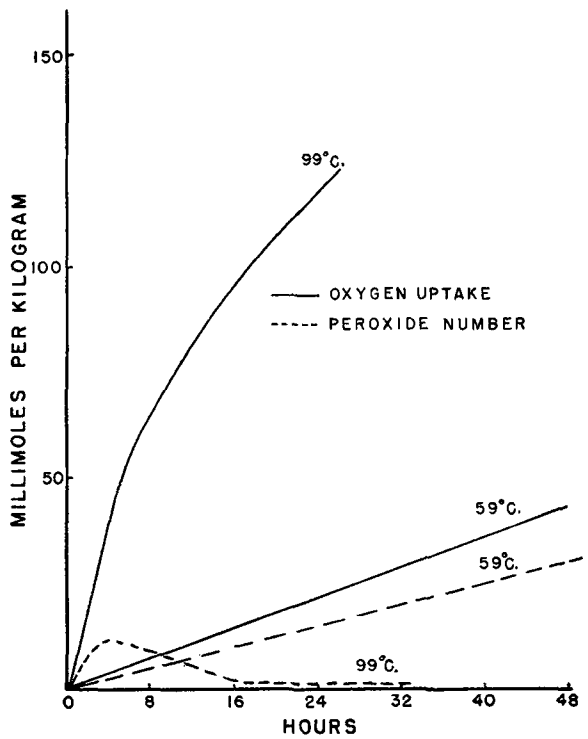

FIG. 3. Effect of addition of 10% phospholipids upon the autoxidation of degummed wheat germ oil.

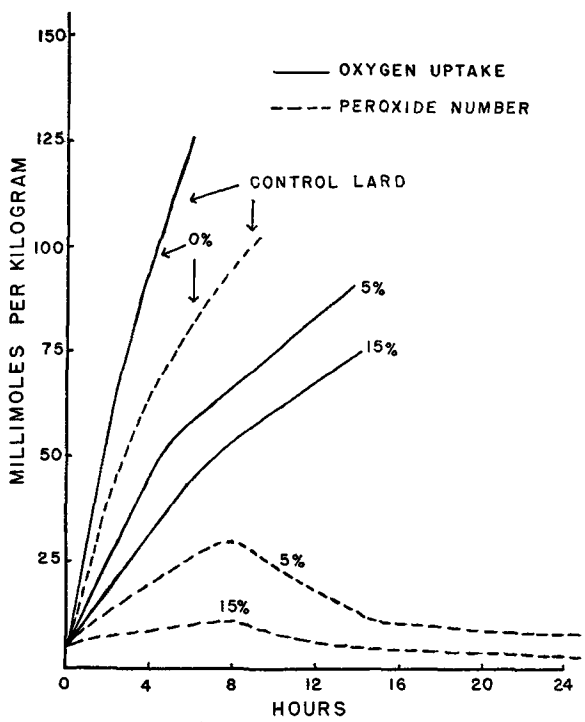

FIG. 5. Effect of 5% and 15% wheat germ phospholipids on the autoxidation of lard at 99°C.

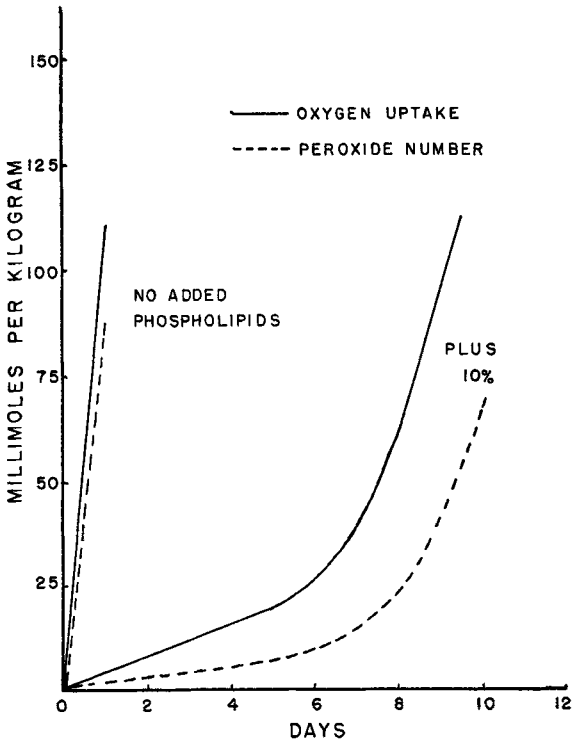

FIG. 6. Effect of 10% wheat germ phospholipids on the autoxidation of lard at 59°C.

lard containing various concentrations of crude wheat germ phosphatides was followed by measuring the oxygen absorption under static conditions in a Warburg respirometer at 59 and 99°C. At 99°C. under the conditions employed, even a 5% concentration of phosphatides largely prevented the accumulation of peroxides in lard (Figure 5). As with wheat germ oil, the peroxide number was no indication of the extent of oxidation. The rate of oxygen absorption decreased as the concentration of phosphatides was increased. At 59°C. a definite protective action was evidenced by a well-defined induction period (Figure 6). At this temperature the phosphatide fraction did not so effectively prevent accumulation of peroxides.

Oxidation of wheat germ phosphatides. Oxygen was bubbled through a 20% cyclohexane solution of wheat germ phosphatides at the reflux temperature

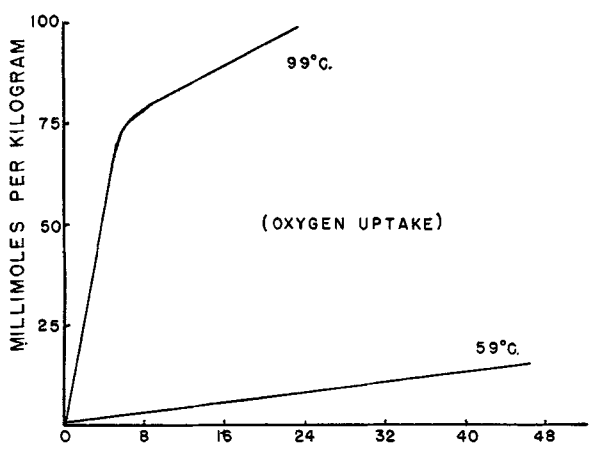

FIG. 7. Autoxidation of wheat germ phospholipids in dibutyl phthalate.

of the solution (approximately 80°C.). Samples were withdrawn periodically for analyses. After more than 72 hours of heating no peroxides were detectable by the usual iodometric methods, and no phosphoric acid was recoverable by water extraction of the cyclohexane solution. A 40% dibutyl phthalate solution of the phosphatides was used for oxygen absorption measurements at 59 and 99°C. Dibutyl phthalate when tested alone absorbed no oxygen at either temperature. The phosphatide solution absorbed oxygen rapidly at 99°C., and slowly at 59°C. (Figure 7). No peroxides were detectable at either temperature. Thus any peroxides which formed were evidently dissipated in some manner by the phosphatides.

Discussion

The lack of agreement between oxygen absorption measurements and peroxide values in the autoxidation of crude wheat germ oil was shown to be due to the phosphatide fraction. The phosphatides apparently reacted with the fat peroxides to form a polymeric substance. This was indicated by an increase in the viscosity of the oil associated with an increase in acetone solubility, followed by the production of an acetone-insoluble plastic mass. The reaction appeared to involve the phosphoric acid constituent since free phosphoric acid performed in an almost identical manner and prevented any appreciable accumulation of peroxides. In lard the phosphatides also appeared to react with fat peroxides to form polymers.

The mode of autoxidation of highly purified wheat germ phosphatides was different from that of neutral glycerides. Since peroxides did not accumulate and free phosphoric acid was not detectable during oxygen absorption, it appears that intramolecular and polymerization reactions involving the phosphoric acid group and the peroxides led to the formation of complex molecules.

Summary

1. In accelerated stability tests wheat germ phosphatides were shown to prevent the accumulation of peroxides in both fresh and partially oxidized wheat germ oil.

2. The phosphatides as well as phosphoric acid apparently reacted with fatty peroxides to form complex polymeric substances.

3. Oxygen absorption by highly purified wheat germ phosphatides proceeded without the accumulation of titratable peroxides and without the liberation of free (water-extractable) phosphoric acid. Polymers appeared to be the chief product of the oxidation.

Acknowledgment

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REFERENCES

1. Calkins, V. P., J. Am. Oil Chemists' Soc., *69*, 384 (1947).
2. Channon, H. J., and Foster, C. A. M., Biochem. J., *28*, 853-864 (1934).
3. Diemair, W., Ludwig, H., and Weise, K., Fette u. Seifen, *30*, 340-54, (1943). (*C.A.* 3498[6], 1944).
4. Dutton, H. J., Schwab, A. W., Moser, Helen A., and Cowan, J. C., J. Am. Oil Chemists' Soc., *26*, 441 (1949).
5. Folch, J., and Schneider, H. A., J. Biol. Chem., *137*, 51 (1940).
6. Lundberg, W. O., Biological Antioxidants Trans. of First Conference, p. 28 (1946), Josiah Macy Jr. Foundation.
7. Olcott, H. S., and Mattill, H. A., Oil and Soap, *13*, 98 (1936).
8. Olcott, H. S., and Mattill, H. A., J. Am. Chemists' Soc. *58*, 2204 (1936).
9. Swift, C. E., Rose, W. G., and Jamieson, G. S., Oil and Soap, *19*, 176 (1942).

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