patent literature. R. Strauss (Seifensieder-Ztg. **62**, 238) recommends chlorinated paraffin for soap and cosmetic preparations. K. Stephan (Chem.-Ztg. **59**, 416-7) discloses that several oils to which 0.2% camphor was added showed no Taufel-Thaler reaction after one year while control samples were all strongly rancid. The camphor may be removed by passing steam through the oil or washing with 90% alcohol. S. Musher (Food Ind. **7**, 167-8) claims good antioxygenic effects are obtained in lard by adding soy bean flour, crushed sesame seed or oat flour. The effect persists even when the flour is filtered from the melted lard, but is greater if the flour is allowed to remain in the fat. H. G. Miller (OIL AND SOAP **12**, 51-2) asserts that cottonseed meal, in addition to being a good stabilizer for cod-liver oil, functions also as a preservative for the vitamin content. E. I. Evans (Ind. & Eng. Chem. **27**, 329-31) demonstrated that vegetable lecithin possesses antioxidant properties in cottonseed oil when the autoxidation is catalyzed by cobaltic oleate. This property is lost by heating to temperatures above 65°.

Methods other than addition of antioxidants, for stabilization of oils are described in patents issued to S. Schmidt-Nielson (Brit. 426,752) and Elact. Ges. elektrische Apparatus G. m. b. H. (Brit. 420,471 and Fr. 766,739) The first patent pertains to the stabilization of marine oils by heating in closed container at 250° for at least 48 hours; while the latter claims good stabilization by passing an electric current through the oil or fat.

M. R. Coe and J. A. Le Clerc (OIL AND SOAP **12**, 231-3) continue their research of the preceding year by supplying evidence confirming a superiority of the green light protection method to those using antioxidants. The work of W. L. Morgan (Ind. & Eng. Chem. **27**, 1287-90) confuses our knowledge of protective wrappers since it does not agree with reports of other investigators. Morgan demonstrated that blue and invisible ultraviolet light materially accelerate development of rancidity; whereas, other visible light, such as red and yellow, have very little effect and he suggests, as a consequence, that rancidity-retarding wrappers may be of any visible color except blue. As a result of the work he has developed a highly protective yellow transparent cellulose film for use in packaging such materials as potato chips, crackers, cake, butter, nuts, etc.

F. R. Robertson and J. C. Campbell (OIL AND SOAP **12**, 234-6) studied the effect of six months' storage of cottonseed oil in containers made of several commercial samples of metals and alloys. The changes in free fat acids, refining losses and color are tabulated. No definite conclusions are drawn except that copper and copper alloys should not be used in contact with cottonseed oil.

The method for recovering rancid fats by treatment with semicarbazide and filtering was investigated by K. Stephan (Chem.-Ztg. **59**, 416-7). A rancid fat treated with one gram semicarbazide and four tenths sodium stearate for each hundred grams of fat showed no Taufel-Thaler or Kreis reaction. The reagent can be recovered from the precipitate by boiling with acid.

The tendency of butter toward fishiness, tallowiness and other oxidative defects was investigated by W. Ritter and M. Christen (Schweiz Milchztg. **61**, 23-4, 31-2, 61-2; 69-70). It was found that the use of cream containing .01-8 mg. copper or 0.5-16 mg. of iron is conducive to formation of the above named defects. Hydroquinone and hydrogen peroxide oppose the process. Vitamin C, maleic acid and aldehydes are inactive. The living *reducto-bacteria frigidum* neutrale, as well as other alkali formers, prevent the tallowiness due to metals. According to G. Van B. Gilmour and P. S. Arup (Ice & Cold Storage **38**, 120) butters of high flavor and high pH (above 6.7) keep well in storage.

[Editor's Note: Due to the length of this paper, it has been divided into two sections. Second section will appear in the May issue.]

ANTIOXIDANTS AND THE AUTOXIDATION OF FATS IV. LECITHIN AS AN ANTIOXIDANT

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AMONG the many substances that have more recently been suggested for delaying the progress of rancidity in fats, vegetable lecithin is probably one of the first (2). It is somewhat surprising that in the past ten years so little information of an experimental nature has been published to support the claims of this substance as an inhibitor and to explain the manner of its action.

In a list of inhibitors and their effect on commercial oleic acid, Trusler (15) found lecithin practically ineffective. Royce (12) included lecithin among the inhibitors which he studied by the methylene blue test. His data showed that in a concentration of 0.2 per cent in finished cottonseed oil, at a temperature of 70° C., lecithin gave an antioxygenic index[1] of 2 by the oven test (organoleptic) and of 3 by the methylene blue method[2]; with hydrogenated shortening 0.1 per cent gave an index of 1.5 by the oven test and 8.3(?) by the methylene blue method; this method gave irregular results with hydrogenated shortening.

Sollmann (13), using the Warburg apparatus for the oxygen-absorption method, reported without supporting data that lecithin inhibited the oxidation of cottonseed oil catalyzed by cobaltic oleate, and that when exposed to oxygen at temperatures above 65° C. for one-half hour it no longer exhibited antioxygenic properties.

Kochenderfer and Smith (8) measured the induction period of the oxidation of lard by the oven test and by the oxygen absorption method, as influenced by two commercial samples of soy-bean lecithin. They obtained indices of 1.7—1.8 by the former method and somewhat smaller figures by the latter. Results obtained from these lecithins after reprecipitation by acetone were variable, and this is not surprising, since by the nature of its prepara-

tion (1) vegetable lecithin is a mixture of substances, and for commercial use is always associated with a carrier.

More recently Evans (4) has found lecithin an excellent antioxidant for cottonseed oil whose oxidation was accelerated by the presence of cobaltic oleate peroxide. His proposed explanation of the effectiveness of lecithin as due to the formation of a compound with the cobaltic oleate peroxide raises a question as to the validity of using such accelerators in the assay of inhibitors for edible fats.

In view of these variable results with a substance which is not a chemical entity, it seemed desirable to investigate more carefully the antioxygenic activity of lecithin.[3]

In our earlier assays of inhibitors[4] we used lard with a small addition of cod liver oil as the test fat mixture; to different lots of lard with varying induction periods an amount of cod liver oil was added such that the induction period was shortened to a convenient interval, six to eighteen hours. Such a fat mixture was quite unaffected by the presence of 0.1 per cent or even 0.5 per cent of lecithin, nor was the induction period prolonged by material that had been purified by acetone precipitation from ether solution, repeated ten times. This precipitation removes fats and other acetone soluble materials from a crude phospholipid. "Pure" soy-bean lecithin, without any carrier, was actually pro-oxygenic.

Since these results were quite at variance with the observations of others on other types of fats, it seemed possible that the effectiveness of lecithin varied with the kind of fat in which it was used. This proved to be the case. With lard alone the following materials were all slightly antioxygenic: brain phospholipid, egg yolk lecithin, soy-bean lecithin and "100 per cent" lecithin without carrier; the last had been purified by four precipitations by acetone from ether solution. The indices obtained with these preparations in 0.2 per cent concentration on lard were 1.3 to 1.6 by the oxygen absorption method at 75° C., and thus agreed with the observations of Kochenderfer and Smith (8). In refined cottonseed oil three commercial lecithins, in 0.1 per cent concentration, gave indices of 2 to 4; one of these lecithins was purified by repeated precipitation with acetone; it still retained its antioxygenic effect. As yet no clear explanation has been given for the variable effectiveness of lecithin in different kinds of fat.

A more important question was to determine what substance or substances in the product known as lecithin might be responsible for its antioxygenic action.

An attempt to isolate the active material in one of the several fractions by partial precipitations with acetone was not successful. The most soluble fraction (lecithin-4) was as active as the least soluble (lecithin-1).

	Oven test, days at 70° C.
Refined cottonseed oil	2½, 2½
Refined cottonseed oil + .05% lecithin-1	10½
Refined cottonseed oil + .05% lecithin-2	9½, 10½
Refined cottonseed oil + .05% lecithin-3	9, 10½
Refined cottonseed oil + .05% lecithin-4	9½, 10

A solution of lecithin in petroleum ether was allowed to percolate slowly through a closely packed column of MgO. As an antioxidant toward refined cottonseed oil and lard the recovered material was equally as effective as the original sample. There was no preferential adsorption of the antioxidant or of possible interfering substances.

The two principal constituents of this phospholipid mixture are supposed to be the classical lecithin and cephalin. According to many investigators a completely definite separation of the two by the insolubility of cephalin in alcohol cannot be achieved. Such separations as we have made by precipitation of phospholipids by alcohol have yielded active preparations in both soluble and insoluble fractions.

The separation and purification of lecithin is best accomplished by precipitation with $CdCl_2$ according to standard procedures. Some pure lecithin was prepared from fresh egg yolks according to the method of Levene and Rolf (9); it was inactive.

Lecithin was also prepared from the small amount of alcohol-soluble material contained in commercial "lecithin" preparations by numerous precipitations with acetone, preparation of the $CdCl_2$ addition compound, rigorous washing of the precipitate with ether, toluene and glacial acetic acid, and regeneration of the lecithin by the addition of ammonia in methanol. The recovered material was ineffective as an antioxidant.

Purified cephalin was prepared from the alcohol-insoluble material by repeated precipitations from ether with alcohol and with acetone. The granular, slightly yellow product was antioxygenic to refined cottonseed oil. One sample, in 0.01 per cent concentration, increased the induction period in the oven from 4 to 8 days.

We are thus inclined to believe that the antioxygenic agent in so-called lecithin is in fact cephalin. The activity of the alcohol-soluble portion (true lecithin) of the mixed phospholipids (commercial lecithin) is probably to be credited to contamination with cephalin.

Various reactions were used in an attempt to determine what particular portion or characteristic of the cephalin molecule was responsible for its action as an inhibitor.

An ether solution of cephalin was hydrogenated in the presence of a platinum catalyst. The recovered product was no longer antioxygenic. Two other attempts to destroy the activity were not as successful, the antioxygenic effect was reduced but not destroyed. Levene and West (10) state that cephalin is hydrogenated with difficulty. Careful bromination of a cephalin fraction in ether and at 0° C. yielded a derivative which was inactive.

A synthetic cephalin was prepared according to Grün and Limpächer (6). The material recovered was supposedly $\alpha\beta$-distearo-cephalin. In solubility it resembled a saturated cephalin prepared by hydrogenation of natural cephalin, but differed from it in melting point. The synthetic material melted below 100° C., that obtained by hydrogenation charred but did not melt at 200° C.

The synthetic product was separated into ether-soluble and ether-insoluble fractions, both of which were slightly antioxygenic to lard.

Cephalin was hydrolyzed by heating with 6 per cent HCl for 25 hours. The ether-soluble fraction of the hydrolysate was entirely soluble in alcohol, that is, it apparently contained no cephalin. The fatty acids were inactive or so slightly antioxygenic as to suggest that the result was due to traces of unhydrolyzed cephalin. The water-soluble fraction, containing presumably the glycerol, phosphoric acid, and amine components, was neutralized and evaporated to dryness; it was invariably inactive. Alkaline hydrolysis seemed more destructive of the phospholipid antioxidant than acid treatment.

The organic base occurring in cephalin is ethanolamine. This compound (Eastman) was added to

lard and to refined cottonseed oil and found to be a pro-oxidant. Ethanolamine combines with oleic acid to form a soap, ethanolamine oleate (16). This substance also was a pro-oxidant toward refined cottonseed oil (0.05 per cent). Its behavior toward lard was variable; added in small amounts (0.02 to 0.05 per cent), it tended to be slightly antioxygenic; in larger amounts it was pro-oxygenic. The inhibitor value of cephalin was apparently not due to the structural portions of the molecule apart from the whole.

Unfortunately, this natural cephalin is not necessary the "cephalin" of the textbooks. Investigators (14) are generally agreed that a pure cephalin has not yet been prepared. A single experiment designed to separate pure cephalin, soluble in hot methanol, from extraneous, hot methanol-insoluble lipids (17) resulted in the isolation of the antioxidant in the methanol-insoluble fraction. Very little methanol-soluble material was obtained. The cephalin fraction was antioxygenic but less so than the fraction insoluble in boiling methanol, which is referred to as "oxidized" cephalin. These results suggest that the antioxygenic action is not a property of a classical phospholipid, but belongs rather to a compound closely associated with cephalin, the identity of which is not yet known.

There seems to be no quantitative relationship between the amount of cephalin used and the protection it affords. This was demonstrated with refined cottonseed oil and also with lard.

	Oven test, days at 63° C.
Refined cottonseed oil	4, 4½, 5, 5
Refined cottonseed oil + 0.005% cephalin	13, 13
Refined cottonseed oil + 0.01% cephalin	8, 11, 11½, 13
Refined cottonseed oil + 0.03% cephalin	10½, 12
Refined cottonseed oil + 0.05% cephalin	11½
Refined cottonseed oil + 0.20% cephalin	13, 13½

	Induction period hours at 75° C.
Lard	12
Lard + 0.02% cephalin	16½
Lard + 0.10% cephalin	20½
Lard + 0.20% cephalin	19½
Lard + 0.50% cephalin	21½
Lard + 1.00% cephalin	23½

At the time these experiments were concluded (October, 1934) they seemed to show that the entire cephalin molecule was necessary for antioxygenic activity; there was no satisfactory explanation for the difference between cephalin and lecithin. Since that time two developments have appeared which suggest a logical explanation for our observations. Eckey and others (3) have demonstrated that phosphoric acid, its acid salts and acid esters, are antioxygenic in salad oils and other fats. Furthermore, by electrometric titration, Jukes (7) has verified a fact (6) which we had not sufficiently appreciated before, namely, that cephalin behaves toward sodium hydroxide as a univalent acid, whereas lecithin has no base-binding capacity. From these observations, it seems clear that the acidic phosphoric acid radicle in cephalin is responsible for its action as an inhibitor. The absence of a titratable acid group in lecithin explains its lack of antioxygenic activity. Our assays on the water-soluble portion of hydrolyzed cephalin were uniformly unsuccessful because these were neutralized before evaporation, and the neutral salts of phosphoric acid are not antioxidants.

The apparent destruction by hydrogenation is explainable on the basis of insolubility; the saturated cephalin was a white, very insoluble powder. It is reasonable to suppose that the antixoygenic activity of any compound is dependent not only on its structure but also on its solubility.

The authors are indebted to Lever Brothers Company for assistance in carrying out this work.

SUMMARY

1. The commercial preparations known as "lecithin" have moderate antixoygenic action on refined cottonseed oil, little effect on lard, and none at all on lard-cod liver oil mixtures.

2. Commercial lecithins contain only small amounts of true lecithin. The antioxygenic agent in these preparations is cephalin. Purified lecithin is not an oxidation inhibitor, but purified cephalin is.

3. There is no quantitative relation between the amount of cephalin used and the prolongation of the induction period.

4. The particular portion of the cephalin molecule responsible for its antioxygenic action is probably the mono-basic phosphoric acid radicle.

[1]The induction period of the fat containing the antioxidant divided by the induction period of the fat alone.

[2]Test made by observing the time for decolorization of a mixture of 25 cc. of melted fat or oil with 1 cc. of 0.025 per cent methylene blue in absolute alcohol.

[3]For various preparations of lecithin we are indebted to American Lecithin Corporation and Ross and Rowe, Inc.

[4]Methods for the determination of the induction period are described in earlier papers from this laboratory (5) (11).

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