Antioxidants for Edible Fats and Oils[1]

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THE subject of antioxidants in fats and oils is so broad that a hurried survey of some parts of the field is all that can be accomplished in a brief space. I propose to outline some of the work carried out at the University of Iowa laboratories which finally led to a tentative classification of fat antioxidants, and to touch upon some developments in the practical application of antioxidants in fats and oils.

Mattill and his co-workers first demonstrated that antioxygenic materials could be isolated from vegetable oils. Professor Mattill's interest in the problem arose from the observation that the addition of lard to a diet complete for rats rendered it deficient in vitamin E (1). This phenomenon was later ascribed to a destruction of the vitamin catalyzed by the oxidation of the lard. In further work (2) (3), it was shown that the stability of a ration could be correlated with its content of vitamin E. That is, sources of vitamin E, such as wheat germ oil, contained antioxidants for lard.

A series of investigations on the antioxygenic activity of properties of known compounds was then initiated (4) (5). An oxygen absorption method was used to measure the lengths of the induction period. The oxidation was accelerated by placing the fat under oxygen rather than air and by making the determinations at 75° C. It may be emphasized that ultra-violet light, metallic catalysts, or temperatures higher than 75° C. were not used in the experiments to be reviewed. Entirely different results might have been obtained under these other conditions.

Lard was used as the substrate fat, and some of the results are shown in Table I. The conclusions to be drawn are, briefly, that a hydroxyl group must be uncombined and attached directly to an aromatic ring in order to exhibit effectiveness. In benzene itself, two or more hydroxyl groups are required, and these are more active where occupying ortho or para positions.

Yamaguchi and Nakamura (6) have described the effects of amines, guanidines, and other nitrogenous compounds on various fats. Marked antioxygenic activity was noted in some instances, but these substances have not yet received as detailed study as the phenolic compounds. In general, amino groups may replace hydroxyl groups without marked loss of antioxidant action.

TABLE I

Effect of Phenolic Inhibitors (0.01%) on Lard at 75° (4)(5)

Inactive Compounds	Antioxidants and Indices[1]			
Hydroquinone diacetate	Pyrogallol	60	1,2,3,4-tetrahydroxybenzene	20
Hydroquinone dibenzoate	Hydroxyhydroquinone	60	Orcinol	4
p-Dimethoxybenzene	Catechol	41	Phloroglucinol	3
Hydroxyhydroquinone triacetate	Hydroquinone	38	β-Naphthol	1.6
Dipyrogallol tricarbonate	α-Naphthol	22	Resorcinol	1.5
1,4-Cyclohexanediol				
Saligenin				
Phenol				
Cyclohexanol				
Anthraquinol				
Cholesterol				

[1] The antioxidant index has been used to indicate the ratio of the induction period of the lard plus added inhibitor to that of the lard alone.

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The results with non-nitrogenous organic compounds suggested that the antioxidant substances which occurred in the vegetable oils were phenolic in nature. Experiments had shown that they could be concentrated in the unsaponifiable fractions of such oils, that they were not sterols, and that they depended for their activity on free hydroxyl groups (7) (8). The name "inhibitols" was tentatively adopted to cover the class of natural compounds which acted as antioxidants for lard, the "ol" ending indicating the presence of the hydroxyl group. In further papers the concentration and properties of the inhibitols were described, but no pure compounds were obtained (9). Following the isolation by Emerson *et al* (10) of vitamin E, samples were kindly furnished by him, and it was found that the pure tocopherols possessed marked antioxygenic activity (11). It appears justified to conclude at this time that some if not most of the antioxidant activity present in the unsaponifiable portions of vegetable lipids can be attributed to the tocopherols.

However, the antioxidant activity of the tocopherols does not parallel their biological activity as vitamin E, as shown in Table II, and therefore the ultimate isolation from vegetable oil unsaponifiable fractions of related substances possessing antioxidant but no biological activity is still a distinct possibility. The lettuce antioxidant, $C_{13}H_{14}O_5$, described by Olcott and Mattill (12) appears to be one such compound, although its existence has not yet been confirmed independently.

TABLE II

Antioxidant Properties of the Tocopherols (11)

	Induction Period[1] Experimental hrs.	Control hrs.	Approximate minimum effective dose mg.
α-Tocopherol	62	15	1-3
β-Tocopherol	96	14	3-5
Tocopherol	166	11	1-3
Lard at 75°			

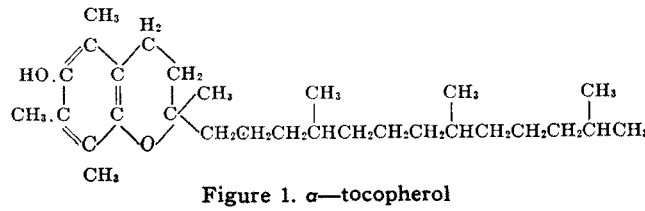


Figure 1. α—tocopherol

Figure 1 gives the accepted structure for α-tocopherol. As was predicted, the compound possesses a free phenolic hydroxyl group attached directly to an aromatic ring. Golumbic (13) has recently shown that synthetic chroman and coumaran derivatives possessing the hydroxyl group but lacking the perhydrofarnesyl chain or the methyl groups of the tocopherols are effective antioxidants. These compounds are ineffective biologically.

Turning now to the vegetable oils as substrate fats rather than as sources of antioxygenic concentrates for lard, it was soon discovered that inhibitol fractions were relatively inactive in the fats and oils from which they had been extracted (14). Table III illustrates the results of an experiment in which an inhibitol concentrate was ineffective in cottonseed oil, whereas in lard

TABLE III

Effect of Inhibitols on Different Fats (14)

Substrate	Per cent of Concentrate Added	Induction Period With inhibitor days	Induction Period Control days
Cottonseed oil	0.05	3	3.5
	0.05	6.5	8
Lard	0.01	11	4
	0.02	9	3

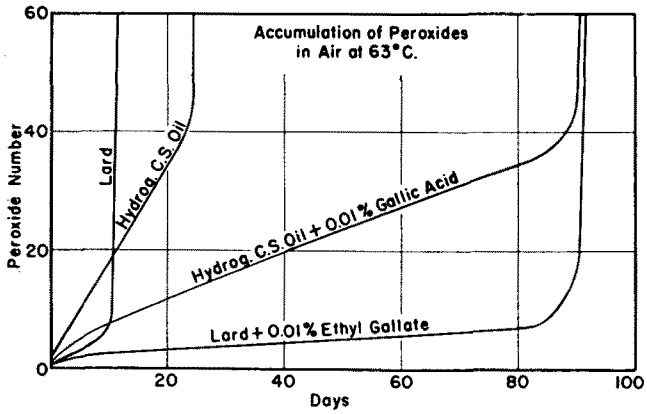


Figure 2.

one-fifth of the amount used more than doubled the induction period.

An essential difference in the behavior of lard and hydrogenated cottonseed oil is illustrated in Figure 2. With the former, the absorption of oxygen and the accumulation of peroxides is very small during the induction period. The addition of antioxidants prolongs that phase with a final, easily detectable change to a period of rapid absorption. With hydrogenated cottonseed oil, the substance accumulates peroxide and absorbs oxygen slowly throughout the induction period. Laboratory assays of antioxidants can be carried out more easily with the lard types of fat than with the vegetable oils since the end of the induction period can be more accurately determined. Other animal fats and also highly purified fatty acids and esters act like lard while the vegetable oils such as cottonseed, olive, peanut, sesame, and others behave like hydrogenated cottonseed oil.

Esterification with ethyl alcohol proved to be a satisfactory method of modifying vegetable oils so that they could be assayed by the oxygen absorption method without changing their inherent reactions toward inhibitors. The fat was refluxed with absolute alcohol containing hydrogen chloride. After dilution with water, the ester layer separated and could be washed free from alcohol, acid, and glycerol.

Like the original fats and oils, these crude ester preparations could not be protected by inhibitols, but they were protected to a remarkable degree by several substances which had been suggested as fat antioxidants but which we had heretofore found relatively inactive in lard. The results of numerous assays are shown in Table IV.

TABLE IV

Antioxidant Effect of Acidic and Phenolic Compounds on the Crude Esters of Hydrogenated Cottonseed Oil (14)

Inactive Compounds		Inactive Compounds	
Hydrochloric acid		Aspartic acid	
Iodic acid		Benzoic acid	
Nitric acid		Phthalic acid	
Boric acid		α-naphthoic acid	
Formic acid		Ethyl oxalate	
Acetic acid		Ethyl malonate	
Succinic acid		Ethyl tartrate	
Adipic acid		Sodium oxalate	
Azelaic acid		Inhibitols	
Active Compounds and Indices			
Sulfuric acid	15-20	Malic acid	8-12
Phosphoric acid	15-20	Maleic acid	4-6
Oxalic acid	15-20	Cephalin	4-6
Malonic acid	10-15	Pyrogallol	26
Tartaric acid	10-15	Catechol	12
Citric acid	10-15	α-naphthol	9
Pyruvic acid	10-15	Hydroquinone	1.5

No general conclusions regarding the activity of inorganic acids could be reached. Although sulfuric and phosphoric acids were active, hydrochloric, iodic, nitric and boric acids were without effect. In the organic acids, those aliphatic dicarboxylic acids of longer chain length than three carbon atoms were ineffective unless hydroxyl groups were present. If the carboxyl groups of an active acid such as tartaric were neutralized or esterified, the activity was lost. Thus, in the case of the acids, the activity seemed to depend upon the presence of free hydroxyl groups capable of splitting off hydrogen ions. What actually happens in the presumably aqueous-free environment of a purified fat is not yet known.

In order to investigate the problem further, the crude ester preparations were carefully distilled *in vacuo*. Ninety-five to 98 per cent of the esters distilled readily and without destruction. When the distilled fractions were used as substrate fats in antioxidant assays, it was found that they reacted entirely differently from the crude esters. They resembled lard, and could be protected by the inhibitols but not by the acid type inhibitors (Table V).

TABLE V

Effect of Inhibitors on the Crude and Distilled Esters of Hydrogenated Cottonseed Oil (14)

Substrate	Inhibitor Added	Induction Period With Inhibitor hrs.	Induction Period Control hrs.
Crude esters	0.02% tartaric acid	100	9
	0.10% inhibitol conc.	9	9
Distilled esters	0.20% tartaric acid	6	6
	0.10% inhibitol conc.	52	6

The distilled esters, then, reacted like all previous purified fatty acids and esters which had been submitted to this kind of assay, and it was apparent that the process of distillation accomplished two things; one, the removal of a substance which prevented the antioxygenic action of inhibitols, and, two, the removal of a substance which permitted or promoted the antioxygenic action of the acid type inhibitors. The first problem, that is, what substance in vegetable fats and oils prevents the antioxygenic action of inhibitols, has not yet been solved. In an attempt to answer the second, that is, what substance promotes the antioxygenic action of the acid inhibitors, a fractionation of the residue from the distillation showed that the inhibitol content of the original oil was the looked-for factor. That is, the inhibitol of the original cottonseed oil stayed in the crude ester layer after the esterification procedure; during the distillation it remained in the residue. When, now, inhibitols and acid antioxidants were assayed together it was found that the acid antioxidants gave a marked increased protection.

TABLE VI

Percentage of Inhibitor Added	Induction Period With Inhibitor hrs.	Induction Period Control hrs.
Distilled Ethyl Esters		
0.04% inhibitol conc.	9.5	5
0.10% tartaric acid	8	5
0.04% inhibitol conc. + 0.10% tartaric acid	148	5
Lard		
0.02% orcinol	75	12
0.10% phosphoric acid	21	12
0.02% orcinol + 0.10% phosphoric acid	314+	12
0.02% ascorbic acid	23	12
0.02% α-tocopherol	75	12
0.02% ascorbic acid + 0.02% α-tocopherol	180	12

The synergism is illustrated in Table VI. In each case, the use of two antioxidants together gave protection in excess of what might have been expected from the results with either alone. It was later found that not only inhibitols, but also certain other phenolic compounds could promote the effectiveness of acid type inhibitors. In particular, and for illustration, the results with orcinol and phosphoric in lard are especially striking.

An attempt to classify antioxidants based upon the work just described is outlined in Table VII. In general it may be said that any acidic inhibitor will promote or energize the action of any phenolic inhibitor. However, numerous combinations have been tried with results that suggest, to date, that the effect of any particular combination upon any particular fat is not yet predictable. The classification will undoubtedly have to be modified as new data become available. It may be repeated that the series of accelerated experiments just described were run at 75° C. under oxygen and that a successful transfer of these observations to practical problems does not necessarily follow.

TABLE VII

Tentative Qualitative Differentiation of Three Types of Inhibitors (14)

	Vegetable Oil	Crude vegetable oil esters	Distilled vegetable oil esters	Lard, lard esters purified fatty acids
Acid type	+	+	—	—
Inhibitols	—	—	+	+
Phenolic type	+	+	+	+

Of interest is the fact that ascorbic acid, vitamin C, possesses antioxidant activity of the acid type while the tocopherols, that is, vitamin E, possess the properties assigned to phenolic inhibitors. Theoretically, combinations of the two should be particularly advantageous antioxidants, and actually the data confirm this assumption (Table VI).

The use of ascorbic acid as an antioxidant in fat emulsions has recently been patented (15). Our first observations on ascorbic acid were, however, made more than three years ago at the University of Iowa. The data in the table were obtained at Mellon Institute.

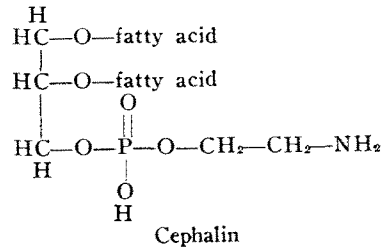
Tentatively the hypothesis may be proposed that acid type inhibitors are not effective unless the protected oil contains native or added tocopherols or other phenolic substances.

Practical Application of Antioxidants

The actual practices of the large producers of animal and vegetable fats are, of course, trade secrets. However, it may be fair to assume that with the more stable oils the trend is not to use added antioxidants but to achieve increased stability by avoiding processes and treatments which would tend to decrease the effectiveness of the natural antioxidants present.

Possibly the oldest of modern patents on the use of antioxidants in edible products is that of Bollman (16) for lecithin. The lecithins on the market at present find some use in this field; they are also used for modifying fats and oils in properties other than stability, for example, to improve emulsification, frying properties, etc. Very carefully purified lecithin possesses no antioxidant activity, but the commercial product is, of course, a mixture of phospholipids, and Olcott and Mattill (17) have shown that the cephalin fraction carries the inhibitor action.

An ionizable hydroxyl group of phosphoric acid is present in cephalin. The same group is not free but combined in lecithin, hence it is assumed that commercial lecithin owes its activity to the presence of an ionizable phosphoric acid group in the cephalin molecule (Fig. 3).



Cephalin

Figure 3.

Parts of the cephalin molecule have separately been patented. Bollman's patent inferentially claims the entire molecule. The phosphoric acid fragment is covered by the patents issued to Eckey and to Richardson, Vibrans, and Andrews (18). Epstein and Harrison (19) have claimed the molecule minus the cholamine and one of the fatty acid radicals, while Royce's patent (20) describes cephalin minus only the fatty acid in the *a* position. Recently four patents covering the use of cottonseed and corn oil phosphatides as antioxidants, emulsifiers, etc., have been granted to Thurman (21). The novelty resides in the claims that these phosphatides are less likely to oxidize or revert because they are more saturated than the soybean product.

In industrial laboratories interested primarily in edible products, naturally occurring materials, especially from food sources, have received most attention since it is unlikely that such substances can have toxic effects. The use of fractions molecularly distilled from vegetable oils has recently been patented by the Eastman Kodak Co. (22). These fractions contain the inhibitols present in the original oil. Gum guaiac was suggested by Grettie (23) and is now being used commercially. Investigations at the University of Chicago have shown it to be entirely non-toxic.

Probably the most publicized study of the practical application of natural materials as antioxidants has been that supported by the Musher Foundation to which some 50 patents have been assigned in the last few years (24). Many of these deal with the use of cereal flours and particularly oat flour, as suggested antioxidants for butter, ice cream, potato chips, and numerous other foods subject to oxidative deterioration (25).

The effective principle in oat flour, or "Avenex," as the commercial product is called, has been investigated by several laboratories. At the University of Iowa, active inhibitol fractions were not obtained, hence this type of antioxidant does not seem to be responsible for the action. Phospholipids may account for part but not all of the activity. The kind of antioxidant recently described by Hilditch and Paul (26) as being extractable from seed cakes only after acid treatment may play a part in the antioxidant activity. The nature of these substances is not yet known. Diemair, Strohecker and Reuland (27) have lately claimed to have isolated the antioxidant from oat flour and describe it as a protein-fat complex.

Musher has also patented combinations of antioxidants, such as extracts of crude sugars and phospholipids, the action of which may ultimately be interpreted in terms of the synergism previously outlined. The actual antioxidants involved in the protection induced in the presence of these complex materials or crude extracts therefrom has, if known, not yet been published.

Hilditch and Paul (26) state that of all the sources which they studied, cottonseed gave the most potent antioxidant activity. We also have found that cottonseed meal is an excellent antioxidant in fats and oils, superior in fact to oat flour in this respect. This activity is independent of the gossypol content, for ether-extracted meats carry the effective principle. The antioxidant in cottonseed meal is soluble in water (28). Further work on its properties is now in progress.

It will be clear that the opportunities for research activities in this field, especially in the study of fundamental reactions, are still practically unlimited.

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Evolution Volumetric Method for Carbon Dioxide

By L. B. HITCHCOCK and R. E. DIVINE

A METHOD for determining combined carbon dioxide in soaps and other detergents was published by the authors of this paper in OIL AND SOAP, January, 1938, Vol. 15, No. 1.

The method was based on the fact that in a system free from gases other than aqueous vapor, carbon dioxide may be liberated from carbonates and distilled into a measured quantity of an alkaline absorbent. The excess of the latter may be accurately titrated.

Further experience with the method as originally described has shown that modification of both apparatus and details of procedure are necessary in order that the results may be consistently accurate.

When the method is applied to detergents containing soap, in order to make sure that no volatile fatty acids distil into the receiver with CO_2, a 3 bulb or 4 bulb Allihn condenser has been substituted for the calcium chloride tube which served as a spray trap in the original apparatus. The tubulure of the condenser has a side tube sealed into it to which the rubber tube leading to the receiver is attached. The separatory funnel through which reagents are introduced is attached to the condenser by a one-hole rubber stopper. The stem of the separatory funnel is prolonged by sealing on a narrow tube which extends down to the center of the lowest condenser bulb when the funnel is in place. This tube must be sufficiently narrow to insure adequate clearance between its exterior and the inner walls of the condenser so that vapors from the boiling evolution flask may condense and reflux without impeding the flow of CO_2 into the receiver.

The evolution flask remains the same as originally recommended, namely a one-liter, Pyrex, round bottom flask with long neck. The receiving flask carries only one connection through which is the passage for admitting CO_2. Two or three pieces of light copper wire are twisted together and disposed vertically in the evolution flask during a test with the aim of moderating or preventing bumping when the acidulated detergent solution is heated under vacuum. A good vacuum is important. When the assembled apparatus is exhausted for a test, the internal pressure should be only a few millimeters of mercury higher than that corresponding to the tension of aqueous vapor at the temperature of the liquid.

A good Bunsen aspirator (water pump) will give satisfactory results, although the Hyvac pump is more certain. A mercury column should be used to indicate the degree of exhaustion, closing off the connection during an actual test. Air leaks are inadmissable. The best way to avoid air leaks is to make sure that all connections are clean and well fitted and to wet rubber and glass surfaces with water before making up connections.

The most satisfactory source of heat we have found is a small argand gas burner with chimney and a regulating lever at the base. This type of burner supplies all the heat necessary for the determination and is adapted to exact control.

A blank test on the reagents used in the evolution flask shows a significant amount of CO_2. For this reason a deduction must be made from the quantity of CO_2 found in a test. It is essential that all reagents used be measured or weighed so that the correction may be valid. The most suitable way of preparing distilled water free from CO_2 is to boil it under vacuum. A large Pyrex round bottom flask is suitable and convenient for this purpose. It may be heated directly with a small bunsen flame. About 15 minutes boiling suffices to remove gases to such extent that bumping takes place.

A test of detergent for combined CO_2 is made as follows: A sample representing 0.200 CO_2 or less is weighed and transferred to the evolution flask and to it are added 400 ml unboiled distilled water followed by 5 ml of absorbent solution. If the presence of bicarbonate is suspected, add 10 ml of absorbent solution. The mouth of the flask is then covered with a small beaker, or a plug of cotton is put into it to prevent circulation of air, and the flask is heated over steam until the sample is dissolved. It is then cooled under the tap until only moderately warm and 20 grams of crystallized magnesium chloride dissolved in 30 ml water are added and mixed in. The flask is now completely cooled and connected with the condenser.