Autoxidation of Fats and Oils

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Products that contain fats and oils turn rancid and deteriorate in other ways when they are exposed to air. The action, known as autoxidation, imparts disagreeable flavors and odors to fats and foods containing them. For a long time baffled investigators spent a vast amount of effort trying to find ways to prevent autoxidation. Specifically, the basic problem was the mechanism of autoxidation—how oxygen attacks a fat. On that point the investigators made several discoveries in 1943. They have developed moderately effective methods of preventing rancidification, and believe now that the solution of the problem is in sight.

It is important to prevent autoxidation because fat and fat-containing foods valued at several billion dollars are produced and marketed annually. The oil, meat, fishery, dairy, and bakery industries suffer serious losses from autoxidation of their products.

Rancidification was an important economic and military problem during the First and Second World Wars, although many modern scientific practices reduced the frequency of its occurrence. Measures that were moderately effective in preserving civilian foods availed little for food that had to be transported long distances and stored in unfavorable climates.

In different fats, and under different conditions, autoxidation produces tallowy, painty, burned, fishy, grassy, and other off-flavors and odors; the senses of taste and smell can detect autoxidative deterioration quite readily. Fats in the early stages of autoxidation are edible, but unpalatable. Thereafter the degree of unpalatability increases until the odor and flavor become so repulsive that the fat cannot be eaten.

Nutritional tests indicate that autoxidation reduces or destroys the food value of fats. For example, the vitamin E content of vegetable oils and the vitamin A content of fish oils are reduced, if not entirely destroyed, during autoxidation. Furthermore, the fatty acid components of fats that are most essential in the diet are the first to be attacked during autoxidation. Rancid fat in diets adversely affects the body's utilization of vitamins from other sources. Finally, there is some evidence that autoxidized fat may exert a toxic action.

NATURAL ANTIOXIDANTS, substances that tend to prevent autoxidation, are present in small amounts in most vegetable fats. Their importance has been realized for a very long time. For instance, the American Indians added oak-bark extracts to the fat of bears to keep the fat from spoiling. Some natural antioxidants have been isolated recently from the fats in which they occur and their chemical structures have been determined. A number of fats and oils contain the same natural antioxidant; some contain one not yet found in any other fat or oil. Fats and oils also differ very markedly in the amounts of natural antioxidants originally present in them.

The most common antioxidant is vitamin E, or tocopherol. Generally speaking, vegetable fats contain appreciable amounts of tocopherols, while animal fats contain them in very low concentrations. Sesame oil contains a

unique antioxidant, called sesamol. Crude cottonseed oil contains a different antioxidant, gossypol.

BESIDES NATURAL antioxidants, fats and oils generally contain phosphatides, complex substances that sometimes act as an antioxidant and sometimes also enhance the activity of other antioxidants present. A substance which has practically no antioxidant activity, but which increases the activity of a true antioxidant, is called a synergist.

Unfortunately, both the antioxidants and synergists are sometimes partly or wholly removed from crude fats and oils during the refining process to which they must be subjected to rid them of objectionable coloring matter and flavorous or odorous constituents. A refined fat or oil may, therefore, require the addition of antioxidants to increase its stability.

When refining is carefully carried out, however, most of the natural antioxidant remains in the refined oil. Under such conditions, there may be no advantage in adding more antioxidant, for it has been found that there is an optimum concentration for these substances in any fat or oil. It is still possible, however, to add synergists to assist the natural antioxidants in protecting the fat. Sometimes, of course, it is advantageous to add both antioxidants and synergists.

The phosphatides and certain other compounds form effective synergist-antioxidant combinations with natural tocopherols. The manner in which synergists act to increase the activity of antioxidants has been investigated, but is not clearly understood. Phosphoric acid, which forms an effective synergist-antioxidant combination with tocopherol, apparently is capable of regenerating tocopherol that has been oxidized. It has been shown that tocopherols in the presence of a synergist are depleted at a retarded rate during autoxidation. One present theory assumes that the antioxidants are continuously regenerated at the expense of the synergists.

The tocopherols, which are only moderately active as compared with certain antioxidants, have some advantages. They impart no color, odor, or flavor to fats. During the autoxidation of fats that contain tocopherols, red substances, known as chroman-5, 6-quinones, are produced, but the color formed is usually not objectionable. Because the tocopherols are not soluble in water and are relatively stable to heat, they generally are carried over into finished food products, as, for example, baked goods, such as piecrust or cakes. Finally, the antioxygenic activity of a tocopherol may be greatly increased when any of a number of synergists is used to increase its activity. These properties indicate that the tocopherols are singularly well adapted to their role as natural antioxidants.

Several effective antioxidants and antioxidant combinations have been suggested for use in artificially stabilizing fats. Only a few are important. The addition of antioxidants to foods is subject to Federal regulations. Their acceptability must be established by toxicological and nutritional tests before their addition is permitted. A long time may elapse between the laboratory discovery and the commercial utilization of an antioxidant.

MANY INVESTIGATIONS of the effect of various antioxidants on fat stability have provided a great deal of information on the properties of antioxidants. Most compounds with appreciable antioxygenic activity belong to the phenols, a group of complex substances including the natural tocopherols—gum guaiac obtained from a tropical tree and nordihydroguaiaretic acid obtained from the creosotebush of the southwestern United States—and the synthetic substance known as hydroquinone.

Adding phenolic antioxidants to vegetable fats which already contain natural tocopherols improves the stability of the fat only slightly. On the other hand, addition of the phenolic antioxidants to fats naturally lacking in

antioxidants, such as animal fats, improves stability appreciably. Moreover, a relatively large group of synergists can greatly enhance the antioxygenic activity of the phenolic antioxidants. The effective members of this group include citric, ascorbic, gallic, and phosphoric acids and the phosphatides, especially the commercial lecithins, such as the soybean, peanut, and rapeseed lecithins.

Propyl gallate, a chemical compound that acts synergistically with tocopherols, is particularly effective in vegetable fats. It is one of the few compounds that are both phenolic antioxidants and synergists. Animal fats and fish oils can be effectively stabilized by the addition of phenolic antioxidants, and even more effectively by synergist-antioxidant combinations. The antioxidants approved for use, subject to change and with certain limiting restrictions, are the tocopherols, gum guaiac, nordihydroguaiaretic acid, butylated hydroxyanisole, thiodipropionic acid, thiopropionic acid plus dilaurylthiodipropionate, and propyl gallate, also the lecithins, and citric and phosphoric acids, which are synergists. The addition of natural food substances is permissible, but generally only mildly effective.

There are several other effective antioxidants whose use in food products has not yet been approved. At the Eastern Regional Research Laboratory work has been done to improve the utility of gallic and ascorbic acids as antioxidants by converting them to esters, which are more soluble in fats than the acids themselves. One recently discovered phenolic antioxidant is norconidendrin, which was derived from the waste liquor from pulping western hemlock by workers at the Southern Laboratory. This compound has antioxygenic activity approximately equal to that of nordihydroguaiaretic acid.

IN PRESENT PRACTICE, autoxidation is prevented mainly by methods of handling, processing, and packaging fats and fat-containing foods. Care is taken to minimize exposure to light, heat, and air (oxygen) and contamination with metallic pro-oxidants, or promoters of autoxidation, such as copper and iron. These precautions call for attention to the design of equipment, to the materials from which the equipment is fabricated, to packaging materials, and to numerous other details. If suitable precautions are observed, many fats, particularly vegetable fats, are sufficiently stable for most uses without added antioxidants.

A large portion of commercially important liquid vegetable oils are hydrogenated, or hardened, to produce plastic fats like shortening and oleomargarine. Although the main objective of hydrogenation is to produce plastic fats, hydrogenated fats are generally many times more stable than the unhardened oils from which they are produced.

Lard and other animal fats are the main ones that require stabilization with antioxidants. Much of the lard marketed today is stabilized with approved antioxidant-synergist combinations selected from those we have mentioned.

An appreciable quantity of fat is marketed in the form of animal-vegetable fat combinations. The animal fat ingredients in such products are stabilized to a marked extent by the natural tocopherols present in the vegetable fats.

THE MECHANISM of autoxidation, or how fats become rancid, has held the interest of chemists for many years.

M. E. Chevreul, a French chemist, discovered a century ago that fats are compounds formed by the reaction of fatty acids and glycerol, or are, as the chemist says, glycerol esters of fatty acids. Scientists also learned that some fatty acids contain carbon-to-carbon double bonds ($>C=C<$) and are therefore chemically related to other compounds that undergo autoxidation.

In 1858, C. F. Schönbein, in Germany, proposed that these carbon-to-

carbon double bonds are involved in the autoxidation of turpentine. The view that double bonds are involved in autoxidation was extended in 1897 by C. Engler and W. Wild, also in Germany. They suggested that autoxidation involves the addition of oxygen to the carbon-to-carbon double bonds of fats producing cyclic peroxides, which may be represented as follows:

$$>C=C< + O_2 \longrightarrow \overset{O-O}{\overset{|\quad|}{>C-C<}}$$

double bond   oxygen   cyclic peroxide
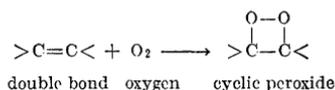
That concept was generally accepted until recently; most investigators supposed that the reaction at carbon-to-carbon double bonds with atmospheric oxygen is a simple addition, analogous to reactions at such bonds with other chemical agents such as bromine and ozone. Also, this theory explained how, during autoxidation, oxygen is consumed, peroxide groups, or at least groups containing active oxygen, are formed, and the carbon-to-carbon double bonds disappear. Actually, these details applied only to the over-all reactions of autoxidation; it is now known that carbon-to-carbon double bonds do not disappear during the initial (or peroxide-forming) stage of autoxidation.

During the next 40 years, while the fundamental reactions of autoxidation remained a mystery except for that theory, scientists learned many useful facts. New data indicated that autoxidative reactions are autocatalytic; that is, they perpetuate themselves at a rate that depends on the amount of oxidation products present at any given instant. The scientists also found that certain substances possess pro-oxidant or antioxidant activity; that is, they accelerate or retard the rate of autoxidation of fats. They also found that fats spoil faster at higher temperatures and can be preserved for a long time if refrigerated. Chemists investigating the effect of light on fat deterioration found that exposure of fats to light, particularly ultraviolet light, promotes

rancidity, and that for maximum stability a fat should be stored in the dark or in an opaque container.
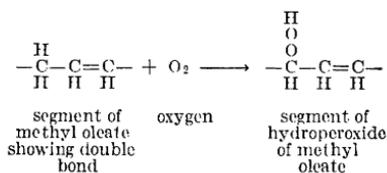
Investigators also developed chemical methods for detecting autoxidation and for measuring the keeping quality, or stability, of fats and oils. They devised improved methods to prevent or retard autoxidation and isolated and identified from rancid fats and oils a number of products formed by autoxidation.

Alfred Rieche at the University of Leipzig and R. Criegee and coworkers, at the Chemical Institute of the Technical University at Karlsruhe, Germany, reported in 1937 and 1939 that the relatively simple hydrocarbon cyclohexene, and the related compounds containing carbon-to-carbon double bonds, form hydroperoxides during autoxidation. In those simple compounds a molecule of oxygen was presumed to add to the carbon atom nearest the double bond and between that carbon atom and the hydrogen atom already attached to it: that is, C–H in cyclohexene became C–O–O–H, the whole reaction being represented thus:

cyclohexene   oxygen   hydroperoxide of cyclohexene
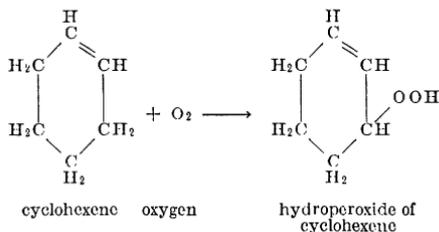
E. H. Farmer and coworkers of the British Rubber Producers' Research Association in England, while studying the autoxidation of various substances with chemical structures related to those occurring in rubber, investigated the autoxidation of the methyl ester of oleic acid, one of the principal fatty acid constituents of fats. They reported in 1943 that the initial product formed during the autoxidation of methyl oleate is indeed a hydroperoxide, which they succeeded in isolating. They believed that the hydroperoxide group is attached to carbon atoms on either side

of the carbon atoms possessing a double bond.

$$-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-\overset{}{\underset{\underset{\displaystyle H}{|}}{C}}=\overset{}{\underset{\underset{\displaystyle H}{|}}{C}}- \;+\; O_2 \;\longrightarrow\; -\overset{\overset{\displaystyle H}{\overset{|}{\underset{\displaystyle O}{O}}}}{\underset{\underset{\displaystyle H}{|}}{C}}-\overset{}{\underset{\underset{\displaystyle H}{|}}{C}}=\overset{}{\underset{\underset{\displaystyle H}{|}}{C}}-$$

| segment of methyl oleate showing double bond | oxygen | segment of hydroperoxide of methyl oleate |

Farmer's description of the isolation for the first time of a hydroperoxide of a fatty acid greatly stimulated investigations of autoxidation and invited reexamination of all of the phenomena of autoxidation. The fact that the autoxidation of methyl oleate occurs with the formation of hydroperoxide without destroying the carbon-to-carbon double bond was confirmed at the Southern Laboratory and in the laboratories of Prof. T. P. Hilditch at the University of Liverpool. Shortly afterwards, two of Farmer's associates, J. L. Bolland and H. P. Koch, and also S. Bergström, of the Nobel Institute at Stockholm, investigated the autoxidation of linoleic acid. This acid, which is also a constituent of many of the common fats and oils, such as cottonseed, soybean, and peanut, differs from oleic acid in having two carbon-to-carbon double bonds instead of one.

In that case, autoxidation occurs initially to form a hydroperoxide, but now one of the two carbon-to-carbon double bonds is shifted along the carbon chain to produce a different kind of linoleic acid. The different linoleic acid contains conjugated carbon-to-carbon double bonds; that is, the double bonds are attached to adjacent carbon atoms, instead of being separated by an intervening carbon atom without double bonds.

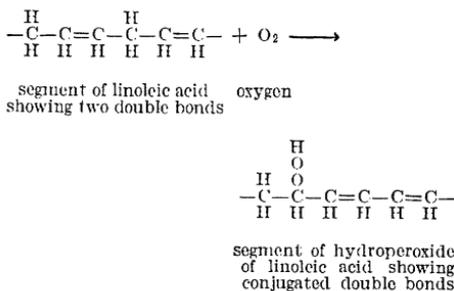The two forms of double bonds can be represented thus:

$$-CH=CH-CH_2-CH=CH-$$

unconjugated carbon-to-carbon double bonds

$$-CH=CH-CH=CH-$$

conjugated carbon-to-carbon double bonds

But let us return to the autoxidation

of linoleic acid. As we have stated, the oxidation of natural linoleic acid is accompanied by a shift of one of the carbon-to-carbon double bonds, with the formation of a hydroperoxide, which may be illustrated as follows:

$$-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-\overset{}{\underset{\underset{\displaystyle H}{|}}{C}}=\overset{}{\underset{\underset{\displaystyle H}{|}}{C}}-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-\overset{}{\underset{\underset{\displaystyle H}{|}}{C}}=\overset{}{\underset{\underset{\displaystyle H}{|}}{C}}- \;+\; O_2 \;\longrightarrow$$

segment of linoleic acid showing two double bonds   oxygen

$$-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-\overset{\overset{\displaystyle H}{\overset{|}{\underset{\displaystyle O}{O}}}}{\underset{\underset{\displaystyle H}{|}}{C}}-\overset{}{\underset{\underset{\displaystyle H}{|}}{C}}=\overset{}{\underset{\underset{\displaystyle H}{|}}{C}}-\overset{}{\underset{\underset{\displaystyle H}{|}}{C}}=\overset{}{\underset{\underset{\displaystyle H}{|}}{C}}-$$

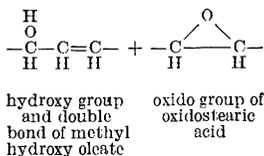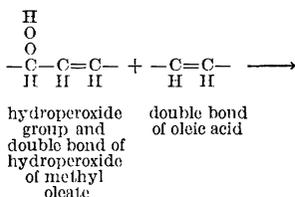segment of hydroperoxide of linoleic acid showing conjugated double bonds

These observations all accorded with Farmer's concept of the way oxygen adds to the unsaturated fatty acids. Additional evidence was soon brought forward in other laboratories to substantiate Farmer's theory and today there appears little doubt that the initially formed products of autoxidation of the principal fatty acid components of fats are hydroperoxides whose structures are reasonably well established.

Having resolved the problem of the first step of the addition of oxygen in the autoxidation of fats, the next problem to be attacked was the fate of the hydroperoxide once it had been formed. It was well known that this compound is not in itself the cause of the off-flavor and off-odor of rancid fats.

As soon as chemists learned how to prepare and isolate the hydroperoxidized fatty acids from rancid or autoxidized fats and oils, it became possible to investigate the purified product itself. At the Southern Laboratory we found that this product continues to react with other substances that are parts of, or are present in, most fats and oils. The first discovery was that the hydroperoxide reacts with other substances containing one or more carbon-to-carbon double bonds. For example, the hydroperoxide of methyl oleate reacts with unoxidized oleic acid or methyl oleate to form oxido and

dihydroxy acids or esters. This reaction may be illustrated as follows:

$$-\underset{\underset{\text{H}}{\overset{\text{H}}{\overset{\text{O}}{\overset{\text{O}}{|}}}}{\text{C}}-\underset{\text{H}}{\overset{}{\text{C}}}=\underset{\text{H}}{\overset{}{\text{C}}}- \quad + \quad -\underset{\text{H}}{\overset{}{\text{C}}}=\underset{\text{H}}{\overset{}{\text{C}}}- \quad \longrightarrow$$

hydroperoxide group and double bond of hydroperoxide of methyl oleate

double bond of oleic acid

$$-\underset{\underset{\text{H}}{\overset{\text{H}}{\overset{\text{O}}{|}}}}{\text{C}}-\underset{\text{H}}{\overset{}{\text{C}}}=\underset{\text{H}}{\overset{}{\text{C}}}- \quad + \quad -\underset{\text{H}}{\overset{}{\text{C}}}\overset{\overset{\text{O}}{\diagup\diagdown}}{\quad}\underset{\text{H}}{\overset{}{\text{C}}}-$$

hydroxy group and double bond of methyl hydroxy oleate

oxido group of oxidostearic acid

The new compounds, however, did not have disagreeable odors or flavors and, therefore, could not be responsible for off-flavors and odors in rancid fats.

The discovery explained why some of the carbon-to-carbon double bonds disappear during rancidification, or autoxidation, of a fat, even though oxygen of the air does not attack the bonds directly.

The second discovery at our laboratory was that the hydroperoxides break into fragments with the formation of aldehydes which do have objectionable flavors and odors. It had long been known that aldehydes are among the products formed in rancid fats, but the ones we isolated turned out to be quite different from those previously imagined. These aldehydes have not only a typical aldehyde group, but also a carbon-to-carbon double bond just like the original fat.

One of the aldehydes isolated from autoxidized, or rancid, cottonseed oil even has two of these carbon-to-carbon double bonds. To date three aldehydes have been isolated from rancid cottonseed oil and one from oxidized soybean oil. They are called 2-undecenal, 2,4-decadienal, and hexanal. Hexanal contains no carbon-to-carbon double bonds. Still other aldehydes are known to be present in rancid fats and work continues to isolate and identify them.

We can now identify some of the important substances formed in rancid

fats and oils, and we also know a great deal about how they are formed. It is not enough to know how and why these substances are formed, however. It is much more important to know how to prevent their formation. This brings us to another phase of autoxidation research—the effect of antioxidants in preventing rancidification.

ACCORDING TO the present theory of autoxidation, the first step of the reaction with oxygen involves only a few activated fat molecules, which react to form an equally small number of hydroperoxide molecules with the simultaneous liberation of energy. The liberated energy is transferred to and activates other fat molecules. The process continues to repeat itself. The chain reaction will continue until all the fat is consumed unless something intervenes to break its sequence. A substance that will break the chain of autoxidation is called an antioxidant.

An antioxidant may be considered as being acted on by the oxygen in preference to the fat, or of using up the energy liberated by the first few fat molecules that react with oxygen. Regardless of how the antioxidant functions, it cannot prevent the oxidation indefinitely, probably because it is itself used up in the process of protecting the fat. But during the time that it does protect the fat only small quantities of hydroperoxides are formed. Thus it prolongs the induction period of the fat, that is, the time before which appreciable quantities of hydroperoxides are formed. During that period, little or no off-flavor or odor can be detected. Once the induction period ends, however, hydroperoxides begin to develop rapidly, and the odor and flavor are increasingly noticeable. In other words, the fat is becoming rancid. Therefore, a measure of the induction period will give an idea of the length of time a fat will keep in ordinary storage.

The chemist has devised a practical method of rapidly determining the keeping quality of fats. It consists of bubbling air through the fat while it is

held at a relatively high temperature (208° F.) and periodically analyzing it for its content of hydroperoxides. A high-quality fat shows little formation of hydroperoxides for an extended period; and then suddenly it begins to form them at a very rapid rate. When that happens, the fat is said to break, and the time taken to reach that point (that is, the induction period) is a measure of the stability of the fat and is related to the time a fat will keep in ordinary storage.

The stability can also be determined by measuring the number of hours required for a specified content of hydroperoxides to be formed when the fat is heated under the conditions previously mentioned. That time, too, is related to the time the fat will keep in ordinary storage.

THE NEED for continued advancement of our knowledge of autoxidation and its attendant problem is generally recognized, and many phases of the problem are currently under investigation in industrial, university, and Government laboratories. Until recently the development of fundamental information on autoxidation and antioxidant activity has proceeded slowly and has depended on empirical, rather than scientific, knowledge. Today, however, scientific research in this field

is based on a sound understanding of the fundamental reactions of autoxidation and antioxidant activity and it may be expected that the multitude of problems pertaining to fat deterioration will be solved in the not too distant future.

C. E. SWIFT *received his higher education at the University of Maryland. His association with the problems of autoxidation began with a fellowship at the Technological Laboratory of the Bureau of Fisheries, College Park, Md., and was continued first as a collaborator with the Bureau of Agricultural and Industrial Chemistry as the Fellow of the National Cottonseed Products Association stationed in Washington, D. C., from 1939 to 1943 and at the Southern Regional Research Laboratory from 1943 to 1948, and subsequently as a chemist in the oil, fat and protein division, Southern Laboratory. He is now with the Bureau of Animal Industry in Beltsville, Md.*

F. G. DOLLEAR, *a native of Illinois and a graduate of Illinois College and Northwestern University, has been engaged in chemical research on fats and oils since 1937, when he joined the staff of the United States Regional Soybean Industrial Products Laboratory in Urbana, Ill. Since 1939 he has been stationed at the Southern Laboratory.*

Methods used in United States for processing principal oilseeds in 1949

[Capitals indicate principal process]

Oilseed	Hydraulic press	Continuous press	Solvent extraction	Forepress and solvent extraction	Double pressing
Cottonseed	X	x	x	....	....
Soybeans	x	X	x	....	....
Linseed	....	X	x	x	....
Copra	....	x	....	x	X
Corn germ	....	X	....	x	....
Peanuts	X	x	....	....	....
Castor	....	....	x	X	x
Tung	....	X	....	....	....
Rice bran	....	x	X	....	....
Safflower	....	X	....	x	....