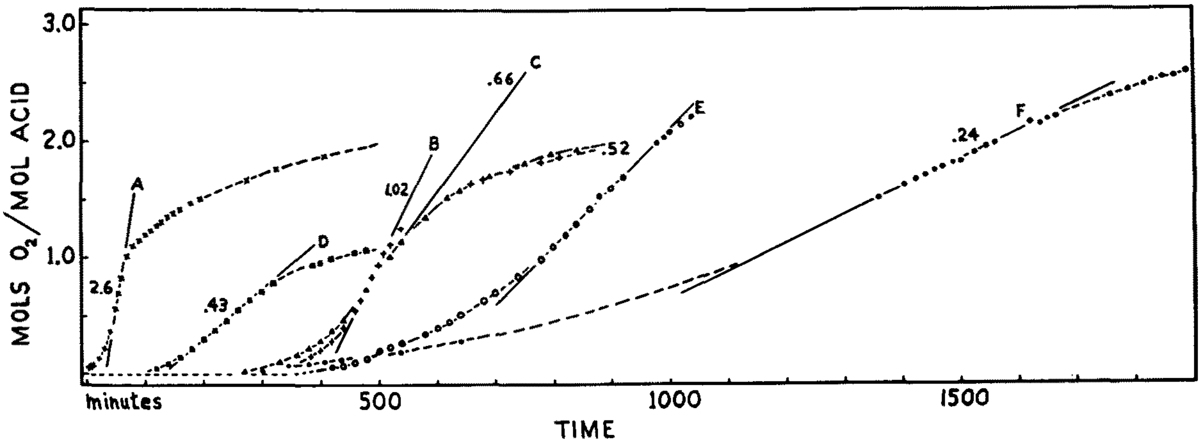

Rates of Oxygen Absorption by Octadecatrienoic Acids and their Esters at 40°

A—alpha Eleostearic acid	*C—pseudo Eleostearic acid*	*E—Linolenic acid*
B—beta Eleostearic acid	*D—Methyl pseudo eleostearate*	*F—Ethyl linolenate*

peated high-temperature distillations is not due to the removal of antioxidants but rather to the accumulation of catalyzing products of decomposition. The present data seem to indicate that pure fatty acids and their derivatives normally have induction periods not due to contaminating inhibitors and that the reaction is therefore autocatalytic. It would follow that in a series of pure compounds under the same physical conditions, the induction periods will fall in inverse order as their maximum velocities of oxygen absorption. This may be impossible to realize experimentally, since identical purities can never be assured. With any given compound, the induction period may obviously be explained on the one hand as the time required for the destruction of the inhibitor in the process of breaking chains in a chain reaction and by normal peroxide molecules, and on the other hand as the interval necessary for the accumulation of a sufficient amount of reaction product for autocatalysis. Solution of the problem whether pure substances normally have inductiton periods awaits the study of the mechanism of oxygen absorption by several samples of the same compound purified in various ways.

Conclusion

1. The maximum velocities of oxygen absorption as measured by the Warburg-Barcroft respirometer at 40° and expressed as mols O_2/mol substance/100 minutes was found for a series of octadecatrienoic acids and their esters to be as follows: *alpha*-eleostearic acid, 2.68; *beta*-eleostearic acid, 1.02; pseudo-eleostearic acid, 0.64; methyl pseudo-eleostearate, 0.42; alpha-linolenic acid, 0.52; ethyl linolenate, 0.24.

2. The data showed the effect of spatial configuration as well as type of unsaturation and functional grouping on the oxidation reaction.

3. The apparently pure fatty compounds were found to have induction periods, pointing to the autocatalytic nature of the oxidation reaction.

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Methods of Measuring the Rate and Extent of Oxidation of Fats

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OUR interest in measuring the rate and extent of oxidation of edible fat is directed toward determining the relative susceptibility of fats to become rancid. Oxidative rancidity is not the only type of fat deterioration which is known but it is most common and the only type that is considered here.

A resumé of our knowledge of the mechanism of fat oxidation, the induction period, autoxidation, and antioxidants as directly related to the methods used to determine the rate and extent of fat oxidation have been presented in other articles in this symposium and this paper will therefore be confined to the methods that have been proposed to measure the oxidation of fats.

The literature on the oxidation of edible fats and oils is extensive and it cannot be completely reviewed here. No attempt will be made to discuss the manner in which ordinary analytical characteristics of fats such as the iodine number, refractive index, acid value, and acetyl value change as oxidation progresses, but it may be stated that these changes are so small that methods for determining the rate of fat oxidation based on them are unsatisfactory. Likewise, many modifications of the methods which will be mentioned for measuring the rate and extent of fat oxidation are omitted in the interest of brevity. For a more comprehensive discussion of the oxidation of fats the reader is referred to Rancidity in Edible Fats by C. H. Lea (1).

In the fall of 1932, the American Oil Chemists' Society appointed a committee to study the methods which have been in use for determining the relative stability of edible fats and oils to determine, if possible, whether one of them could be standardized sufficiently to permit its adaption as a standard method. Before any of the methods described in the literature were examined by the committee, a survey was made of the laboratories making stability tests on edible fats and oils to determine which one of the tests that had been used would lend itself best to committee study. Up to the present the results of that survey have not been published, and they have therefore been made the basis for the present communication.

The practical value of the various chemical tests which have been devised to determine the extent to which a fat has become oxidized and for estimating its relative keeping quality depend on how well they can be correlated with organoleptic rancidity. A test that cannot be correlated with organoleptic rancidity is not of any very great utility or service.

KREIS TEST

The best known and probably the most widely used test for rancidity at present is the Kreis (2) test. As used by Kreis, 2 ml. of oil was shaken with concentrated hydrochloric acid and then with a benzene or ether solution of phloroglucinol. If the acid layer acquired a permanent violet or red color which was not discharged by addition of water, the fat was considered rancid. The technique of carrying out the Kreis test has been modified a number of times but the basic reaction remains unchanged. The Kreis test was investigated by Kerr (3), who recommended the use of a 0.1 percent ether solution of phloroglucinol in the place of the 1.0 percent solution used by Kreis. He also recommended carrying out the test with two dilutions of the fat with mineral oil, viz., one part of fat with nine parts mineral oil, and one part of fat with nineteen parts mineral oil, thus making the test roughly quantitative. Kerr and Sorber (4) reported further work on the Kreis test which was intended to make it more quantitative. According to these authors, "the depth of color is roughly but not exactly proportional to the degree of rancidity." To determine the depth of color it was recommended that the original fat be diluted with mineral oil until the phloroglucinol reaction is just faintly positive. The intensity of reaction or degree of rancidification is reported in terms of the highest dilution at which the reaction is obtained. Holm and Greenbank (5) also attempted to make the Kreis test quantitative. They proposed a modified method which depended on determining the quantity of an ether solution of a fat which would react with phloroglucinol to produce a color matching a standard methyl red solution. More satisfactory than any of these dilution tests is the one reported by a committee of the American Oil Chemists' Society (6) in which the Kreis test color is matched against Lovibond color glasses in a simple tintometer.

Although the Kreis test is of value in determining the state of oxidation at the time the test is made, it does not give any indication relative to the time which will elapse before a fat becomes organoleptically rancid. It cannot, therefore, be used in estimating quality.

Another test that has been developed to indicate rancidity is known as the von Fellenberg (7) test. It is a test for aldehydes and is dependent on the development of color when an acid solution of fuchsin which has been decolorized with sodium sulfite is shaken with a fat. The greater the intensity of the color produced, the greater is the concentration of aldehydes present. This test has no advantage over the Kreis test and therefore has never been very generally used.

Schibsted (8) also proposed a method of testing for aldehydes for use in testing butter. His experimental data indicated that by using a new rosaniline reagent the test was over twenty times more sensitive than the Kreis test or the Schiff test and that it was specific for aldehydo-fatty acid glycerides. The results of this test were expressed in arbitrary specific color units, called "fat aldehyde values." Although the test may be more sensitive than the Kreis test, it provides essentially the same information as the Kreis test and for this reason is not in general use. The uses to which tests of this type can be put are quite limited and, as previously stated, it is much more important to know something about the keeping quality of a fat rather than its state of oxidation especially when nothing is known about its history or how long it has been in storage.

ISSOGLIO-KERR TEST

In 1916 Issoglio (9) published a method for estimating the degree of rancidity of a fat. He showed that the steam distillate of rancid fats reduced acid potassium permanganate. The amount of oxidation as measured by the reduction of the acid potassium permanganate he called the "oxidation number" and defined it as the number of milligrams of oxygen necessary for oxidizing the organic compounds distilled with steam from 100 grams of fat. Issoglio's method was modified by Kerr (10), who determined the oxidizable material in a water extract of fats rather than the oxidizable materal in a steam distillate. Other than the fact that Kerr's modification is a little easier to carry out, it has no advantage over the original Issoglio test.

These tests indicate the state of oxidation of a fat but provide very little if any information about its keeping quality because it is usually not known how long the fat has been held before it reached the state of oxidation indicated by the tests that have been described. Since nothing is known about the age of the fat when tested, positive reactions obtained by these tests cannot be used to predict how much longer a fat will keep before it becomes organoleptically rancid.

As research on edible fats and oils progressed, the demand for an accelerated test which would indicate the relative keeping quality of these fats and oils became more urgent. Consequently, in the last fifteen or twenty years a number of tests for determining the relative keeping quality of edible fats and oils have appeared.

AERATION METHODS

In 1923 Bailey and Ebert (11) proposed a method in which the fat to be examined was spread evenly on filter paper and heated in a glass tube at 92°C. A slow current of air was passed through the tube and into a carefully prepared solution of Schiff's reagent. The time required for the appearance of a pink tint was defined as the accelerated rancidity value. The test is difficult to standardize and the results are often erratic. To make the results comparative, a standard fat must be included with each series of fats examined. Although not in general use, five years ago it was used, apparently with success, in the laboratory of a company using a large amount of both animal and vegetable shortening.

In 1931 Grettie and Newton (12) reported a method for determining the relative keeping quality of fats and oils based on the work of Issoglio and of Bailey and Ebert. Air was passed over the fat spread on filter paper maintained at 100°C. After passing over the fat on the filter paper, the air carrying the volatile products of oxidation was bubbled through a measured volume of standardized potassium permanganate solution for a definite length of time. The potassium permanganate solution was then titrated against oxalic acid to determine the amount of volatile organic compounds liberated from the oxidizing fats. This method looked very hopeful for a while but it never developed into a satisfactory procedure for determining the relative keeping quality of edible fats.

PHOTOCHEMICAL METHODS

The year previous to that in which the method just described was published, Greenbank and Holm (13) published a paper on the rate of reduction of methylene blue dissolved in a fat or oil when catalyzed by light. They suggested that a measure of the rate of reaction of the initial oxidative processes may be utilized to determine the relative susceptibility of fats and oils to additional oxidation. This method was further developed by Royce (14), whereupon a manufacturer of scientific instruments announced that a satisfactory method for determining the relative keeping quality of fats and oils had been developed. However, when an attempt was made to produce satisfactory apparatus, many difficulties were encountered and the apparatus and method could not be standardized. The manufacturer then admitted that the apparatus was not satisfactory.

OXYGEN ABSORPTION

Probably the first attempt to measure the rate at which a fat absorbs oxygen consisted of determining the change in weight of thin layers of the fat or oil when exposed to air under controlled conditions of temperature and time. This method, of course, could not take into account the volatile products lost during oxidation so the increase in weight was really a measurement of the difference between the oxygen added and the volatile products lost. It did not prove to be sensitive enough to show an increase in weight during the induction period and at the beginning of the autoxidative period—the places where the rate of oxidation is of most importance in determining the keeping quality of edible fats. Although the method has been carefully tested, it is of little value in studying edible fats.

Oxidation methods based on measuring volumetrically the amount of oxygen absorbed by a known amount of fat aged under controlled conditions of temperature and pressure have proved very useful in determining the relative keeping quality of edible fats. Holm and Greenbank (15) have described both the apparatus and the methods which may be used for measuring the rate at which fats take up oxygen. They employed both static and stirring apparatus. Many variations have been introduced by investigators who have used oxygen absorption methods in studying the keeping quality of edible fats. Recording apparatus designed to give a continuous record of the induction period and the beginning of autoxidation are in use. When recording devices are used, experiments may be made continuously day and night and the amount of oxygen absorbed by a measured amount of fat can be calculated for any length of time during the experiment.

A study of the rate of oxygen absorption by oils and unsaturated fatty acids using the Barcroft-Warburg technique was first reported in 1931 by Jány (16). No one seems to have applied this technique to the study of the oxidation of fats and oils at other than approximate body temperature until this year. Recently Johnston and Frey reported preliminary experiments at the Detroit meeting of the American Chemical Society on the rate of oxidation of fats and oils in which they used the Barcroft-Warburg technique to determine the rate of fat oxidation at temperatures up to 110°C. They stated "that autoxidation induction periods can be measured with considerable precision at any temperature up to 110°C., and possibly higher. Accurate control of temperature, pressure, oil surface, agitation, and other factors results in precision of the order 1 to 3 percent in estimating induction periods at 100°C. for sesame, cottonseed, and corn oils." Although this technique should prove very useful as a research tool, it probably will not become a general method or a routine test for estimating the relative keeping quality of fats and oils.

Apparatus designed to determine the relative keeping quality of fats based on the volumetric measurement of the rate of oxygen absorption is used by a number of laboratories with satisfactory results. It appeared at one time that this procedure might possibly prove to be the most useful method for measuring the relative keeping quality of edible fats, however at the present time the peroxide or active oxygen test, which will be discussed later, is more generally used.

OXYGEN ABSORPTION—PEROXIDE METHOD

It has been known for many years that an organoleptically rancid fat, when shaken with an acid, liberates a peroxide. The use of this reaction as a test for rancidity was not successful until after Lea (17) in 1931 showed that when a rancid fat is dissolved in a mixture of chloroform and glacial acetic acid it quantitatively liberates hydrogen peroxide almost instantaneously and that if the reaction takes place in the presence of potassium iodide, iodine is liberated and can be titrated with standardized sodium thiosulfate. Later in 1931 Taffel and Revis (18) described a method based on this reaction which served as a means of determination of rancidity in fats and oils. Based on the work of Lea and of Taffel and Revis, Wheeler (19) described a method for determining the susceptibility of salad oils to oxidation by bubbling air at a uniform rate through the oil at 100°C. and determining the rate at which peroxide formed in the fat. King, Roschen and Irwin (20) simplified Wheeler's technique and designed equipment for using the accelerated active oxygen test for determining the relative keeping quality of edible fats and adapted it to use as a routine test.

In using this test, 20 ml. of fat are measured into each of three one-inch diameter test tubes. One of the tubes is placed in a constant temperature bath at 98°C. and air bubbled through it at the rate of 2.33 ml. per second. Exactly one hour after the first tube was started aging, the second tube is started and followed another hour later by the third tube. At regular one-hour intervals the tubes are inspected for rancid odor. When the first tube becomes definitely organoleptically rancid, all three tubes are removed from the bath and the peroxide determination is made on them. The peroxide value is taken as the final measure of rancidification. Many tests on lard show that it is organoleptically rancid under the conditions of this test when the peroxide content is twenty or more milli-equivalents per 1000 grams of fat. The keeping quality of lard by this test is therefore the total number of hours of incubation required to produce a peroxide value in the fat of twenty milli-equivalents or more per 1000 grams of fat.

When using this test for determining the relative keeping quality of fats and oils, it is important to remember that different types of fats and oils do not become rancid at the same peroxide concentration when tested under identical conditions. Under the conditions of this test, the best data (21) available indicate that lard is organoleptically rancid at 20, hydrogenated cottonseed oil at 75, compound at 100, and cottonseed salad oil at 125 mille-equivalents per kilogram.

The active oxygen test is now used in more laboratories than all the other methods combined for estimating the relative keeping quality of edible fats and oils. In the hands of a careful laboratory worker results can be checked quite satisfactory. Likewise, analyses made in different laboratories check quite well and the test has proved very useful in estimating the relative keeping quality of edible fats.

There is very good correlation between data on the relative keeping qaulity of fats as determined by the active oxygen test and data by the oxygen absorption methods.

Another test which should be mentioned for determining the relative keeping quality of fats is known as the Schall test and it is quite generally used in bakery laboratories. It consists of aging fifty grams of fat at 145°F., in a suitable container with a loose fitting lid, until it becomes organoleptically rancid. Organoleptic examination is made daily and the number of days required for the fat to become rancid is taken as the index of keeping quality.

EFFECT OF INHIBITORS

Some comment is required on the subject of determining the relative keeping quality of fats to which an antioxidant has been added. Depending on the nature of the antioxidant, accelerated keeping quality tests made at 90 to 100°C. may give misleading results because an antioxident like lecithin (22) is not effective at high temperatures but pronounced antioxidant properties are observed at temperatures below 65°C. No general procedure has been developed which is applicable in such cases, and the only way to determine the effectiveness of an inhibitor is to test the fat thus stabilized under the conditions which it is going to be used.

Finally, although research workers in the field of fat chemistry are aware of the need of better methods for determining the relative keeping quality of edible fats and oils, there have been no new tests reported which appear at the present time to be superior to either the oxygen absorption or active oxygen tests.

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