Problems in the Evaluation of Fat Stability*

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THE importance of knowledge concerning the stabilities of food fats is well established. Rancidity in fats not only renders food unpalatable but also can be responsible for the partial destruction of the essential fatty acids (1) and of other dietary nutrients such as vitamins A (2), E (3), and perhaps D (4) and certain members of the B complex (1).

It is well at the outset of this discussion to define the terms rancidity and stability. Rancidity has been employed in the past in both a general sense, to indicate an off-flavor, and in a specific sense, such as the hydrolytic spoilage of butter (5). Fats may develop off-flavors and odors through the absorption of odors, the action of enzymes, the action of micro-organisms, and through atmospheric oxidation. For the purposes of the present discussion we shall limit the term rancidity to apply to the development of objectionable odors and flavors through oxidative changes. Experimentally this condition is usually found shortly following the end of the induction period. If the oxygen uptake of a fat is plotted against time, there will be found an initial period during which the rate of increase in oxygen uptake is low, after which the rate will increase in a rather sudden fashion. This condition is shown as a sudden rise in the curve. Shortly after this point is reached, the sample becomes organoleptically rancid. The effect of an antioxidant is to lengthen the induction period hence it delays the onset of rapid oxygen uptake. Similarly, we shall consider that stability refers to a measure of the extent to which the fatty substance resists the development of oxidative rancidity, and it thus is a measure of the length of the induction period. It is usually expressed as the time that elapses under specified conditions before the fat becomes rancid. This definition implies that there is a reliable test for the condition of rancidity. Such is not the case. Without stopping here to summarize the available literature, we shall simply call attention to the fact that in the final analysis rancidity must be detected through organoleptic observation. This type of test is subject to all the weaknesses inherent in a test involving personal judgment. Variabilities in the taste and odor sensitivities of persons in different laboratories, their previous taste experiences, the prevailing condition of health and taste apparatus, and the condition of the respiratory tract—all these factors lead to a lack of uniformity of judgment. No one chemical test has been devised which can accurately measure and correlate all the factors which act simultaneously to produce the odors and flavors we call rancidity. Of the various tests which have been submitted for the detection of oxidative rancidity, the determination of "active oxygen" or peroxide content seems to give rather good correlation of data. During the oxidation of a fat certain oxygen compounds are formed which are "active" in the sense that they are capable of liberating iodine from potassium iodide (6). The iodine may be quantitatively determined, and it thus becomes a measure of rancidity.

IT would seem then that to determine the stability of a fat one would merely determine the peroxide value at arbitrary intervals, and when the value would rise to a given point, the sample would be considered rancid. It is the purpose of this paper to point out some of the difficulties involved in such a procedure from the standpoint of obtaining useful data.

It should be borne in mind that the rancid odors are not considered as being due to the peroxides themselves but to the breakdown products resulting in part from their action. The rate of breakdown depends in part on the temperature so that rancidity is detectible at various peroxide values depending on the temperature of the tests. The peroxide content may rise to very high values at refrigerator temperatures before the breakdown becomes sufficiently rapid to be noticed organoleptically. We have noticed that in shelf storage tests at room temperature peroxide values rise to several times the values observed in accelerated tests before rancidity develops. We have noticed also in the testing of certain antioxidants that extreme rancidity develops even though the peroxide value is very low—less than 10 m.e./kg. The current active research involving the addition of antioxidants may develop this situation still further.

In considering the problem of determining stability, we are confronted with the situation that fats become rancid so slowly under ordinary storage conditions that months or even years would be required to learn the stability of a given sample for that set of conditions. An accelerated method of producing the development of rancidity is needed which will enable one to determine relative stabilities.

Several methods for this purpose have been proposed and are in use. The following quotation from Lea is of interest (5):

> Basically all of the methods which have been employed in the determination of susceptibility (to oxidation) are similar in principle. Oxidation is accelerated under carefully controlled conditions, while still preserving as far as possible the original relations between the various samples, until the time necessary for the development of spoilage has been reduced from months or weeks to days or hours. Increase in the rate of oxidation is usually brought about by heating at some constant temperature between 40° and 100°C. in an atmosphere of air or oxygen. In a few cases exposure to light or to catalysis by traces of metallic salts has been employed, often supplemented by heat. The progress of the accelerated oxidation is then followed by smell and taste, by direct measurement of the weight or value of oxygen absorbed, by changes in some physical characteristic of the fat, or by chemical estimation of the products of the reaction. It is extremely important, with all these accelerated methods, to calibrate the method with the particular type of fat being studied.

PERHAPS the simplest test is the Schaal oven test (7) or some modification of it, in which the fat is merely placed in a warm oven (60°C.) and examined for indications of rancidity at regular intervals. This method has the disadvantages that

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differences are found due to the large personal errors involved, the difficulty of obtaining reproducible data, and the fact that a long time is necessary to obtain results. The personal errors may be offset to some extent by using relatively large numbers of samples and plotting the peroxide change with time. To learn the stability of a given sample by this method may require a time of from a few days to several months. Another widely used method is the so-called "Swift," or active oxygen method (8), in which carefully cleaned air is bubbled through the fat, which is maintained at the temperature of boiling water. A recent modification of this test employs a higher temperature (110°C.) in order to speed the development of rancidity still further. The samples are tested organoleptically at frequent regular intervals, and when the air which has passed through the samples assumes a rancid odor, chemical determination of peroxide content is made on them. This method has the disadvantage that the conditions are drastically different from those of ordinary storage in that the air is continuously bubbled through the heated fat so that equilibrium is maintained between the fat system and the environment. This is not the case in a packaged fat. There is also the difficulty that rancidity is not detected at the same peroxide level in all fats. In lard, for example, rancidity is considered to occur at a peroxide content of 20 milliequivalents per kilogram of fat. The peroxide value must reach 100 in hydrogenated or blended shortenings before organoleptic indications of rancidity are usually observed under these accelerated conditions (9). It is therefore essential that the method be calibrated for the type of fat being studied.

Various methods have been devised for the determination of stability based on the oxygen absorption by the fat. That is, the fat is allowed to deteriorate in a closed system containing air or oxygen, and the amount of oxygen absorbed is plotted against time. In this way, it is possible to determine the length of the induction period. These methods have the disadvantage of being rather cumbersome and difficult to operate.

The Barcroft-Warburg constant volume manometric apparatus has been applied to stability studies (10, 11, 12), and has been studied in our laboratories to a considerable extent (12). Disadvantages of this method are the long time required for a sample to become rancid (the time at 70°C. is about five times as long as that required for the A O M), and the fact that it is not possible to make organoleptic observations without disrupting the experiment. A further disadvantage of this type of test is that the volatile products of decomposition may interfere with manometric readings. The results of our work indicate that the relative data obtained with the Barcroft-Warburg apparatus at 70°C. correlate fairly well with storage tests at room temperature on lard to which no antioxidant is added, and on lard to which lecithin alone is added.

With lard to which d-isoascorbyl palmitate or d-isoascorbyl monostearate had been added the results on the Barcroft-Warburg apparatus were in better agreement with storage tests than were the data obtained by the active oxygen method. The d-isoascorbyl esters in concentrations of 0.01%-0.10% in lard behaved as antioxidants with the active oxygen method and as either pro-oxidants or antioxidants, depending on concentration, with the Barcroft-Warburg apparatus. Storage data agreed with the Barcroft-Warburg results. We learned in further work on this problem, that the presence or absence of moisture in the air used for the active oxygen method of testing is sometimes of considerable importance (13). An untreated lard yields the same stability data whether the air used is wet or dry. If the lard contains certain materials added as antioxidants, much higher stabilities are found with moist air than with dried air. These materials include d-isoascorbyl palmitate, ascorbic acid, d-isoascorbyl monostearate, triethanolamine, and certain other substances. On samples of this type the dry air data have been more reliable than the wet air data as an indication of storage behavior. These results offered an explanation as to why the Barcroft-Warburg apparatus yielded more reliable data than the active oxygen method as normally employed without drying the air. In the study of certain antioxidant materials the B-W apparatus was supplied with dried tank oxygen. When the antioxidants used were nordihydroguaiaretic acid, resin guaiac, or tocopherols, the moisture made little or no difference.

The Kreis test (14) should be mentioned here as a method of the determination of stability. This test has been very widely used as a means of detecting rancidity. The difficulty with it has been that many fats which are not rancid will give a positive Kreis test. If the test is used for the determination of stability, however, by plotting the intensity of the Kreis reaction against time, curves may be obtained which are quite similar to those for oxygen uptake and development of peroxides (7), and the length of the induction period may thus be ascertained.

There are other methods of detecting products of deterioration in fats, which may be used as stability tests if the increasing intensities of the reactions are plotted against time. All of these methods, including the Kreis test, have the disadvantage that the plotting of a curve for each sample is somewhat laborious.

THE final test for any stability data is of course shelf storage. There are many problems in the determination of shelf storage stability. Some of the factors which will affect the stability data are:

1. The temperature of storage. It is more or less obvious that the higher the temperature, the shorter the time that will be required before the fat becomes rancid.
2. The presence or absence of light. It has been shown quite definitely that fats will develop off-flavors sooner in the presence of light than they will in the dark (15).
3. Wave length of the light. It has been observed that certain wave lengths of light will cause rancidity to develop in a shorter time than will other wave lengths (16, 17).
4. The type of package is quite important, not only from the standpoint of whether or not the package will admit light, but if it does admit light, what wave length of light will pass through. In addition to the packaging consideration as to the light we have problems regarding the material of the package. Certain metals will greatly influence the development of rancidity. Another very important consideration is the permeability of the package to the fat. Work has been done in our laboratories which indicates quite clearly that if a package allows fat to ooze out onto the exterior surface of that package, the rancid odor will develop very quickly. Now of course this does not mean that the entire mass of fat contained will become rancid that quickly, but the judgment of the person examining the fat will be influenced by this rancid smell on the outer surface.

5. Another item to be considered in studying shelf storage is the state of fullness of a sealed package. There are reliable data which show that a package half-filled with fat develops rancidity in a shorter time than does fat in a package which is full (18).
6. The presence or absence of oxygen in the package is of importance. An inert atmosphere will cause higher stability figures to be obtained than if the atmosphere overlying the fat is oxygen or contains oxygen.

The amount of air whipped into the fat is also of importance. It is common in commercial practice to include air or an inert gas in the fat as a means of obtaining a lighter product. This air is intimately mixed with the fat molecules, and it is quite reasonable to suppose that it will influence the stability data obtained.

It is assumed in all the discussion in this paper that the technician is practicing meticulous cleanliness of all materials which come in contact with the fat during testing. In the absence of extreme cleanliness it is useless to apply any test at all.

A DISCUSSION of this type should include mention of a problem which is particularly important in the current studies on the evaluation of antioxidants in the stabilization of edible fats. Even though, by some of the methods described here, it is possible to determine the stability of a fat in question, the stability data so obtained cannot be applied to the food products made from the fat. It has been the experience of a number of people working on antioxidants that, while a fat may be stabilized to a very high degree as judged by accelerated methods of testing, the food products prepared from this fat may have a stability only slightly greater than that of food prepared from the unstabilized fat. Here again we are faced with the problem of determining the stability in such a manner that the data will be useful regarding the final product which we intend to use.

Our method of determining the stability of pastry is to place a number of broken pieces of the product in 4-oz. mayonnaise jars with screw tops and store them at room temperature or in the Schaal oven in the absence of light. At regular intervals, samples are removed, tested for peroxide content, and examined for organoleptic indications of rancidity. It is usually found that the stability data as obtained on the fat before its use in cooking is of very little value in predicting the stability of the pastry or other food material prepared from the fat in question.

In summary, it should be stated that just as there is no completely reliable chemical test for rancidity, there is no completely reliable laboratory test for stability. Rancidity is ultimately determined by taste and smell, and stability is ultimately determined by placing the fat in storage and allowing it to become rancid. Even then, the food products prepared from the fat may not possess a stability even remotely like that of the fat from which they were prepared. In spite of these inadequacies in our laboratory methods, however, much valuable time is saved through accelerated tests, and much information is obtained which serves as a guide for planning a relatively small number of well-chosen experiments on an elaborate scale for the final investigations. This was especially true during the war-time emergency when we needed to use these rapid tests for evaluating materials for their antioxidant effectiveness.

By means of the accelerated tests described and with full knowledge of their limitations the chemist is making rapid progress in food stabilization.

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Methods of Sampling Bulk Oil With Particular Reference to Bleeder Samples

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THE subject of bleeder samples arises more often in connection with bulk oil shipments than with tank cars or land storage tanks and requires special thought when intended for ship's tank deliveries. When considering the results of this method in comparison with core samples taken by other recognized means, such as the sectional zone sampler or thief in illustration, numerous factors must be taken into account and understood before correct conclusions can be reached.

First and foremost, however, it should be emphasized that nothing can take the place of sound judgment and technical understanding in the use of these devices and methods based on wide and diversified experience in this field (5). Knowing when and how to apply particular methods or equipment and when their limitations make their use inadequate is a fundamental necessity in the science of sampling if dependable results are to be expected. Applying any recognized method of sampling in itself does not assure accuracy in all cases; it is the character of the equipment, its appropriate application to the particular circumstances involved and the ability to secure mathematical proportions representing all