Report of the Fat Stability Subcommittee of the Fat Analysis Committee—1956: Active Oxygen Method for Determining Fat Stability

IN 1953 A SUBCOMMITTEE was formed to reinvestigate the well-known A.O.M. test with the view of attempting once again to develop a method which would be acceptable as an official method of the Society. Following is the final report of the work accomplished by this subcommittee. A considerable amount of time and work has been spent in an effort to provide an acceptable method for the evaluation of keeping qualities of fats. The procedure included in the attached report represents, in the opinion of the subcommittee, the best practice known to this day. The A.O.M. method is widely used in industry both in the United States and abroad. Admittedly this method does not come up to the standards of precision and accuracy for which the A.O.C.S. strives. However, because of the wide use of this method and because it is the best procedure known at this time, the committee feels that the fat and oil industry would be best served by adoption of the following procedure as a method of the Society.

Historical. In 1933 King, Roschen, and Irwin (1) simplified and standardized an earlier method of D. H. Wheeler (2) to give a practical test for the relative stability of different fats against oxidation. This method, based upon determining the peroxide oxygen accumulated in a fat during aeration at a specified temperature, was first called the Swift's Keeping Quality test or SKQ, from the laboratory in which it originated. Over the years since, it has become known by the more generic name of Active Oxygen Method, generally abbreviated to A.O.M.

Between 1933 and the present day many investigators (3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 17, 24, 25, 26, 27, 28, 29, and others) have published data which demonstrated the shortcomings as well as the advantages of the method. Although the method remains today very much the same in principle and procedure as it was first proposed, some modifications have resulted from subsequent publications. The more outstanding changes have been in the method of cleaning equipment, the increased awareness of the importance of temperature control, and the method of determining end-points. Fore *et al.* (27) demonstrated that inaccuracies can result from the use of chromic acid as a cleaning agent for the sample tubes and proposed the use of synthetic detergents alone as cleaning agents. Freyer (10) and Mehlenbacher (15) clearly showed the importance of correct temperatures while Riemenschneider (17) and Moore and Bickford (28) proposed changes in the method of determining the end-point of the determination.

Further discussion of the information contained in other papers is outside the scope of this report. It is sufficient to say that all were considered by this committee in arriving at the procedure which will be proposed.

Early Committee Work. Shortly after the publication of the paper by King, Roschen, and Irwin a committee of this Society was appointed (3) to study the method with the thought of developing a procedure suitable for adoption as an official method.

However neither the 1934 committee, nor subsequent committees through 1937, were able to obtain satisfactory agreement among all collaborators. As a result the Uniform Methods Committee refused (30) to recommend adoption of a suggested method, even on a tentative basis, despite the fact that two committee reports (5, 8) recommended its adoption with the comment that "in spite of its limitations

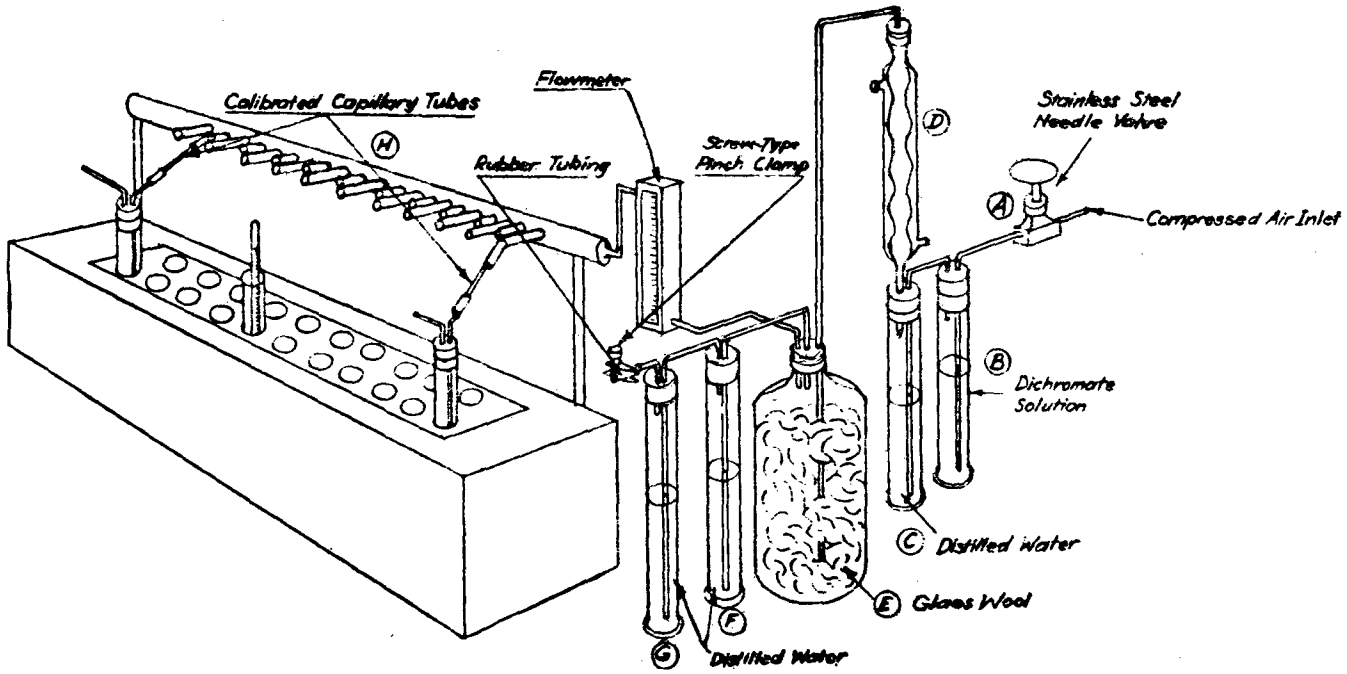

FIG. 1. A.O.M. stability heater with air purification and distribution assembly.

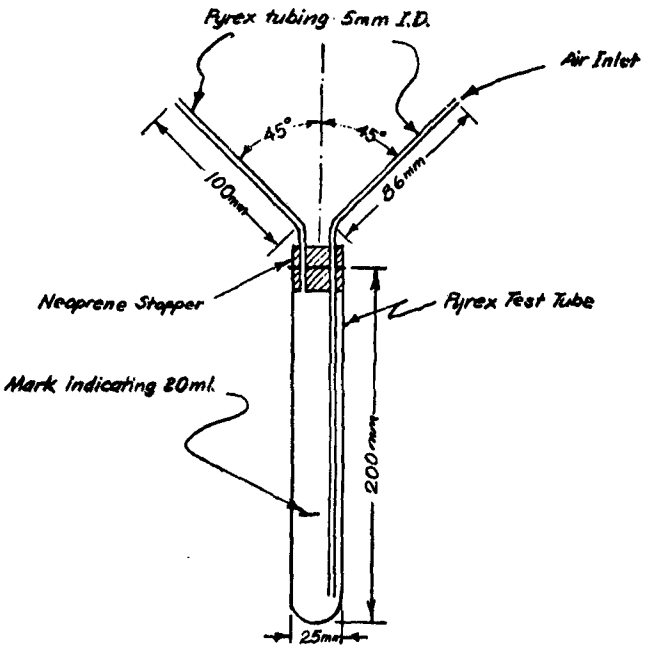

FIG. 2. A.O.M. sample tube.

it is the best method known." All of this early work was reviewed and summarized by Freyer (11).

No further work on the A.O.M. test was undertaken by the Society until 1944 when the question was reopened (31). A report of that committee shows that samples were distributed to 23 laboratories. The results were better than those of the earlier committees, but several laboratories varied as much as 20% from the accepted average. The only recommendation made was that more study was necessary.

In 1945 samples were distributed, this time to 10 laboratories. The average agreement was better than was found in the previous year, but some collaborators still varied 20% or more from the accepted average. The 1945 committee made no recommendations regarding adoption of an official method, but at the request of the Quartermaster's Corps a method was published (32) which represented the best procedure known to the committee.

After 1945 no further committee work appears to have been done on the method until formation of the present subcommittee early in 1953 although the subject was mentioned in reports of the Fat Analysis Committee.

Despite the lack of any official status and its known shortcomings the A.O.M. procedure has continued to be used, and its use has spread until it is undoubtedly the most widely known method for determining the resistance of a fat to oxidation, at least in the United States. The method is used both in research work and in the control laboratories of practically all processors and large consumers. Figures based on the results of the test are often found as part of purchasing specifications.

This has resulted in a rather odd state of affairs in that the Society has no official procedure for performing one of the most widely used analytical determinations.

Present Committee's Work. As a result of the situation outlined above, the Uniform Methods Committee requested that the Fat Analysis Committee reopen the subject, and the present subcommittee was established (33).

It was soon apparent that this subcommittee faced a more difficult task than is usually encountered in considering a method for official status. Unlike a new or little used procedure, some modification of the original A.O.M. method has been used for more than 20 years in laboratories operated by dozens of companies. There has been wide variation in the tendency to accept or utilize the improvements suggested by the later publications. As a result each laboratory has accumulated a large volume of data based on some particular modification of the original method. Since the basic procedure is quite empirical, any standardization of the method at this late date must necessarily invalidate these accumulated data to some extent. Nevertheless the subcommittee would have been lax in its duty if it had ignored changes which have been suggested by the many researchers who have published data on the subject.

In view of the controversial nature of the subject great care was used in selecting subcommittee members so that all branches of the fatty oil industry would be represented. The first step taken after the subcommittee was formed was to mail a questionnaire to all laboratories known to be concerned with fat analysis. Of the 80 questionnaires mailed, 46 or 57% were returned. Meanwhile each committee member reviewed the literature and familiarized himself with the data reported in the various publications. Later, at a meeting in New Orleans in May 1953, a tentative procedure was drawn up. This procedure was based on the information contained in the literature and on the replies to the questionnaire mentioned above. Collaborative samples were sent out to all committee members. On the basis of the results obtained on these samples, slight modifications were made and additional samples were distributed. In all, four sets of samples were analyzed, the results of which are shown in Tables I through IV. The end-point (expressed as milliequivalents of peroxide per kilogram of fat) for cottonseed oil and hydrogenated vegetable shortening was 125. For lard the end-point was 20 in Tables I and II, both 20 and 125 in Table III, and 125 in Table IV. The tabulated results show

TABLE I

Series I. Collaborative Samples

Laboratory	Prime steam lard	Liquid cottonseed oil	Hydrogenated vegetable shortening
1	12, 12	11, 11	78, 79
2	11, 11	11, 11	79, 78
3	11	11	78
4	12	11	75
5	10	11	78
6	12, 10	12, 10	76, 79
Average	11.2	11.0	77.8

TABLE II

Series II. Collaborative Samples

Laboratory	Prime steam lard	Liquid cottonseed oil	Hydrogenated vegetable shortening
1	7, 6	12, 13	77, 76
2	6, 6	11, 12	75, 75
3	6, 6	10, 10	77, 78
4	7	11	74
5	6, 6	11, 12	74, 74
6	7, 7	12, 12	83
Average	6.4	11.5	76.3

TABLE III

Series III. Collaborative Samples

Laboratory	Prime steam lard		Liquid cottonseed oil		Hydrogenated vegetable shortening	
	20 m.e.	125 m.e.				
1	8, 8	9, 9	12,	12	37,	41
2	6, 6	7, 7	10,	10	36,	36
3	8, 8	10, 10	12,	12	40,	40
4	9	11	12		38	
5	6, 6	8, 8	11,	11	36,	36
6	7, 7	8	12,	11	33	
7	8, 8	9, 9	12,	13	41,	41
8	5, 5	6, 6	10,	10	35,	36
Average	7.0	8.4	11.3		37.6	

TABLE IV

Series IV. Collaborative Samples

Laboratory	Prime steam lard		Liquid cottonseed oil		Hydrogenated vegetable shortening	
1	4.6,	4.9	10.0,	10.0	30,	30
2	5.2,	5.2	9.3,	9.5	28,	28
3	5.3,	5.8	11.6,	12.1	30,	32
4	5.0,	4.9	10.5,	10.3	25,	27
5	4.5,	4.4	9.3,	9.5	29,	26
6	5.1,	5.3	9.3,	9.3	30,	31
7	4.9,	5.0	9.5,	9.3	27,	28
8	5.3,	5.3	9.5,	9.5	28,	27
Average	5.0		9.9		28.5	

TABLE V

1945 Report of Committee on Analysis of Commercial Fats and Oils

Fat Stability Test Subcommittee

Laboratory	Sample			
	2	3	4	5
8	30	45	34	35
2	22-20-26-21	46	31	28
7	29	45	33	30
9	28	45	35	30
12	35	48	40	34
6	31	52	35	33
4	28	47.5	31	31
5	26	....	31	33
11	25	42	29.5	25.5
10	24	47.5	32	21
Average	27	46	33	30
Av. deviation	3.5	1.9	2.3	3.2

the time in hours required for the sample to reach the end-point.

The data for Series I and Series II represent results on different portions of the same samples. The rather large differences between the average results for lard is probably caused by deterioration during storage between experiments.

Series III and IV represent results on different portions of a second set of samples. Series IV differed from the other three in that the samples were aerated with pure oxygen instead of air.

The better precision obtained among the various laboratories on the samples in Series I was apparently coincidental inasmuch as it cannot logically be attributed to better procedure or technique.

From these results it can be seen that the agreement among collaborators, while far from perfect, is better on long stability samples than was obtained by previous committees. The improvement can best be seen by comparing the data reported by the 1945 committee (32) as shown in Table V with the data in Tables 1 through IV. Taking as an example Sample No. 5 in Table V, the variations between extremes is 14 hrs., whereas on samples of comparable stability in Tables I through IV the maximum variation between extremes is only 8 hrs.

On samples of relatively low stability the variation among collaborators is still rather great on a percentage basis although not great in actual hours. For example, in the poorest case the high was 9 hrs., the low 5 hrs., or an average of 7 hrs. This is thought to be caused by slight fluctuations in temperature or other variables which tend to average themselves out on long stability samples but which have an exaggerated effect on short stability samples.

After careful study of the above results at the Minneapolis meeting of the Society in November 1954, it was decided that probably nothing could be gained by further collaborative work. Accordingly it was agreed that the composite method mentioned previously should be recommended to the Society for adoption as an official method.

Discussion of the Proposed Method. Before giving details of the proposed method, there are three points which should be mentioned. They are the inclusion of a new type of heating apparatus on a purely optional basis, the somewhat detailed method of tube cleaning, and the method of determining the end-point for the test.

The new heating apparatus is described briefly in the proposed method, and it is described in detail in a separate publication by the originators. It is emphasized here that its use is entirely optional and that any type of heater which will meet the performance requirements of the method will be satisfactory. However it is the opinion of this subcommittee that temperature fluctuations in the test sample are responsible for many of the undesirable variations in results. It is the unanimous opinion of all who have tried it that the aluminum block type of heater is superior to any other type currently in use in uniformity of temperature and in simplicity of operation and maintenance.

The washing procedure involves the use of solvent to remove excess fat and films of oxidized oil, followed by washing in hot detergent and rinsing with distilled water. Some question has been raised regarding the necessity for using solvent. If only animal or hydrogenated vegetable fats were involved and all tubes were cleaned immediately after each use, it is possible that detergents alone, with a double washing technique, would suffice. However it is important that any official technique should cover all materials to which the test is applicable. If very greasy tubes are placed in a detergent bath, an oily layer will collect on top which may contaminate the tubes as they are removed from the bath. After determinations on high iodine value oils the tubes often are covered with a film of oxidized or polymerized oil, which makes it almost impossible to clean the tubes using detergent alone. The subcommittee evaluated five detergents. Two of these gave rise to erratic results while the three listed in the method did not affect the results even when added to a tube in which a test was being run.

For an end-point the subcommittee proposes to use a single peroxide value of 125 milliequivalents for all fats. The reasons for this are:

1. Several workers (20, 21, 22, 23, 28) have shown that the traditional end-point value of 20 m.e. for lard is probably too low when added antioxidants are present, as is the case with most present-day animal fat products.
2. The already wide variety of blends of animal and vegetable fats is steadily increasing. To attempt to classify all such products to conform with a double end-point system would be impossible.
3. Even among vegetable fats there is a wide variation in the peroxide value at which organoleptic rancidity occurs (34, 35). For example, coconut oil is generally rancid

at 20 m.e., babassu at 40 m.e., hydrogenated cottonseed oil (shortening) at 80 m.e., liquid cottonseed oil at 125 m.e., etc.

Regardless of whether the sample is animal, vegetable, or a blend, after the onset of organoleptic rancidity—usually corresponding to the end of the induction period—the peroxide value increases so rapidly that the curve of peroxide value *vs.* time becomes almost vertical (12, 14, 16, 18, 19, 28). Furthermore, between 100 m.e. and 200 m.e., the rate of increase for different fats is remarkably constant. Consequently it seems that greater convenience, better over-all accuracy, and less confusion would result from using a single end-point at 125 m.e. than from a multiple end-point system.

Obviously, for research purposes, in the study of uncommon fats or in testing new antioxidants, a more accurate method for determining the length of the induction period should be used (17). However it is generally not feasible to include in any official method all the details required by the numerous applications to which the method may be put. The best that can be done is to tailor the method to give acceptable results on as wide a variety of materials as possible.

Proposed Method

FAT STABILITY

Active Oxygen Method

Definition. This method measures the time (in hours) required for a sample of fat or oil to attain a predetermined peroxide value under the specific conditions of the test. The length of this period of time is assumed to be an index of resistance to rancidity. The exact relationships between peroxide value and such qualities as shelf-life, actual rancidity, and oxidative stability have not been established.

Scope. Applicable to all normal fats and oils of animal or vegetable origin intended for human consumption (Note 1). Not applicable to fatty acids.

General Precautions. This procedure is highly empirical, and close attention to details is required if reproducible results are expected. The two most likely sources of error are inadequate cleanliness and inefficient temperature control. All equipment must be scrupulously clean. Do not use chromic acid or other acid-cleaning agents because their final traces are difficult to remove. Even distilled water is a potential source of error if it contains traces of heavy metals, particularly copper. The bath or heater must be calibrated by checking the temperature of an actual sample in each sample tube opening under the specified test conditions, including aeration. After the heater has been shown to be satisfactory in this respect, the actual temperature during operation must be measured by a thermometer in a sample tube containing the recommended quantity of oil. The oil in this tube should be changed often enough to prevent gelation.

A. APPARATUS (Figures 1 and 2)

1. Constant temperature bath or heater (Note 2), which will maintain all samples at a temperature of 97.8°C. ± 0.2°C.
2. Air-distributing manifold constructed of stainless steel, nickel, aluminum, or glass. The capillaries must be calibrated to permit the same flow (± 10%) through each outlet when the total flow is adjusted to 2.33 ml. per tube per second.
3. Air purification train:
   a. Air-inlet tube from compressed air source equipped with stainless steel needle valve.
   b. Air-washing column: hydrometer cylinder 50 mm. o.d., 375 mm. high, containing 2% $K_2Cr_2O_7$ in 1% H_2SO_4. Fill to *ca.* 25 cm. depth and replace after 72 hrs. of continuous operation.
   c. Air-washing column: hydrometer cylinder 50 mm. o.d., 375 mm. high, containing distilled water. Fill to *ca.* 25 cm. depth. Change the distilled water at the first appearance of yellow color.
   d. Water-cooled condenser, Allihn 5-bulb type, 300-mm. jacket.
   e. Trap, wide-mouth 16-oz. bottle containing glass wool.
   f. and g. Pressure regulating columns: hydrometer cylinders 50 mm. o.d., 375 mm. high, containing distilled water. Fill to *ca.* 20 cm. depth.
   Pressure regulation may also be obtained through the use of a suitable pressure regulating valve.
   h. Manifold.
4. A source of low-pressure, clean, oil-free, compressed air. An individual air compressor of the pistonless diaphragm type is preferred to the use of industrial compressed air.
5. Thermometer accurately calibrated, which will indicate the temperature to within ± 0.1°C. in the range of 95°C. to 110°C. A.S.T.M. #40 C is satisfactory.
6. A gas flow meter, preferably of the calibrated, conical tube type.
7. Test tubes, Pyrex, 25 mm. x 200 mm. For convenience these tubes may be calibrated and etched at the 20-ml. level. Each tube is provided with a two-hole neoprene stopper and aeration tube as shown. An all-glass assembly, such as Martin M 7920, or equivalent, is satisfactory.
8. Tube-cleaning bath, consisting of an iron or stainless steel pan or sink, heated with steam coils (no copper tubing) or gas and of sufficient area to permit laying the required number of aeration and test tubes on the bottom.
9. Test-tube brush, fan tip, nylon.
10. Tongs, stainless steel or nickel-plated, suitable for handling tubes in hot detergent solution.
11. Apparatus as described in A.O.C.S. Method Cd 8-53 for for peroxide value.

B. REAGENTS

1. Petroleum ether, A.O.C.S. specification H 2-41.
2. Acetone, A.C.S. Grade.
3. Detergent for cleaning glassware which will leave no contaminating residue. Alconox, Dreft, and Vel have been tested and are known to be satisfactory, but others may be equally good.
4. Reagents as described in A.O.C.S. Method Cd 8-53 for peroxide value.

C. CLEANING SAMPLE AND AERATION TUBES

1. Melt and drain off as much of the fat from previous determinations as possible. Wash off the remaining fat with a suitable solvent. Petroleum ether is satisfactory if the cleaning is done immediately after the preceding determination and the fat involved is of 100 iodine value or less; otherwise acetone must be used.
2. Prepare a 1% solution of detergent, and heat almost to boiling in the cleaning bath. Rinse out each tube with the hot detergent solution, brushing briefly with a nylon brush. Then place the tubes in the hot detergent solution in such a manner that the tubes are full and completely covered and so that no air bubbles are trapped within. Place the fat-free aeration tubes with stoppers in the hot detergent bath in such a manner that they are completely covered and so that no air bubbles are trapped within. Boil all test tubes and aeration tubes with stoppers vigorously for 30 min. Brush each test tube vigorously with a nylon brush, rinsing twice in the hot detergent solution. Rinse thoroughly with tap water, followed by distilled water, and place upright in a test tube rack. Fill with distilled water and soak at least one hour. Rinse the aeration tubes thoroughly in tap water, followed by distilled water, and place upright in a clean, two-liter beaker of distilled water in such a manner that the long straight tube and the stopper are covered. Allow to soak for at least one hour. At the end of the soaking period, rinse both test tubes and aeration tubes once again with fresh distilled water, drain on clean filter paper, and dry in an oven at 100°–105°C. Arrange washing and rinsing schedules so that test tubes and aeration tubes are dried at the same time. Assemble as soon as dry, and store in a dust-free location.

D. SAMPLING

Because of peculiarities inherent in this procedure, special precautions are necessary in obtaining and transporting samples.

Where packaged fats are involved, the sample should consist of an unopened package if possible. Where this is impractical, samples must be removed from large containers or processing equipment with clean sampling devices only of stainless steel, aluminum, nickel, or glass.

Samples of solid fat should be taken at least 2 in. from the walls of large containers and 1 in. from the walls of small containers. If liquid oil is poured from a container, the pouring spout or lip should first be thoroughly cleaned, using a clean cloth moistened with acetone.

After removal from packages or processing equipment, samples should be transported or stored only in glass containers cleaned as described in D above, or in new tin containers. Under no circumstances should sample containers have plastic or enameled tops or covers with paper or waxed liners.

Samples should be protected from contact with heat and air as much as possible.

E. PROCEDURE

1. Unless already completely liquid, the sample should be melted at a temperature not more than 10°C. above its melting point. Pour 20 ml. into each of two or more sample tubes (Note 3). Pour carefully into the center of the tube so that none of the fat comes in contact with the top of the tube and subsequently the stopper.
2. Insert the aeration tube assembly, and adjust so that the end of the air-delivery tube is 5 cm. (2 in.) below the surface of the sample.
3. Place the tube and sample in a container of vigorously boiling water for a period of 5 min. At the end of this time remove the tube from the water, wipe dry, and transfer immediately to the constant-temperature heater maintained at 97.8°C. Connect the aeration tube to the capillary on the manifold, having previously adjusted the air flow rate, and record the starting time.
4. The figure to be reported as the A.O.M. stability value is the time (to the nearest hour) required for the sample to attain a peroxide value of 125 milliequivalents (m.e.) and should be the average of two samples. This value should be determined as follows. A short time before the end-point is reached (Note 4), determine the peroxide value according to A.O.C.S. Official Method Cd 8-53 with the exception that the sample weight should be 1 g. instead of 5 g. If this determination indicates that the peroxide value is between 100 and 200 m.e., another peroxide value determination should be made immediately, using a 5-g. sample. If the peroxide value so obtained should be above 200 m.e., the sample must be discarded and another determination started (Note 5).

   If the pilot determination indicates a peroxide value below 100 m.e., estimate when a value of 100 m.e. will be reached, and at that time make another determination on a 5-g. sample.

   Make a second determination on a 5-g. sample from the same tube exactly one hour after the first.

   This should give two peroxide values between 100 and 250 m.e. Use rectangular co-ordinate paper to plot these two values against their respective aeration times in hours. The A.O.M. stability value is the time in hours at which a straight line connecting these two points crosses the 125 m.e. co-ordinate. Repeat this procedure on the duplicate tube and report the average of the results.

F. NOTES

1. This method was designed for oils and fats normally consumed by humans including those containing approved antioxidants within established limits of concentration. The procedure up to the determination of the end-point (E-4) may be suitable for evaluating or comparing fats for animal feeds, inedible greases, soapstocks, and fats containing new antioxidants. However there are insufficient data available regarding the peroxide value *vs.* time curves for such materials to permit establishment of a definite end-point.
2. In view of the variety of existing baths and heaters in service the committee does not feel justified in specifying one particular type at this time. One has been shown to have certain distinct advantages(36). This is an aluminum block which utilizes thermostatically controlled, electric heat.
3. At least two samples must be run for each determination. If the product is an ordinary material so that not more than two 1-g. samples will be required to establish the 100 m.e.–200 m.e. range, two tubes are sufficient. However, in the case of an unknown or unusual material, three tubes should be used, one to serve as a guide to establish the 100 m.e.–200 m.e. range and the other two for making duplicate determinations of the final end-point. It is usually advantageous to have the starting times for two or more tubes spaced one or two hours apart.
4. With some experience the approach of the end-point can be judged by the odor of the effluent air from the sample tube.
5. It is desirable to heat and aerate samples continuously until the end-point is reached. Where this is not practical, remove the tube from the heater, chill immediately, and hold below 10°C. until ready to start again. The regular procedure should be followed starting at E-3.

G. PRECISION

1. The coefficient of variation, that is, the standard deviation expressed as a percentage of the A.O.M. value, is approximately 13.4%. This signifies that a maximum variation between laboratories of ± 25 might be expected on a 100-hr. sample, or ± 2.5 on a 10-hr. sample.

[Received February 27, 1957]

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