An Oxygen-Absorption Method for Examination of Fat*

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VARIOUS investigators have described the use of oxygen absorption measurements for comparing fats with respect to their resistance to oxidation. In the development of the method and apparatus described in this paper the aim was to get improved convenience and economy in routine or semi-routine testing of numerous samples. Since the test as developed along these lines has been giving useful results over a period of years, it may become useful in other laboratories where more than a few samples have to be examined.

As ordinarily used, the method is to determine the time required for a fixed weight of fat to absorb a fixed volume of oxygen when held under controlled conditions in air without illumination. Thus, one point on the curve for oxygen absorbed vs. time is determined; the volume of oxygen absorbed by the fat at this end-point is such that the test may be said to measure the induction period of the fat. If desired, a modified apparatus can be used to determine several points instead of one point on the curve.

The essential parts of the apparatus are the absorption flasks and their attached manometers with sealed-in electrical contacts, an oil bath, continuously agitated and thermostatically controlled to maintain a uniform temperature within ± 0.1°C. or better, fittings to support the flasks and manometers in the oil bath, and a multiple-pen operations recorder with appropriate electrical connections to the manometers. In one laboratory in which the test is used the oil bath accommodates 32 flasks.

Flasks and Manometers. Two styles of manometer will be described, the first of which has had the most use and is the less costly; the second style probably is an improvement although experience with it is not yet extensive enough to prove that it gives more accurate results.

Style 1. This is illustrated in Figure 1. The absorption vessel is a 50 ml. Erlenmeyer Pyrex flask sealed to the female portion of a 19/38 Standard Taper interchangeable ground glass joint with a flared lip to allow use of a mercury seal. The standard taper joint connects the flask to a side arm which is sealed to the manometer, made of 7 mm. Pyrex tubing with 2 mm. bore. Electrical contact with the mercury in the manometer is provided at points A and B by means of No. 28 B. & S. tungsten wire sealed into the glass. To provide a sturdy connection the tungsten wire is cut off with only a short length extending outward from the wall of the tube; several turns of fine copper wire are wrapped around the tube, making contact with the tungsten. The wire is then coated with solder, to which the lead wires can easily be attached.

The manometer is supported in the oil bath by a phone plug which fits the jack shown at F. The jack fits through a hole in the L shaped piece of heavy copper strip E. A thin copper strap, C, passes around the manometer tubes and is fastened to E by two small stove bolts. A spacer of laminated plastic between the two arms of the manometer at D allows the bolts to be drawn up tight to make a rigid assembly. A copper wire is soldered to contact B and to the strap C, to connect to one side of the jack. Another wire leads from A to the other contact of the jack.

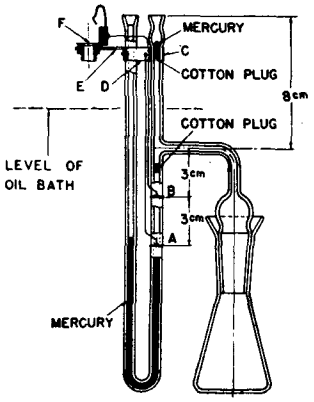

FIG. 1

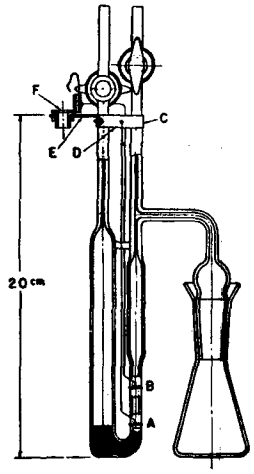

FIG. 2

A cotton plug in the manometer tube above contact B prevents the mercury from being drawn over into the absorption flask. Another plug just below the bulb at the top of the same arm of the manometer together with a small quantity of mercury placed in the bulb provides an automatic valve to allow air to escape when the system is placed in the hot oil bath and to prevent air from entering when the pressure falls due to absorption of oxygen. (Such a valve is not suitable when oxygen instead of air is used in the test since in that case the pressure can fall enough to suck the mercury through the cotton.)

Style 2. The manometer shown in Figure 2 was designed to eliminate the effects of changes in barometric pressure during a test and to allow the use of atmospheres other than air when this is desired. The absorption flask and the method of supporting the assembly and making the electrical connections is the same as described above. Both arms of the manometer have stopcocks, with oblique bore, which are closed after the assembly has come to constant temperature in the oil bath. The side of the manometer away from the flask is made of wide tubing to provide a reservoir which remains at approximately constant pressure during the test. The other side of the manometer has a 5 mm. bore and the quantity of mercury is such that air will slip through from the reservoir before mercury is drawn over into the absorption flask.

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Oil Bath. Various styles of bath would be suitable. A well-insulated rectangular iron tank, 18"x30"x15" deep, with an agitator at the center, driven by a gear--in-head continuous duty motor has given good service. Heat is supplied by a continuous heater and an intermittent heater controlled by a mercury- toluene regulator controlling a power relay. Temperature variation can be held well within ± 0.1°C. Two bars of cold rolled steel ¼"x2" are supported across the bath, 12 inches above the bottom. Each is drilled and tapped to hold two rows of eight phone plugs which serve as supports for the manometers and as electrical connections. Insulated wires lead from the phone plugs to the recorder.

Mineral oil is used in the bath which is held at a level about 1 inch below the steel bars that carry the phone plugs. No. 10C Switch Oil (General Electric Co.) or its equivalent has been satisfactory. With the bath operated continuously at 80°C. this oil can be used for a year or more without replacement.

Recorder. An instrument that will record the time when the circuit is completed in each of the individual manometers is required. An Esterline-Angus Model AWT Operations Recorder, with a chart speed of ¾ inch per hour, is satisfactory. Units having up to 20 pens each can be purchased; twin units have up to 40 pens.

Procedure. The oxygen absorption test may be made with the fat simply spread over the bottom of the flask, or the fat may be held on a support of some kind. Both tests have been found to be useful although they do not necessarily give results that show the same ratio between two given fats. A good support is pure silica sand, sized as will be described.

The fat sample to be tested is melted, and 1 g. ± 0.05 g. is weighed into the flask. If the sand is used, a 12½ gram portion of it is first weighed into the flask, and the melted fat is dropped upon it as it is weighed into the flask. The fat is then spread over the sand more uniformly by stirring the mixture with a thin glass stirring rod.

The joint that attaches the flask to its manometer is held tight either by a spring clip, or by a shrunk-on ring of cellophane made by cutting away the top portion from a cellophane bottle cap. In the latter case, the moist ring of cellophane is slipped over the male portion of the joint, which is then lubricated lightly with stopcock grease. The flask is then attached, a little mercury is put into the trough formed by the lip of the flask, and the ring is slipped down so that the lower edge will grip the lip of the flask and the upper portion will grip the bulge in the glass above the joint. The cellophane ring is then allowed to dry and shrink before the assembly is placed into the oil bath.

With some joint lubricants it is possible to dispense with the mercury seal. A product sold by the Scientific Glass Apparatus Company as "Joint-Lube No. 18712, Yellow Label" has given satisfactory seals without mercury.

The flask-manometer assembly is placed in the oil-bath simply by pushing the jack down onto one of the phone plugs previously described. Each plug has a number which corresponds with the number of one of the pens on the recorder. A record is put into a notebook showing the sample number of the fat being tested, the number of the flask-manometer assembly, the number of the recorder-pen to which it is connected and the date and hour when the flask is put into the bath. With the type of manometer having the automatic valve this completes the operation except for transferring to the notebook the record from the recorder showing the date and hour when the fat has absorbed enough oxygen to cause the mercury to be drawn up to the upper contact. The number of hours to the nearest quarter hour elapsed is recorded as the result of the test.

Exposure of the fat sample to light during weighing and the other operations should be kept to the practical minimum. The oil bath should be placed where strong light will not strike it.

Cleaning of Flasks. The following procedure is recommended. Wash the flask by shaking with warm soap suds. Soak for 1 hour in hot 5% caustic soda solution. Rinse with water and soak for an hour or more in cleaning solution. Rinse thoroughly with distilled water and invert over a steam jet for 30 minutes or more, then dry thoroughly. The steam should be free from impurities such as rust and oil and preferably is generated in glassware in the laboratory; otherwise it is better simply to rinse thoroughly with distilled water.

The manometers may simply be wiped and inspected to make sure that they are in good operating condition and placed in a rack for re-use. If oil gets into the cotton plugs they must be renewed.

Calibration of Manometers. As ordinarily used, the manometers are calibrated to close the circuit when the 1 g. sample of fat has absorbed 3 ml. of oxygen, calculated to 0°C., 760 mm.

Each absorption flask is numbered to correspond with the number of the manometer with which it is to be used. The volume of the flask-manometer system is then determined as follows: With about the normal quantity of mercury in the manometer, but with the cotton plugs removed, weigh the flask and manometer together, the joint being lubricated. Fill the flask and the parts of the manometer between the flask and the mercury with water up to within 1 cm. of the top of the manometer tube. Weigh the whole again. Calculate the volume from the weight of the water and its temperature.

From the volume obtained calculate the pressure difference that should exist when the fat has absorbed 3 ml. of oxygen. This will be about 50 mm.

The manometer is then cleaned thoroughly and dried. Approximately the right quantity of clean mercury is put into the manometer and cotton plugs are inserted in the manometer arm nearer the flask, one half-way between the side-arm and the top contact, the other just below the bulb at the top of the manometer tube. Mercury is then placed in this bulb, sufficient to fill it not more than ⅓ full.

The quantity of mercury in the manometer is then adjusted as follows: Connect the manometer in a vertical position to a large suction flask to which are also connected an aspirator and a plain open U-tube manometer with a millimeter scale. Make a temporary electrical connection to the recorder or to a relay. When suction is drawn on the flask very slowly, the pressure at which the circuit is made, causing the recorder or relay to operate, can be read on the plain U-tube manometer. The quantity of mercury is then adjusted so that the circuit is made at the calculated pressure-difference. A cotton plug is put into the top of the other arm of the manometer

to keep out dust and prevent spilling of the mercury.

Preparation of Sand. For some purposes it appears preferable to have the fat spread over a support rather than in a single layer on the bottom of the flask. For this purpose glass-makers' sand has been used, sized by sifting through silk screens and selecting the portion that passes a No. 1XX screen (opening, 0.0157 inch) and is retained on a No. 6XX screen (opening, 0.0092 inch). This is approximately equivalent to selecting the portion that passes a 40-mesh testing sieve and is retained on 60-mesh. Metal screens should not be used since they can contaminate the sand with pro-oxidant material.

Discussion

End-point of the Test. This is placed at 3 ml. oxygen absorbed per gram of fat in preference to a smaller quantity so that the rate of absorption at the end of the test will be relatively rapid. Thus errors introduced by changes in barometric pressure occurring during the test will be smaller than they would be if the end-point were taken at 1 ml. O_2 per gram. If a much greater absorption is taken as end-point, it sometimes happens that the fall in pressure in the flask, due to absorption of oxygen, ceases before the mercury reaches the contact: this probably is caused by evolution of gaseous oxidation products by the fat. It would therefore be necessary to complicate the operation by putting an absorbent in the flask to take up volatile oxidation products if the end-point selected were much greater than 3 ml. per gram.

Temperature. A temperature of 80°C. has been found to be convenient. Temperatures much above 100°C. probably are not practical for regular use because deterioration of the oil in the bath would become too rapid.

Reproducibility. It is advisable to run tests in duplicate. Results of individual tests seldom differ by more than 20% from the average result obtained by running a large number of tests on the same fat on a number of different days. There still is room for improvement here.

Representative Results. A few typical results are given in Table I.

TABLE I

Typical Oxygen Absorption Tests at 80°C.
(Hours required for 1 g. fat to absorb 3 ml. O_2 from air)

	Without sand	With oil spread over sifted glass-makers' sand
Salad Oil (winterized cottonseed oil)	70	20
Refined, deodorized cottonseed oil	63, 66	14, 16
Vegetable shortening (unhydrogenated oil base)	59	
All hydrogenated shortening	130	50
All hydrogenated shortening, bulk type	290	125
All hydrogenated shortening (long-keeping type)	Over 400	
Lard. A random sample from market (tests made on 5 different days)	53, 54 48, 50 61, 55 59, 60 58	
Lard. Another random sample from market	26, 27 28, 37	5½ 5¼, 5¼
Purified methyl esters of cottonseed oil	3.8, 3.5	1¼, 1¼
Purified methyl esters of cottonseed oil with 0.01% phosphoric acid added	3.8, 4.0	
Purified methyl esters of cottonseed oil with 0.15% alpha tocopherol and 0.01% phosphoric acid added	58, 65	
Purified methyl esters of cottonseed oil with 0.1% catechol and 0.01% phosphoric acid added	285, 291	
Purified methyl esters of olive oil	17, 17, 20 16, 18	5¼, 5¾, 5¼ 6, 6
Purified methyl esters of long-keeping type of all-hydrogenated shortening	79, 82, 86	13, 15
Same esters with 0.1% gamma tocopherol and 0.01% phosphoric acid added	477, 490	345, 352

Summary

Apparatus and procedure for testing fats by the oxygen absorption method are described. These were developed to reduce the labor required for testing numerous samples. Over a period of years they have given useful service.

Some Aspects of Recent British Studies on Antioxidants*

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WARTIME conditions of food processing, transport, and storage offer many additional problems to those encountered in normal times. One of the major difficulties is that products liable to oxidative spoilage must often be stored for long periods, and protection by gas packing imposes an additional burden on the restricted supplies of tinplate.

This paper will describe and summarize certain aspects of the research work on antioxidants carried out during the war period in Great Britain by a team of workers belonging in part to the Food Investigation staff of the Department of Scientific and Industrial Research and in part to other organizations, notably the Hannah Dairy Research Institute in Ayr, Scotland.

The immediate necessity was to discover, if possible, some effective antioxidant for each type of food requiring protection, such antioxidant also having to be non-toxic at the levels used and free from any objectionable taste or odor. Long range research, for example on the mechanism of antioxidant action, could not be included in such an emergency program

* Presented at the Conference on Problems Related to Fat Deterioration in Foods, under the auspices of Committee of Food Research, Research and Development Branch, Military Planning Division, Office of The Quartermaster General, in Washington in June, 1945.