An Accelerated Stability Test Using the Peroxide Value as An Index

A. E. King, H. L. Roschen, W. H. Irwin, Swift and Company Laboratories, Chicago, Ill.

A vast amount of research work and experimentation has been done on the problem of fat spoilage, the reactions involved, and the various tests bearing on rancidity.

It is impossible, in a paper of this kind, to give a comprehensive bibliography or to mention more than a small portion of the work done. However, some of the more pertinent references are given. In recent articles, Vibrans[27] and Wheeler[22], list a large number of references and discuss the work of various investigators briefly.

The work covered by this paper grew out of a demand for a quick method for comparing the stability or keeping qualities of lard samples. At present several methods for determining the stability of fats are in use in the baking and packing industries, all of which are open to one or more objections.

The methods in common use are:

1. *Incubation Tests*—such as Schaal's and similar tests:

In these tests the fats are exposed to the air in a heated incubator and examined organoleptically at regular intervals until they become rancid. Such tests are objectionable because of the time involved, and because the results are dependent entirely on personal judgment.

2. *Oxygen Absorption Tests*[1 to 12 inclusive]:

In these tests either the amount of time required by the fat to absorb a certain amount of oxygen, or the amount of oxygen absorbed in a fixed time is taken as indicative of the keeping qualities of a fat. These methods require complicated and expensive apparatus and produce results which are influenced by changes in absorption rate due to changing pressure and the presence of volatile oxidation products.

3. *Color Reaction Tests*—such as the Kreis[13], modifications of it[14-15] and the Schiff tests[16-17].

These are tests which produce a color reaction in the presence of the aldehyde products of fat decomposition and have been shown to give no indication of the keeping qualities of a *fresh* fat.

4. *Tests Depending on the Collection of the Volatile Aldehyde Products of Decomposition of Incubated Fats and the Determination of them Quantitatively as an Index of the Keeping Quality of the Fat Tested*[16, 23].

An arbitrarily set rancid point depending on a certain amount of aldehyde collected in a definite time cannot be specific for all fats, and in many cases serious errors may be made in grading fats on this basis.

In general such tests are difficult to carry out and are not sufficiently sensitive to detect small differences in the products tested.

Many other tests for stability have been described in the literature, but are at present unimportant commercially.[24, 25, 26]

Oxidative decomposition of a fat is characterized by two chemical changes which are of importance in the accelerated keeping test about to be described. Various aldehydes, some of which are volatile and are probably responsible for the odor called "rancid" are produced and identify the fat as rancid. Peroxides are formed and can be determined in the fat. The quantitative determination of peroxides in fats has been the subject of much work and investigation[18 to 22 inclusive]. The method of Wheeler[22] for the quantitative determination of peroxides in fats and oils has been used in the work herein described and the incubation apparatus used is a much simplified adaptation of that used by him in studying corn and cottonseed oils.

The principle of the method is briefly as follows: By aerating a sample of lard in a test tube held at constant temperature in a bath, the aging of the lard is greatly accelerated. By starting 3 or more portions of the sample at intervals of one hour, the oldest portion becomes rancid (to organoleptic tests) while those which were started later are still in their induction period. Since the personal factor renders organoleptic tests, at best, open to suspicion, the results are not based on the odor of the treated fat samples alone, but on the peroxide content of them after treatment.

The peroxide content of fats and oils at the inception of a rancid odor, varies with the type of fat or oil, and with the conditions of storage. Edible oils of high iodine number usually have a high concentration of peroxides at the start of rancidity. In the drying oils, oxidation produces high concentrations of peroxides; however, due to polymerization, rancidity is seldom produced. Fats and oils containing quantities of natural or added antioxidants also have a high concentration of peroxides at the rancid point. Fats of low iodine number have a relatively low concentration of peroxide present at the point where rancidity is apparent. Kilgore, in a recent paper[28], has shown that cottonseed oil can become rancid at a very low peroxide concentration when the air supply is restricted and the factors of light and temperature are favorable to rancidity. The peroxide content at which a fat becomes rancid is therefore governed by several factors. The nature of the fat, the temperature at which it is held, the amount of oxygen available, the surface exposed, the amount of agitation, the presence or absence of light, and perhaps other factors, have a bearing on the peroxide value at the point of incipient organoleptic rancidity. It is only by adhering to certain fixed conditions for the carrying out of this accelerated test that a peroxide value can be established which coincides with the organoleptic rancid point.

The peroxide content of an average sample of pure lard at the point of incipient organoleptic rancidity, under the accelerated aging conditions of the test outlined, was determined to be approximately 20 milli-equivalents of peroxide per 1,000 grams of fat. This number, while not representing the exact rancid point in all cases, is very close to it for the conditions of the test described in this paper, and no pure lard samples have been found which had peroxide numbers very far from this amount, when the rancid point was reached.

The following gives the method of treatment, details of the apparatus and notes on the determination. However, the amount of sample, temperature, the rate of air flow, etc., in this work has been arbitrarily fixed. It is quite probable that for certain types of fats and stabilized shortenings these conditions could be modified to advantage. However, in all the work herein described, the method was followed in detail.

Incubation.—Constant Temperature Bath:

This bath consists of a water jacket in which water is kept at the boiling point, with a reflux condenser keeping

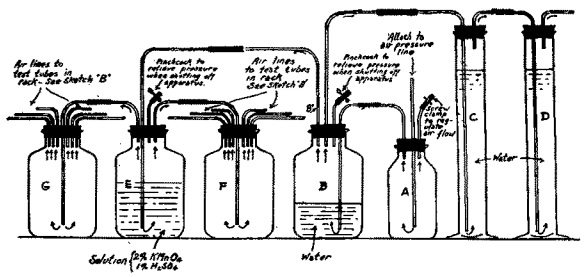
the volume constant. This water jacket encloses a mineral oil bath in which is a rack accommodating 8 inch by 1 inch test tubes. At Chicago this jacket of boiling water maintains the oil bath at a constant temperature of 208° F. This temperature has been selected as being the one obtained with this type of apparatus at most points. At any point not obtaining the temperature of 208° F. the water in the jacket may be modified either by using alcohol to lower the boiling point or by adding glycerin to elevate it.

Aerating Train.—This part of the apparatus is designed[23] to furnish a fixed volume of air to each test tube in the bath. The arbitrarily established volume of air required to pass through each tube is 2.33 ccs. per second. Air is supplied to the unit from compressed air service lines. Where compressed air is not available it can be obtained by the use of an aspirator pump operating in a bottle. The details of the aerating train are shown in sketches A and B.

Sketch A

Keeping Test Apparatus—Air Flow Diagram.

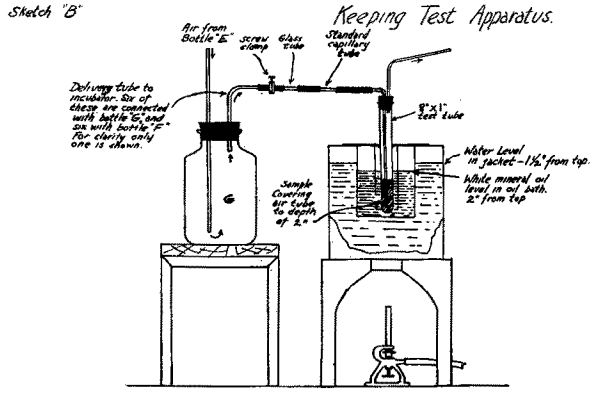
A - Trap to remove water, oil, etc. from incoming air. B - Water wash for air. The space above the water in B is kept under constant pressure sufficient to by-pass air through the water columns C and D at a steady rate. The air is regulated by a valve on the supply line and the screw clamp on A. The air to be used for adsorption flows thru the tube F to bottle E where it is washed in acid permanganate and passes into portions F and G which act as manifolds to distribute the air to the test tubes in the incubator.

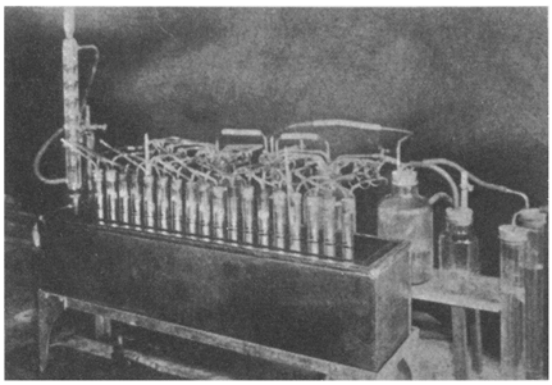


The air flow is standardized as follows: Capillary tubes about 2 inches long are cut from one piece of *thermometer* tubing. Each capillary is then standardized to pass the same amount of air as the others in the apparatus at any given pressure. Any convenient means may be used to make this standardization. To increase the flow the capillaries may be ground down on a fine emery wheel. The capillary tubes are installed in the apparatus, 20 ccs. of lard placed in each test tube and the apparatus brought up to temperature. Bottle B is filled with distilled water to about one-third of its capacity. Bottle E is filled a little over half full with a 2 per cent solution of potassium permanganate containing 1 per cent of sulphuric acid. The apparatus is connected as shown in sketches A and B, with the exception that a meter is installed in the air line between bottle B and bottle E. The amount of water in cylinders C and D is then adjusted until the air passing through the meter is equal to 2.33 ccs. per second for each test tube. When this point is reached bottles B and E, and cylinders C and D are marked with a file mark and the liquid level maintained at this mark thereafter. The amount of air by-passed through cylinders C and D should be sufficient to form a steady stream of bubbles which is almost continuous. The capillary tubes used in several apparatus built by the authors have been of such an air flow capacity that the total height of water columns C and D has been approximately 20 inches. In gauging the apparatus with the meter as above, it must be remembered that the air is being measured under slightly increased pressure and the actual amount of free air passing through each tube will be slightly greater than 2.33 ccs. per second. The permanganate used in bottle E should be changed once each week. To obtain correct regulation of the air passing through the apparatus it is advisable to use the screw clamp on bottle A, by-passing a little excess air. In case of breakage of any part of the apparatus a part of identical measurements should be used wherever it has any influence on the air flow. This applies particularly to the capillary tubes used and the delivery tubes used in the test tubes. The end of the delivery tube in the test tube should be 2 inches below the surface of the lard.

Sketch "B"

Keeping Test Apparatus.





Keeping Test Apparatus

Incubation of Sample.—Measure 20 ccs. of melted lard into each of three 8 inch by 1 inch test tubes, which for convenience should be calibrated at the 20 cc. level, Heat one of the tubes to approximately 208° F. by immersing in hot water. Place in the oil bath, which has previously been brought to a temperature of 208° F. by maintaining the water jacket at the boiling point, and make necessary connections. Start the air flow, making a record of the time. Stopper the second and third portions and hold at room temperature until the time arrives for their incubation. Exactly one hour after starting the first portion, start the second portion of the sample in a similar manner. One hour later start the third portion. At regular one hour intervals inspect the first portion for odor. When it has become definitely rancid as indicated by odor, remove all three test tubes and immediately weigh 5 gram portions for the peroxide determination. The peroxide determination is taken as the conclusive test for rancidity since organoleptic tests vary with the individual. When the lard has a rancid odor it has been found that the peroxide content is 20 milli-equivalents per 1,000 grams of sample or greater. As the keeping test of the lard, report the number of *hours* required to produce the *first titration* equal to 20 milli-equivalents of peroxide or more per 1,000 grams in the sample being tested.

Determination of Peroxides.—A 5 gram (± .05) portion of fat is weighed into a 200-300 cc. Erlenmeyer flask and dissolved in 30 cc. of a mixture of 60 per cent acetic acid and 40 per cent chloroform. To the solution is then added 0.5 cc. of saturated KI solution and the flask shaken until the solution becomes clear. After about 2 minutes, 30 cc. of water is added and the mixture titrated with either .01N or .1N solution of sodium thiosulphate, using starch solution as an indicator. The flask should be shaken vigorously near the end of the titration to liberate all the iodine from the chloroform layer. The number of milli-equivalents of peroxide present per 1,000 grams of sample is calculated from the amount of sodium thiosulphate solution required to titrate the iodine liberated. Results may also be expressed in mill-moles per 1,000 grams of fat. For convenience, both methods of calculation are given below:

$$\text{Milli-equivalents per 1,000 grams} = \frac{\text{cc} \times \text{N}}{\text{gm}} \times 1{,}000$$

$$\text{Millimoles per 1,000 grams} = \frac{.5 \times \text{cc.} \times \text{N}}{\text{gm}} \times 1{,}000$$

cc. — cc. thiosulphate
N — Normality of thiosulphate
gm — Grams of oil
1 milli-mole = 2 milli-equivalents

Typical peroxide values and interpretation of results are illustrated below.

Sample No.	No. of hrs. aerated	Peroxide content in milli-equivalents per 1,000 gms.	Reported keeping Test (hrs.)
.....	3	3.2	)
1.....	4	6.2	5)
.....	5	78.5	)
.....	6	6.0	)
2.....	7	12.0	8)
.....	8	22.2	)
.....	8	5.8	)
3.....	9	17.8	10)
.....	10	60.0	)

This method can be used to develop graphs showing the exact breaking point, or point at which rancidity occurs. If this is desired it will be sufficient to plot the number of hours of aeration against the peroxide content. For the purpose of making such a graph a large number of determinations will have to be run so that one sample can be removed at the end of each hour. For the regular test the three tube system described is preferred, it being shorter and more practical for a large number of samples. By this method only the breaking point, or point at which rancidity is apparent is obtained. From this point on the peroxide content of the lard increases very rapidly and on a graph shows a mounting line which is almost vertical. The curve eventually flattens out and finally indicates a lowering peroxide content in the more advanced stages of fat decomposition.

Absolute cleanliness is necessary in order to obtain results which are of any value. Each time a test tube and delivery tube are used they must be thoroughly washed and immersed in a chromate sulphuric acid cleaning solution, preferably over night. They should then be rinsed thoroughly with distilled water and dried in an oven before re-use. The capillary tubes should be inspected daily as any small particles of dirt reduce the volume of air passing and destroy the accuracy of the whole apparatus.

Occasionally a fat which foams will be encountered. In this case a final air tube with small bulbs blown in it is used instead of the straight tube. A tube suggested by Dr. F. C. Vibrans of the Institute of American Meat Packers has been found to stop the most persistent foam and to prevent any loss through the air discharge tube. This tube is shown in Figure I. It will be found very convenient to determine the condition of the sample at

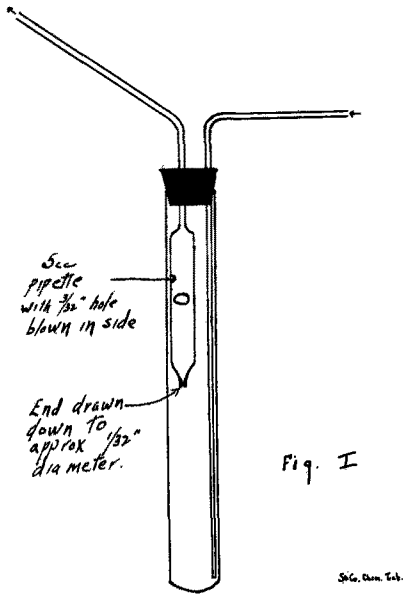


Fig. I

intervals by holding the nose at the outlet tube while the samples are being aerated.

The thermometer used to determine the oil bath temperature should be surrounded entirely by oil and should not touch the metallic part of the bath at any point. A slight day to day variation in this oil bath temperature may possibly be noted, due to changes in atmospheric pressure, however, if this change is no greater in magnitude than one-half degree it should not influence the results appreciably.

A heavy grade of highly refined mineral oil such as Stanolax is most satisfactory for use in the oil bath. Oils less highly refined give off vapors which make it difficult to observe the odor of the lard being treated. A slanting copper shield placed on the top of the bath beneath the outlet tubes also serves to deflect mineral oil vapors if any, to the rear of the apparatus where they will not interfere with organoleptic tests as aeration proceeds.

Light has the property of accelerating oxidative rancidity. The apparatus should not be mounted in a location where it is exposed to direct sunlight. When the rack is only partially filled with samples the unused spaces should be covered by pieces of sheet iron or copper slightly larger than the holes.

When a sample has a keeping test greater than the length of a working day, that is, over 8 hours, it is necessary to interrupt the determination and resume it the following morning. When this is necessary, the samples should be taken from the oil bath on the even hour and chilled immediately in a bucket of cold tap water. The air delivery tube and the stopper are not removed. After chilling, the samples are placed in a refrigerator or cooler over night. There is reason to believe that holding samples in this manner over night gives a slightly lower keeping test than if the sample is carried on to rancidity without interruption. However, if the above precautions are observed it should not affect the results greatly.

The apparatus can be operated over night, if desired. For over night operation the water bath should be elec-

trically heated by means of a space heater or hot plate. For day operation gas heat is satisfactory.

Tests made in widely separated laboratories using this method and apparatus show very good agreement. The reported results from six laboratories on two check samples of Prime Steam Lard are given below:

	Laboratories					
	1	2	3	4	5	6
Sample A—hrs.	7	7	6	7	6	6
Sample D—hrs.	2	2	2	2	2	2

Samples of lard to which an antioxidant had been added were tested by five laboratories with the following results:

	1	2	3	4	5
Sample B—hrs.	20	22	20	20	21
Sample C—hrs.	12	13	10	11	12

While the above results agree within fairly close limits they are remarkably good when it is considered that in some cases the samples were held 6 weeks under refrigeration before the apparatus was assembled to make the determination.

A more recent set of figures obtained at six laboratories where the samples were handled as promptly as received, are given below:

	Laboratories					
	1	2	3	4	5	6
Sample D—hrs.	7	6	7	6	6	7
Sample E—hrs.	5	4	5	5	5	5

All of the above samples were put up at laboratory No. 1 and sent by Parcel Post to the other laboratories located at points 300 to 800 miles from the shipping point. The fact that the samples were without refrigeration for two to three days in transit may account for some of them being slightly lower in keeping qualities than the samples held at laboratory No. 1.

In order to check the correlation of this method with other keeping test methods in common use, a program of cooperative work between the laboratories of the Institute of American Meat Packers, Armour & Co., Wilson & Co., and Swift & Co., was arranged. Each laboratory used the test or tests in daily use by them, Swift Laboratory using the keeping test and apparatus herein described. The results are tabulated below. Samples A and D are ordinary lard, Samples B and C are lard containing an antioxidant.

Sample	Laboratories						
	1		2	3		4	
	(test a) hrs.	(test b m.m. per kilo	test c) days	(test d) hrs.	(test e hrs.	test f hrs.	test g) days
A.....	7	5.0	5	14.3	17.0	38	18
B.....	20	2.75	12	35.2	35.0	75	39
C.....	12	3.5	8	21.7	28.3	52	31
D.....	2	23.5	3	6.0	7.5	9.4	8

Test a—Test as described.

Test b—20 gram samples are aged in an incubator equipped with an air circulating fan, without agitation, at 70° C. for three days and the peroxide concentration determined. Results are expressed in millimoles per kilogram of fat. Stability of a fat is supposed to be inversely proportional to amount of peroxides developed.

Test c—20 grams of fat are incubated at 70° C. in an incubator equipped with a fan for circulating air. Samples are observed daily until rancid to smell. Results are expressed in number of days required to become rancid.

Test d—Oxygen absorption method. A special incubator is used to enclose the flasks containing the fat samples, holding them at 90° C. The flasks are connected with recording manometers outside of the incubator. The figure reported is the time in hours required for 10 cc. of fat to absorb 30 per cent of its volume of oxygen from the air contained in the flask with it.

Test e—Same as Test d.

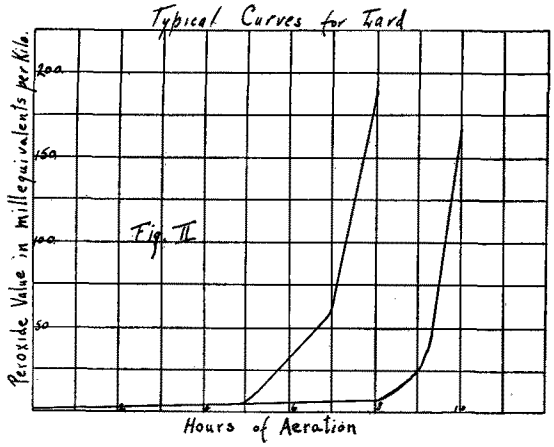
Test f—10 grams of sample are held in an oil bath, thermostatically controlled at 79° C., until rancid. The rancid point is checked by determining the peroxide value. No agitation or aeration is used. The results indicate the number of hours required to reach the rancid point.

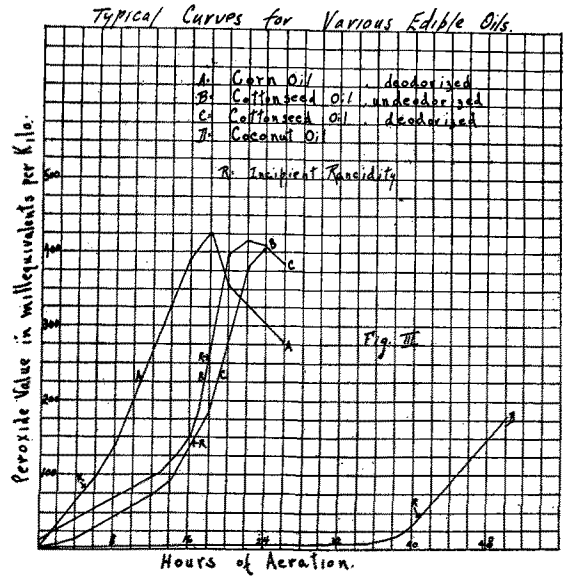
Test g—Schaal Test—50 grams of sample are incubated at 60° C. and smelled daily until rancid. The results shown indicate the number of days required to produce rancidity apparent to the sense of smell.

The cooperative study indicates beyond a doubt that samples tested by the method described in this paper are classified in the same order as they are by all the other tests used in the cooperating laboratories. It has a combination of several advantages which none of the other methods in use have:

1. Results are obtained on lard in a working day.
2. The apparatus is inexpensive to construct.
3. The manipulation is simple and can be carried out by an analyst of ordinary ability without the introduction of the personal equation.
4. A large number of samples can be handled on a single apparatus.
5. Samples are taken to the rancid point and observations at some intermediate point are not used as stability indicators.
6. The method is invaluable as a means of securing data for graphs, etc., showing the relation of peroxide number, color, odor, etc.

Up to date the work with this method has been largely confined to the study of lard and oleo oil. In an experimental way it has been applied to corn, cottonseed and coconut oils and also to cottonseed oil shortenings of various kinds. In all of the products mentioned the rancid point occurs at a peroxide number higher than in lard or oleo oil. In some cases the point of organoleptic rancidity is difficult to determine due to natural oil odors which mask rancidity until it reaches an advanced stage. By determining the average peroxide content at the rancid point for any given class of fats or oils this method can be applied in the same manner as for lard. On fats of considerable stability, two hour intervals are more satisfactory and on materials of unusual keeping qualities three to five hour intervals should be used. Typical curves showing the peroxide value at various stages in the aging of lard, corn oil, cottonseed oil, and coconut oil are shown in Figures II and III.

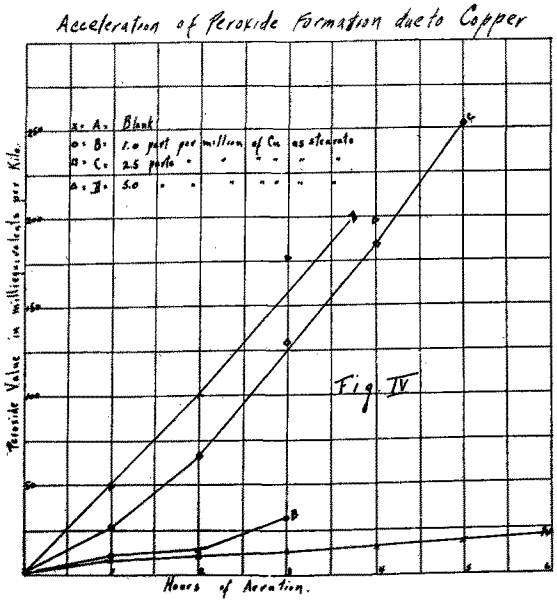




In the experiments made it was found that certain types of hydrogenated shortening had stability of such an order that 50 to 100 hours of aeration were required to produce rancidity. Some work was done in an effort to find a controllable catalyst to accelerate the action so that the time could be shortened. This work is still being carried on. Most of the work done to date has been on the use of metals and metallic soap catalysts.

Strips of copper, brass or monel metal placed in the test tubes during aeration produced marked acceleration of rancidity. Solutions of the stearates of copper and nickel in chloroform are also being tried and other experiments with metallic soaps are being carried out.

A study made of the effect of small quantities of copper stearate added to the fat gave some rather surprising results. The copper stearate was dissolved in chloroform in such proportions that the amount desired could be obtained in a 2 cc. portion. The results obtained are



shown in graphical form in Figure IV and indicate that the presence of copper in a fat, even in the proportion of one part per million has a marked accelerating effect on its oxidative decomposition.

The effect of copper is so great that it may be impossible to control it for use as a catalyzer in an analytical procedure. It is hoped that an investigation of other metallic soaps will produce a controllable catalyst, the use of which will permit this method to be applied to shortenings of great stability so that reproducible results can be obtained in a working day.

SUMMARY

A quick stability test for lard is described which depends on the peroxide content for identification of the rancid point. By its use ordinary samples of lard can be evaluated for stability in a working day. Oleo oil also is being tested by this method. It is applicable to edible fats and oils and hydrogenated shortenings. Typical peroxide curves for various fats and oils are shown. The effect of copper in accelerating oxidative rancidity is discussed.

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Soybeans of High Oil Content Urged for Carolinas, Virginia

Farmers in North Carolina and South Carolina and Virginia who plan to grow soybeans for a cash crop in response to the demand for export and for oil crushers will find most suitable for that market the yellow-seeded varieties which are high in yield of oil, W. J. Morse, soybean specialist of the U. S. Department of Agriculture, says.

In this region, increased exporting and crushing activity has improved the demand for the high oil yielding varieties. The growing of soybeans as a cash crop has proved popular with many farmers because the beans can be grown after an early grain or truck crop has been harvested.

Among the varieties which are most favored by buyers for oil or export purposes are the Mammoth Yellow, Haberlandt, Dixie, Herman, and Toki. Farmers who want soybeans for forage crops usually grow the Tarheel Black, Biloxi, Laredo, Otootan, and Mammoth Brown varieties, but these do not yield as well nor produce as much oil as the varieties recommended for high oil content.