Stability and Rancidity

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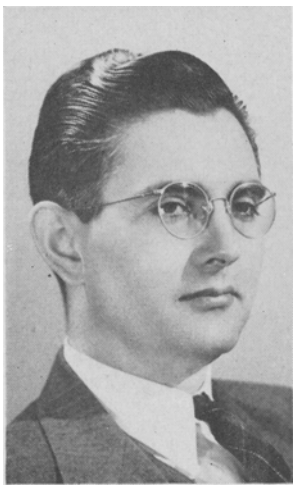
THE DETECTION of advanced stages of rancidity in a fat or fatty food has never been a problem for people with normal olfactory senses. The sharp pungent odors mixed with certain stale and musty odors have provided tell-tale evidence of rancidity throughout the ages. That this is not always regarded as undesirable is evidenced by the fact that some peoples have deliberately aged fats before using them. The present-day preferences for products with fresh bland flavors and odors require that we have ways and means for evaluating keeping quality and incipient rancidity.



L. R. Dugan Jr.

The history of objective tests for rancidity and for keeping quality begins over 50 years ago with development of the Kreis test (12). Then the oxygen absorption methods for determining the resistance of a fat to rancidity were developed. The baking industry developed the oven test for rancidity, which became commonly known as the "Schaal Oven Test" (10) although there is no published reference by the originator. Great strides forward were made with the development by Lea (14) and later by Wheeler (29) of quantitative methods for peroxide determination. These were followed by the accelerated method known as the "Swift Test" or the "Active Oxygen Method" by King, Roschen, and Irwin (11). The rapidity and reproducibility of this method coupled with the organoleptic correlation of rancidity with developed peroxides made it extremely useful for evaluation of the resistance of a fat to rancidity and especially for the evaluation of the relative effectiveness of antioxidants in fats. Many early workers recognized the presence of aldehydes and/or ketones in autoxidized fats and attempted to develop objective tests based on them. It was only after the development of quantitative colorimetric tests for carbonyl compounds in low concentrations that substantial agreement between carbonyl content and organoleptic evaluation of rancidity was accomplished.

The most commonly used methods for assessing stability or rancidity of a fat or fatty foods are the: active oxygen method, Schaal oven test, thiobarbituric acid test, carbonyl test, and peroxide value.

The Active Oxygen Method. This method is based on the principle that the aging and rancidification of a fat is greatly accelerated by aeration in a tube held at a constant elevated temperature. While an organoleptic evaluation may be used to indicate the rancid point, the results are based on the peroxide content of the fat after correlation with organoleptic rancidity.

The apparatus consists of a constant temperature bath and an aerating train contrived to deliver a continuous flow of air to the sample at a constant rate. Initially the heat was supplied by an oil bath surrounded by a hot water jacket maintained at a constantly boiling temperature. Recent refinements use an oil bath with a stirrer and a thermoregulator for precise temperature control. The aerating train has a bottle with water through which air is passed into another bottle containing 2% potassium dichromate with 1% sulfuric acid. These bottles serve as scrubbers to purify the air before it is led into the manifolds, to which the sample tubes are connected. A tube leads from the scrubber to two cylinders connected in series and each filled with water to the height necessary to provide a suitable pressure head for forcing air through each tube leading into the sample tubes. The rate of air flow was established at 2.33 cc. per second through each sample. This flow rate is controlled by the pressure head and by inserting a piece of capillary tubing in the line leading from the manifold into the sample tube. The capillary tube is cut to length according to the size of the capillary to permit the established rate of air flow.

The determination may be made by measuring 20 cc. of liquid fat into three 8-in. by 1-in. tubes. One tube is heated to temperature in an external water or steam bath and then placed in the oil bath; and the air tube is connected to the tube which projects through the sample to near the bottom of the sample tube. The time of the beginning of air flow is recorded. At suitable time intervals of 1 or more hours, the remaining tubes progressively may be started. When the effluent air from the first tube has the characteristic rancid odors, a sample may be taken from each tube for a peroxide determination. By a proper choice of starting intervals for the tubes containing a given sample, it is possible to bracket the end point of 20 milliequivalents of peroxide per 1,000 g. of fat (animal fats) and then by periodic sampling from the remaining tubes accurately to determine the time for development of the necessary peroxide content. Variations of this method are practiced. A commonly used variation is that reported by Riemenschneider *et al.* (23), which uses only one tube and periodic sampling of 0.2-g. samples for determination of the peroxide value. These workers introduced the all-glass aeration tubes in place of the rubber-stoppered test tubes and added a water condenser and extra air distributing bottle to prevent entrained moisture in the air-distributing system from clogging the capillary orifices.

A major contribution to application of the A.O.M. to control work was made by Mehlenbacher (16), who found that the time for rancidification could be shortened by a factor of 2.5 by elevating the incubation temperature to 110°C. It is obvious from this that temperature control is critical for reproducibility of results since only a small change in temperature may introduce a wide variation in end-point.

There are numerous factors which can influence the rate of rancidification of a fat, and the temperature effect cited is one. Other factors are light, availability of oxygen, surface factors, the presence of natural or synthetic antioxidants, the nature of the fat, and trace metal or metal contact catalysis. For reproduci-

bility it is necessary to make all conditions as standard as possible.

A most important factor is absolute cleanliness of all glassware that comes in contact with the fat. Early workers used a dichromate-sulfuric acid cleaning solution with numerous steps of soaking and rinsing in distilled water. Subsequently it has been learned that boiling the glassware in a solution of one of the numerous heavy-duty detergents, followed by rinsing and boiling in distilled water and then an overnight soak in distilled water, will clean the glassware and eliminate the possibility of trace quantities of chromium adsorbed on the surface of the glassware. That trace metals, especially copper, will affect the rate of rancidification was shown in the initial paper by King *et al.*

The peroxide values achieved by the A.O.M. at which a fat will be rancid by organoleptic evaluation varies with the nature of the fat. It has been accepted that the end-point for lard is a PV of 20 m.e./kg. Vibrans (28) reported PV's of 20 for lard, 75 for hydrogenated cottonseed oil, 100 for compound, and 125 for cottonseed salad oil. General practice now is to use a value of 20 for lard and 100 for vegetable oils and hydrogenated fats.

The presence or absence of antioxidants may influence the peroxide value at which organoleptic rancidity may be determined. This is shown by the fact that frequently it is not possible to detect a rancid odor in a fat treated with (BHA) butylated hydroxyanisole until the peroxide value has exceeded 25. It may be that the effect exhibited by the peroxide *versus* time curve with a given antioxidant is responsible since these curves vary. An antioxidant, such as propyl gallate, gives a peroxide curve which remains essentially flat until the end of the induction period and then rises abruptly, showing a very marked acceleration in rate of production of peroxides. An antioxidant such as BHA will exhibit a gradually increasing peroxide value until the end of the induction period, at which point a marked acceleration of rate of formation ensues. It is probable that organoleptic evaluation is dependent on carbonyls, and these may be present in quantity sufficient for evaluation only when a marked acceleration of oxidation has begun. Moore and Bickford (17) have noted that, in lards treated with various antioxidants, the rate of peroxide formation typical of the end of the induction period does not become apparent until the peroxide value is well past 20 when certain antioxidants are used. They have proposed that all end-points be based on a peroxide value of 100, at which point the induction curve for all systems they tested was practically a vertical line.

The A.O.M. provides a simple and reproducible method for accelerating the time for a fat to become rancid. It is useful for comparing the stability of one fat with another and for determining the relative effectiveness of antioxidants and other treatments designed to improve stability.

The Oven Test. The oven test was developed by the biscuit and cracker industry to provide a relative rating to the shortenings used (10). Since it is run at temperatures only moderately greater than those found in ordinary storage conditions, it provides an index of keeping quality which more nearly rates a product as a user will find it.

The test is normally accomplished in an oven with forced draft ventilation at 63°C. or 145°F. The sample is stored in beakers covered with watch glasses or in glass jars with loose-fitting caps. (In the author's laboratory it has been found that Bakelite caps with the liners removed are quite satisfactory.) Keeping time is measured as the number of days to detect rancidity by organoleptic evaluation. It is possible to use the peroxide value as an index of rancidity, but it too must be used judiciously and according to the nature of the fat or food being tested. Absolute cleanliness of the glassware is quite as important in this method as in the A.O.M.

In addition to the fact that the oven test more nearly simulates rigorous natural storage conditions than the A.O.M., there are other features which should not be overlooked. Among these are the fact that a minimum of equipment is needed, and little if any technical skill is required. Another feature is that it is useful for revealing flavors and odors other than rancidity which may occur due to the nature of the fat, faulty processing, or contamination during processing.

A frequently asked question is "what is the relation between ordinary shelf storage, oven storage, and the A.O.M. value of a fat?" There is no direct and precise answer to this. The relation varies with each fat and the treatment it has had. Claims have been made that 1 hr. by A.O.M. is equivalent to 15 days of shelf life of a fat. The author has observed that frequently the A.O.M. value will be approximately one-fifteenth the value in hours that the oven test will give. Variations noted however have been from 10 oven hrs. per A.O.M. hour to more than 20 oven hours per A.O.M. hour. Riemenschneider *et al.* (24) found that, on the basis of protection factors, oven tests were in general agreement with those obtained by the A.O.M. They noted also that protection factors vary with the initial stability of the lard and are valid only when in the same substrate. Another factor not to be overlooked is the fact noted above that longer incubation periods at lower temperatures tend to bring out odors and flavors which will show a fat to be unfit for many uses even though it is not rancid either by peroxide value or by organoleptic evaluation.

The Peroxide Value. The peroxide value of a fat has long been associated with its state of rancidity. As stated above, the peroxide value attained when a fat is judged rancid by sensory evaluation has been correlated under certain conditions for given fats. In lard and other animal fats, a fat will normally be judged rancid by odor and taste when it has attained a peroxide value of 20 m.e./kg. under conditions of the A.O.M. test or under milder conditions of storage. This indicates that up to this point there is probably no appreciable loss of peroxides due to decomposition or secondary reactions. This does not mean that flavor changes are not occurring in the fat but only that they are not yet those flavors and odors typical of rancidity.

It was the development by Lea (14) and the modification by Wheeler (29) of iodimetric methods for precise determination of peroxides that made possible the reproducibility and accuracy of the A.O.M. test for stability of a fat.

The method of Lea involves adding 1 g. of potassium iodide to 1 g. fat and then adding 20 ml. of acetic acid-chloroform mixture (2:1 by volume). The solution is heated to boiling, which is continued for a half a minute. The tube is rapidly cooled, and the

contents are poured into 30 ml. of water and titrated with sodium thiosulphate solution.

The Wheeler method uses 3–10 g. of oil or melted fat in 50 ml. of a mixture of glacial acetic acid and chloroform (3:2 by volume). One ml. of a saturated solution of KI is added, the flask stirred by a rotary motion and let stand 1 min. (The author uses 2 min. reaction time and a 5-g. sample of fat). One hundred ml. of distilled water are added, and the liberated iodine is titrated with a standard solution of sodium thiosulphate.

In either method the peroxide may be reported as millimoles per kilogram of fat or as milliequivalents per kilogram of fat. The latter unit is most commonly used in the U.S.A.

The peroxide value is useful as a measure of rancidity or degree of oxidation in a fat under certain conditions where there is not cause for serious decomposition of the peroxides or where peroxidation does not result primarily in cross-linking rather than fissioning of reacted molecules. The peroxide value is not a useful measure of the stability of a fat. Any fat may have a low peroxide value when freshly prepared but have a very short keeping time. On the other hand, many antioxidants will permit the buildup of a low level of peroxides, which will stay constant and the fat remain nonrancid for quite extended periods of time thereafter. In a similar sense the fat extracted from a fatty food may indicate by its peroxide value the extent of oxidative change, but unless the pattern for peroxide buildup is known for this food under the conditions of holding, it will have little meaning with regard to stability time until the time the value is equivalent to that for rancidity.

The Carbonyl Test. It has long been recognized that the odors and flavors associated with typical oxidative rancidity are mostly due to carbonyl type compounds. The shorter chain aldehydes and ketones isolated from rancid fats are due to oxidative fission and were associated with advanced stages of oxidation. Tests with reverted oils and later tests with rancid fats have shown that carbonyl type compounds are developed in low concentrations quite early in the oxidative process. The Kreis test is a very sensitive test for early indications of the oxidative process. According to Powick (21), the substance responsible for this reaction is epihydrinaldehyde. The Shibsted (25) test was an early attempt to use the formation of carbonyl compounds as evidence for rancidity in fats. Neither of these tests was too reliable. The Kreis test is so sensitive that it indicates a state of deterioration of the fat not necessarily consistent with the stability of the fat as measured by other means and should not be used in lieu of the peroxide value or other tests.

Numerous workers have attempted to make quantitative evaluations of rancidity through use of carbonyl tests. Few were successful however until the publication by Lappin and Clark (13) of a quantitative colorimetric method for carbonyl compounds based on the formation of a colored quinoidal ion of a 2,4-dinitrophenylhydrazone in a solution of base. Pool and Klose (20) applied chromatography and the color reaction with base to the determination of monocarbonyl compounds in rancid fats. Use was made of the Lappin and Clark reaction by Neumer and Dugan (18) for evaluation of volatile carbonyls from dry dog food under accelerated testing conditions and modified for use in the extracted fat. An extensive study and application was made by Henick *et al.* (7). Their method involved the formation of the 2,4-dinitrophenylhydrazones in benzene solution with trichloroacetic acid as catalyst so that the entire determination can be made in benzene. It is possible by their method to use the absorbances at 430 and 460 mμ for the simultaneous determination of saturated and allenic carbonyl content. An excellent application was also made by Chang and Kummerow (4). They bubbled a stream of nitrogen through 100 g. of fat at 83 cm.^{3}/min. under specified conditions, trapped the volatiles, and used the Lappin and Clark reaction to assign a carbonyl index to an oil. The carbonyl indices were found to agree with the degree of reversion and rancidity of the oils as determined by organoleptic evaluations. The carbonyl index of an oil after aging at 60° or 100°C. gave a good indication of the flavor stability of the oil.

The Thiobarbituric Acid (TBA) Test. The 2-thiobarbituric acid or TBA test was proposed for the evaluation of quality of dried milk and other dairy products. Sidwell *et al.* (26) studied the use of the TBA test as an agent for the measurement of fat oxidation in animal and vegetable fats.

The test is performed by treating the fat in benzene or chloroform solution with TBA reagent in aqueous acetic acid solution. After shaking, the aqueous layer is separated off and heated in a boiling water bath for 30 min. to develop the red color. After cooling, the absorbancy at 530 mμ is read against distilled water. The intensity of the red color developed is a measure of the degree of oxidation. It was noted that when a soybean oil and a cottonseed oil were compared, the peroxide development in the two fats was not greatly dissimilar, but the TBA colors both in the fat and in the volatiles from the fat were greater with soybean oil than with cottonseed oil at a given stage of oxidation. This was deemed significant in the light of the fact that soybean oil will develop undesirable flavors, such as those of reversion, at peroxide values as low as 2.5 m.e./kg. whereas cottonseed oil does not revert. From their studies with volatiles from butter oil it was suggested that TBA possibly reacts with both carbonyl and noncarbonyl compounds since it was found that the TBA values of the volatiles increased progressively with time of oxidation whereas the corresponding carbonyl values reached a constant level early in the oxidation period. It was also found that sensory evaluations by a panel showed a direct relationship between TBA values and off-tastes and odors in stored butterfat and that the TBA values were more directly related to storage temperatures than were peroxide values. Turner *et al.* (27) have used the TBA test to measure rancidity in frozen pork. They found that the TBA test provided a more reliable index of the age and quality of frozen pork than other chemical tests for fat rancidity. A significant positive correlation was obtained between taste test acceptability scores for wieners and pork patties and the TBA value of pork used. They noted that sucrose and some compounds in wood smoke will react with TBA to give a red color so that cured and smoked meats require corrections for the sugar content and for smoke constituents in the outer layers.

Another application was made by Asköe and Madsen (1), who found from 3,803 samples that the TBA reaction can be used to detect oily and fishy off-flavors

in bacon and that the results were in good agreement with tasting tests.

Caldwell and Grogg (3) have applied the TBA test recently to cereal and baked products. They used a chromatographic column packed with cellulose powder to remove the yellow colors resulting from reaction of TBA with sugars or other reacting materials and measured the red colors developed. They found a correlation in oat cereal and oatmeal cookies between rancid odors and optical density levels of 0.25 or greater for the solutions containing the TBA colors.

Other Methods. Other methods and techniques may be used to indicate the presence of oxidation products of fats and therefore may hold promise for future application. Specifically one may refer to ultraviolet and infrared spectrophotometry and polarography.

It has been known for a number of years that the primary products of autoxidation of fatty acids with multiple double bonds are hydroperoxides with the double bonds being conjugated. This conjugation is readily determined by absorption in the ultraviolet at 232 to 233 mμ.

The infrared spectrophotometer has been used to finger-print the various kinds of functional groups that are to be found in oxidizing fats. These include such groups as the hydroperoxyl, hydroxyl, carbonyl, and the geometric configuration of the double bonds. Henick (8) noted that the steam distillate from autoxidizing milk fat contained materials which gave spectral changes in the infrared region for carbonyl compounds before a trained flavor panel could detect them in the fat. The changes in flavor were correlated with specific absorption bands and were found to vary considerably before any appreciable change in the peroxide value.

The use of polarography to measure the products of oxidation of fats and fatty acids was introduced by Lewis and Quackenbush (15). Later studies (30) have revealed that the polarograph measures the hydroperoxides essentially quantitatively and can be used for the qualitative and quantitative determination of reducible oxygen groups in a molecule. It was shown by Ricciuti *et al.* (22) that the polarographic method compared favorably with the Wheeler iodide and with the stannous chloride methods for determining hydroperoxides in high purity systems. In samples of lower purity and in autoxidized methyl oleate the chemical methods gave higher results. They proposed that the polarographic method may give more reliable results in impure systems because it is more specific for hydroperoxides than the chemical procedures.

Evaluation of Methods. The methods for determining rancidity in and stability of fats and fatty foods offer a variety of choices, and it occasionally may seem to pose a problem as to which method to use. The choice may be dictated by equipment available, by the skill and training of personnel, or by the nature of the problem.

The evaluation of an antioxidant requires consideration of several factors. First of all, it is necessary to determine how a fat treated with antioxidant will behave under conditions accelerating the tendency to become rancid. This will involve A.O.M. tests and possibly the Oven Test. These tests are limiting in that they reveal how the fat alone behaves but do not reveal whether the antioxidant has other desirable properties such as "carry-through" to protect against rancidity in foods made with the treated fat. Antioxidants such as BHA and BHT (butylated hydroxytoluene) do not provide A.O.M. stability equivalent to that provided by others such as propyl gallate, but they have superior carry-through activity, especially when used in combination, while propyl gallate has little carry-through effect. It is therefore necessary to prepare foods and determine the stability of the foods. The Oven Test is most commonly used for this purpose since the other tests such as TBA and carbonyl have not been fully investigated for many applications. These tests appear to have promise for this purpose however.

The carbonyl test and the TBA test have features which make them attractive for evaluation of flavor stability of fats and fatty foods prior to the development of true rancidity. These are demonstrated by the TBA test for evaluation of fishy flavors in bacon, for evaluation of frozen pork to be used in sausages and bacon, and for flavor evaluation of fats which contain linolenic acid, such as soybean oil. Applications of the carbonyl test, such as the total carbonyl or the simultaneous determination of the saturated and allenic carbonyls, will be meaningful in some systems. The determination of volatile carbonyls for feedstuff stability and for correlation with reverted or other off-flavors in oils provides objectivity to tests which have heretofore been dependent on subjective tests. A significant development is the study by Henick *et al.* (9) with cottonseed oils. The sensory rancidity of oils nonrancid, just rancid, and slightly rancid was evaluated by a trained panel. The oils were also analyzed for peroxide, conjugated diene, saturated and unsaturated carbonyl, free fatty acid, and TBA reactables content. A statistical analysis showed that only peroxide, conjugated diene, and total carbonyl contributed to a correlation with rancidity. It may well be that some fats and foods made with these fats will show a correlation also with the TBA test.

Methods now available make possible the objectivity for determination of rancidity and of stability which was long sought. New methods and new applications of existing methods will extend this objectivity in the years of the immediate future.

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Performance Testing[1]

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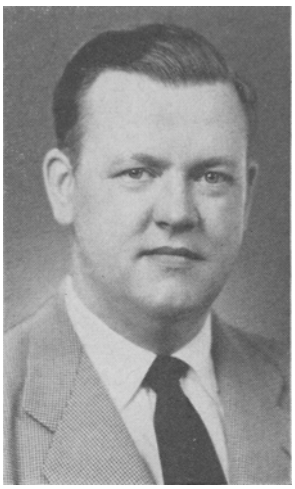
PERFORMANCE TESTING of fats and oils can be considered to be divided into three basic categories: handling, quality, and stability. Although this discussion will refer primarily to the field of dry mixes, it will be seen that the considerations involved could be generalized to include other phases of the shortening industry. Since the third category, stability, is discussed thoroughly in another paper in this series, only the first two categories will be discussed here.



E. C. Swanson

In the category of handling performance, plastic range is quite important in the light of present practical technology. With improved technology and development of present knowledge it is quite likely that plastic range may not be so important in the future as it is today. The plastic range governs the ease with which the shortening is blended with the other dry ingredients of the mix. Every organization has set up its own definite temperature limits within which the various shortenings may vary. The methods of measuring these temperature limits are discussed adequately in other papers in this series.

Votation is responsible for the physical condition of the shortening which, in turn, affects the ease with which the shortening can be incorporated into a mix. Physical condition, within reasonable limits, does not appear to be a significant factor after the shortening has been incorporated into the mix. Texture of the shortening becomes important chiefly when excessive graininess occurs. Graininess obviously exerts a marked influence on the uniformity of the mix.

Another generally used criterion of handling performance is the penetration test. Penetrometer readings however are quite meaningless unless they are correlated with dilatometer measurements. For example, two shortenings which exhibit identical penetration values may contain widely differing amounts of liquid fat. Such differences are important to product quality under given manufacturing conditions. Although processing conditions can be adjusted for various liquid oil levels, obviously such variations affect the routine of processing control.

Because of the tediousness of dilatometric measurements such tests are not readily adaptable to rapid routine control although the testing can be accelerated by setting up a variety of baths. In order to use penetration data in their most reliable form a "grease absorption" test is being developed for the determination of the liquid content of shortenings based on the capillary action of the liquid oil.

With reference to the second phase of performance testing, quality, there are analytical tests galore. Only those which are most readily adaptable to rapid routine control, particularly with reference to the field of dry mixes, will be discussed.

An important test of the quality of a shortening is its water-emulsifying ability. In this test water is dripped from a burette into a standardized amount of fat during mixing in standard mixing equipment. This gives a measure of the "emulsifiability" of the shortening. The greater the amount of water incorporated before the emulsion "breaks," the greater is the emulsifying power of the shortening.

Although shortenings generally contain relatively little water, the analysis of the moisture content serves as an analytical check on the quality of the product.

The determination of free fatty acid is an important test of the quality of shortening, irrespective of the reason for any occurrence of high free fatty acid

[1] Edited by C. W. Hoerr from tape recording of oral presentation by the author.

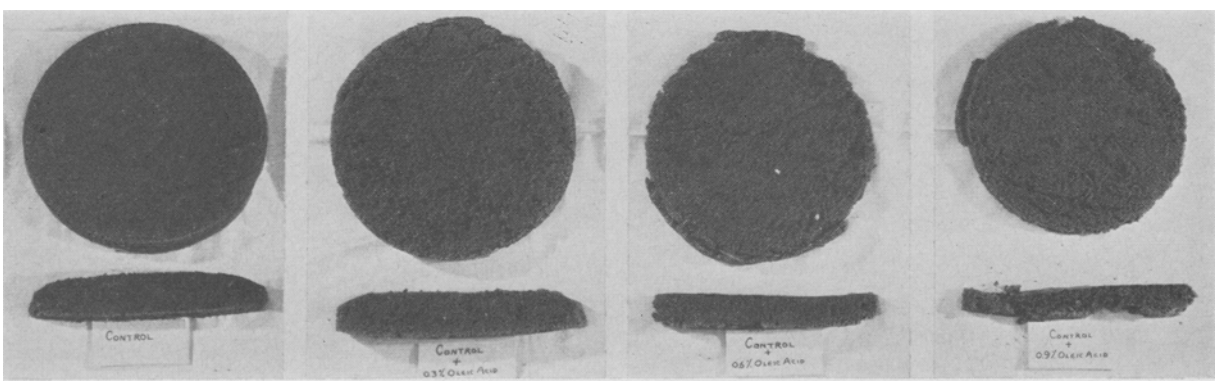


FIG. 1. The effect of 0, 0.3, 0.6, 0.9% oleic acid added as free fatty acid to chocolate cake mix (left to right).