CERTAIN FACTORS INFLUENCING THE OXIDATION OF BUTTER FAT

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INTRODUCTION

During storage, substances containing fat tend to undergo chemical changes resulting in objectionable flavors. The nomenclature describing these flavor defects varies to some extent with different industries. For example, in the general field of fats and oils the term "rancidity" designates these off-flavors irrespective of the chemical changes involved. In the dairy field, however, differentiation is made depending upon the specific chemical reaction. Here the term "rancidity" is used to denote only those flavors resulting from a hydrolysis of fatty glycerides with the ultimate production of free fatty acids, whereas the terms "oxidized" and "tallowy" are used to characterize those flavors resulting from the oxidation of the unsaturated fatty acids.

Economic losses resulting from chemical spoilage of butter during storage has stimulated considerable research on fat oxidation. Investigators have observed that metals, light, air, acidity of the cream, salt, storage temperature, and chemical composition of the fat are among those factors affecting chemical deterioration of the unsaturated fatty acids. The knowledge gained through these investigations has resulted in numerous changes in commercial practices. For example, replacement of copper equipment with stainless steel has resulted to protect the product against metal contamination. Also, particular attention has been given the protection of fats from excessive exposure to light and air. Accurate standardization of cream acidity, salt control, and the use of low storage temperatures are all practiced to improve the keeping quality of butter.

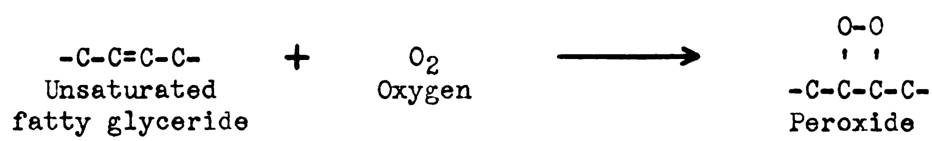
Even though many studies have been conducted dealing with fat oxidation, there are certain phases which are in need of further investigation. There is a necessity of further studies upon the development of a simple, reliable, acceleration method for determining the stability of fats toward oxidation. Also, within recent years, certain changes in manufacturing methods have occurred due to the development of new equipment as well as to an effort on the part of plant operators to hasten the processing procedure. One such change has been in the direction of high temperature heat treatment of the cream. In some cases, these transitions of processing procedure have come about without much consideration of their possible effect upon the keeping quality of the product during storage. Because of the incompleteness of available information regarding the influence of certain of these changing trends in processing procedures upon the chemical deterioration of the fat, studies in this connection seemed especially appropriate.

REVIEW OF LITERATURE

1. Mechanism of Fat Oxidation

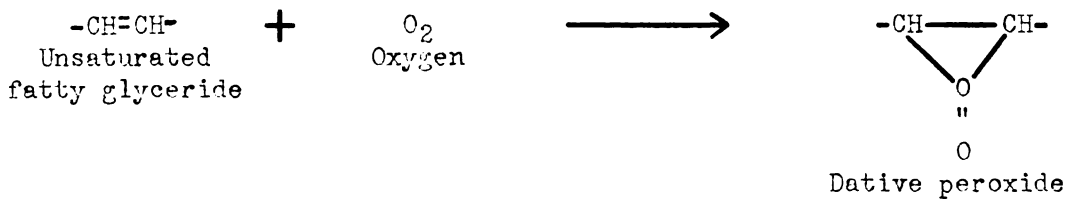
Many theories have been presented concerning the chemical changes involving the oxidation of butter fat. Although there are differences of opinion as to the first products of oxidation, all workers are in agreement that the double bonds of the unsaturated fatty acids are the points of oxidation.

Peroxides: Holm (50), Powick (91), Smith and Wood (112), and Tschirch (122) believe the first reaction is a formation of fatty acid peroxides by direct combination of the active oxygen with the double bonds of the unsaturated fatty glycerides. This reaction is presented by Holm (50) as follows:



These workers assume that after their formation, the peroxides then rearrange into moloxides and ozonides with subsequent formation of aldehydes, acids, and other substances.

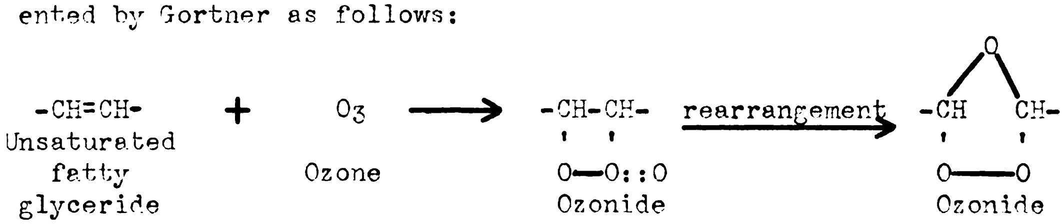
Staudinger (113) and Miles (78) are of the opinion that the first step in the oxidation process is a formation of a highly reactive substance referred to as a moloxide or dative peroxide. Miles (78) suggests that, in addition, there is a connection between the energy content of the molecular valence electrons and the oxidation reaction. The first step in the oxidation is thought to be a combination of the valence electrons of the fat molecule to the active oxygen molecule as follows:



These unstable dative peroxides are thought immediately to rearrange to form more stable peroxides or other compounds.

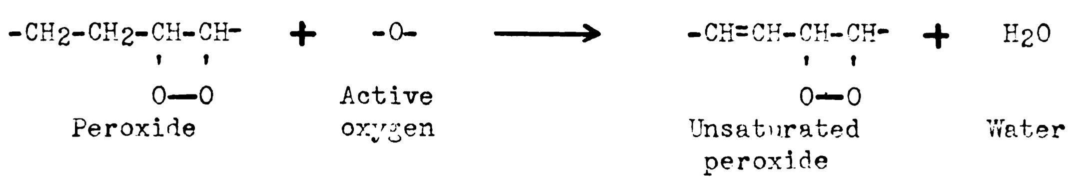
Oxides: Browne (11) suggests a combination of oxygen with the double bond forming a fatty oxide with a simultaneous liberation of an atom of active oxygen. The active oxygen is then thought to act upon the glyceride causing the formation of free fatty acids, aldehydes, carbon dioxide, water, and other by products.

Ozonides: Gortner (41) suggests the possible formation of ozonides by the addition of ozone at the double bonds. This reaction is presented by Gortner as follows:

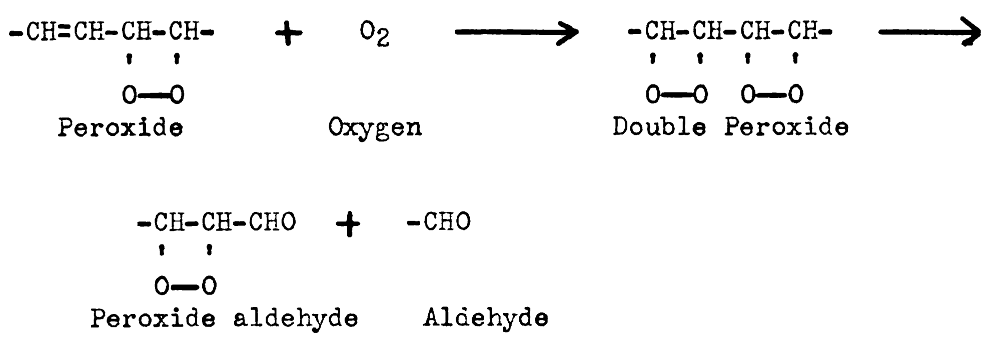


These ozonides, since they are highly reactive, will readily decompose into hydrogen peroxide, acids, and half aldehyde acids. Other workers (106) suggest the breaking of peroxides into oxides and a molecule of active oxygen, the active oxygen then combining with another peroxide forming an ozonide which rearranges into acids and aldehydes.

Water formation: In a more recent study, Mattill (75) found water to be an important oxidation product, and presents the following hypothetical equations:



The new double bonds formed by the above reaction are believed to undergo further oxidative changes, thus:



The mechanism of fat oxidation has been summarized by Mattill (75) who states: "No all-inclusive theory of autoxidation can yet be formulated. Once the obscure induction period is past, events seem to follow thick and fast in greatest confusion, but like the devastating speed and apparent disorder of a blitzkrieg, everything nevertheless goes according to plan. The plan is flexible and is in part dictated by the events themselves. Further careful study is needed to indicate what the events are and why they take place."

II. Accelerated Methods for Determining the Stability of Fats.

Even though many methods have been devised and used to determine the relative stability of oils and fats toward oxidation, one is yet to be found that is entirely reliable. However, all fat stability tests that have been used to any great extent are basically the same. The oxidation is accelerated under carefully controlled conditions, thus reducing the induction period from several months or weeks to a few days or even hours. The catalysis of the oxidation process is usually achieved by heat, utilizing either a hot liquid bath or a hot air oven, although in some instances some catalyst other than heat may be used; i.e., light or metals.

Fat stability apparatus method: One method used in commercial laboratories for determining the relative stability of different fats was devised in Swift's Laboratories by King et al (62). In this method the samples of oil or fat to be tested are placed in to a test tube which is held in a constant temperature oil bath. Washed air is passed through the liquid fat at a constant rate in order to maintain an air-saturated condition in the oil. Samples are tested at definite time intervals to determine the end of the induction period. This method has been used successfully by Bull (17, 18, 19), Musher (83), and Stebnetz and Sommer (114, 115), and Freyer (40) reports on the use of this apparatus in eleven commercial laboratories.

The precision of the fat stability apparatus to detect the relative stability of different fats toward oxidation has been a matter of considerable question. The result of a three year committee study presented by Freyer (40) indicates that the testing of cooperative samples in 11 different laboratories by this method gave inconsistent results. Even by allowing 17 per cent average tolerance of induction period time, about one-fourth of the laboratories reported results outside of this liberal range. Four of the laboratories participating presented consistently uniform data, but these laboratories were among the first to adopt the use of the method and, therefore, had considerable more experience. Two of the laboratories reported inconsistent results at first, but after using the method for about a year, reported results that were in agreement with the other laboratories. However, two laboratories failed to show acceptable results even after two years experience. Freyer (40) suggests that the lack of agreement between the different laboratories was apparently due to insufficient experience with the method and feels that even though the method is not perfect and has many limitations it still is the best accelerated procedure known for determining the relative stability of fats and oils toward oxidation.

Hot air oven method: Barnicoat and Palmer (4), Bird (8), Dahle and Nelson (33), and Ritter and Nussbaumer (96, 97, 98, 99, 103, 104) have made use of hot air oven methods to determine the susceptibility of fat toward oxidation. Ritter and Nussbaumer (96, 97, 98, 99, 103, 104) held fat for eight hours in an oven at 104° C. in 10 cm. petra dishes. At the end of the eight hour incubation period the peroxide value was measured to determine the relative stability of the fats.

In their work with the oxidation of butter fat Barnicoat and Palmer (4) decanted the butter oil into straight-sided crystallizing dishes. These dishes were then heated in an air-stirred water-jacketed oven thermostatically controlled at 80° C. for six hours. The aldehyde value was then used to determine the degree of oxidation of the oil. Because of the simplicity of the method, Bird (8) made use of an oven method in his studies of the stability of vitamin A-containing fish oils. In this work he exposed small, fairly uniform samples of fish oil to the atmosphere at temperatures above normal. Samples were taken at intervals from the series and vitamin A determinations made to determine the stability of the fish oil.

Dahle and Nelson (33) used a 60° C. hot air oven in their oxidation studies of butter fat. Equal volumes of fat were placed into test tubes which were held in the incubator in a basket. Although the temperature of different tubes did not vary as much as one degree, the induction period of different tubes varied as much as four days. In this connection, Lea (70) points out that temperature control is essential in all rapid oxidation methods where elevated temperatures are used and further states that 1^{o} C. variation in temperature may introduce as much as a 10 per cent error.

III. Method of Measuring Fat Oxidation.

Considerable effort has been expended to establish a method for quantitatively determining the oxidation of fats. In general, it may be said that a method for detecting fat oxidation should have the following characteristics: (a) simplicity, (b) reliability, (c) specificity in reaction, and (d) sensitivity to such a degree as to detect minute oxidative changes in the fat, if possible, before the end of the induction period.

Numerous methods have been used to detect the oxidation of fats and oils but many have failed to conform, even within reasonable limits, to the qualifications listed above. Among the less satisfactory methods may be included tests for unsaturation, the acid value, and the Kreis test.

Investigations of Brown and co-workers (15), Nelson and Dahle (84), Overman and associates (87), and Swanson and Sommer (118) indicates that changes in iodine absorption of butter fat expressed as iodine number do not correlate with initial oxidative changes responsible for flavor changes. However, Swanson and Sommer (118) found that a lowering of the iodine number of the phospholipid fraction accompanies the development of oxidized flavor in milk. Briggs (10) found close relationship between the iodine number decrease and oxygen absorption of butter fat. Henderson and Roadhouse (49) show a relation between iodine number and induction period of fat from cows on normal and submaintenance rations. Stebnitz and Sommer (115) show that, in general, the greater the unsaturation, as measured by iodine number or thiocyanogen-iodine number, the less stable is the fat toward oxidation. Wiley and Gill (126) point out that the thiocyanogen number, as determined by the usual method, is more inaccurate than the iodine number to determine the degree of unsaturation.

Attempts have been made by numerous investigators to use the acid value as a means of detecting the end of the induction period (6, 10, 125). Generally, these workers have concluded that this method is not sensitive in detecting the end of the induction period.

The best known and probably the widest used qualitative test for detecting oxidative changes in fats and oils is the Kreis test. The Kreis test has been investigated by Holm and Greenbank (51), Kerr (60), Kerr and Sober (61), Mattill (75), and Powick (91) with the conclusion that this test cannot be used as an accurate quantitative method. The accuracy of the test for detecting oxidation of fats, unless the color is examined spectroscopically, is questioned by Taffel and Revis (120) and Mattill (75) because the reaction is not specific for epihydrin aldehyde.

Other methods have been tried but their merits have not been entirely established. Holm (50) used a dye reduction method in his studies of keeping quality of butter. Bickford and Markley (7) and Greenbank and Holm (45) developed methods for determining the rate of oxidation of vegetable oil by making use of a fading methylene blue chloride-fat system. Another method utilized by Clark and Rugg (21), in working with soy bean oil, made use of a special drop-spreading pressure method to detect oxidation. It was found that the readings increased continually with the drop-spreading pressure method, whereas the peroxide value increased to a maximum and then decreased with increasing oxidation.

Thus far only three methods have been found to possess at least the major desirable characteristics of a useful method for measuring fat oxidation; namely, (a) the peroxide value, (b) the fat-aldehyde value, and (c) oxygen absorption.

Peroxide value: The peroxide method is based upon the measurement of peroxides by titration of the iodine freed by the fat in a chloroform-acid solution. Taffel and Revis (120) utilized a method for measuring the peroxides in fat based upon the reaction presented by Lea (67) and perfected by Wheeler (124) into the present commonly used peroxide method. Greenbank and Holm (47) used a modification of this method. Much use has been made of this method to detect oxidative changes of fats and oils (3, 33, 37, 52, 53, 63, 68, 69, 70, 71, 91, 97, 99, 101, 102, 111, 115, 117, 124, 125).

Several workers have found this method to be a desirable means of determining the degree of oxidation of fats (8, 10, 23, 46, 62, 72, 96, 98, 100, 116, 120). The early work of Taffel and Revis (120) and Wheeler (124) reveals that reproducible results can be obtained with this method. Wheeler (124) found the development of peroxides to proceed any appreciable change in iodine number of cottonseed or corn oil during oxidation.

Briggs (10) found that during the induction period of butter fat, changes occurred which resulted in the formation of small amounts of peroxides. It was concluded that the estimation of these peroxides gave the best indication of the extent of oxidation. Stebnitz and Sommer (116) observed that the production of peroxides ordinarily precedes the appearance of a tallowy flavor in the oxidation of butter fat; however, the tallowy flavor was not always apparent at the same peroxide value. These investigators believe the peroxide value to be an excellent criterion of the degree of oxidation, especially to detect the end of the induction period.

Ritter and Nussbaumer (100) concluded that the best method of determining the keeping quality of butter fat is to determine the time required for the peroxide content to reach a value from five to ten. Lowen et al (72), working with fish oil, observed a close correlation between increases in peroxide value and the development of off-odors. Bird (8) found peroxides to develop in fish liver oil at the same time the vitamin A is destroyed and concluded from this that either a peroxide or vitamin A determination was a satisfactory index of oxidation.

Others have found limitations of the peroxide method (4, 10, 21, 32, 72). Dahle and Josephson (32) found no relationship between the peroxide value of stored butter and the score of the butter. Barnicoat and Palmer (4) concluded that the peroxide test was of little value when applied to butter. Studies of Briggs (10) indicates that the development of peroxides do not correspond well with the absorption of oxygen. Stebnitz and Sommer (116), Clark and Rugg (21), and Wheeler (124) point out that the peroxide value goes to a maximum and then, upon further oxidation, decreases.

Fat-aldehyde method: Another determination of fat stability that has received considerable attention recently is the fat-aldehyde test, devised by Schibstead (108). This method is based upon the color intensity produced by rosaniline hydrochloride combining with a fatty glyceride aldehyde through the SO_2 linkage. Schibstead (108) found considerable correlation between the oxygen absorbed by butter fat oxidized at 100° C. and the fat aldehyde value. These findings are presented in the following table:

Oxidation of butter fat at 100° C.

Duration of heating (hours)	Oxygen absorbed per gram of butter fat	Fat aldehyde value
0	0	1.2
5	14	740.0
10	20	890.0
15	30	965.0
20	38	257.0
25	-	258.0
30	40	106.0
35	-	38.0

Extreme oxidation in this case resulted in decreased aldehyde values. Lea (70) states that from the theoretical standpoint the fat aldehyde test should provide the best measurement of oxidation.

This method has been used by numerous workers in fat oxidation studies (4, 70, 108, 125). Wiley (125) found fair correlation between the aldehyde values and the peroxide values of stored butter but decided that the aldehyde value was the most sensitive in detecting oxidative changes. In his work with the oxidation of stored butter, this worker observed that, on the average, low oxidation values were secured from high score butter and high oxidation values for low score butter, but that individual trials showed wide variations in fat-aldehyde values. Barnicoat and Palmer (4) determined the fat-aldehyde test to be only an empirical test, accurate to within about five per cent.

Oxygen absorption methods: Many investigators have used oxygen absorption methods of determining the stability of fats (10,37, 39, 44, 46, 52, 57, 74, 77, 79, 87, 108). Overman et al (87) in their studies of storage butter found a definite relationship between the quality of butter and the rate of oxygen absorption by the butter fat. Greenbank and Holm (44) devised a method, which was later improved by Mattill (74) and again by French, Olcott, and Mattill (39) of automatically measuring the beginning of oxygen uptake. The latter workers (39) considered samples of fat to have equal stability if a difference of not over one hour induction period time existed between the two samples.

Evans (37), Johnston and Frey (57), Monagahn and Schmitt (79), and Meyer, Kass, and Burr (77) have used the Barcroft-Warburg apparatus to study the oxygen absorption of fats and oils. This method is essentially the method devised by Greenbank and Holm (44) with the exception that a constant temperature water bath is used. The samples are mechanically agitated and the volume of oxygen absorbed is measured accurately with carefully graduated manometers at definite time intervals. Recently, Johnston and Frey (57) made a special study of this method of measuring the induction period of fats, especially at temperatures between 50° C. and 110° C. From their extensive investigations, these men concluded that it was possible to measure, with considerable precision, the induction period of fats at any temperature within the range studied. A comparison of the induction period of sesame and cottonseed oils indicated that results obtained would agree within one to three per cent. Vibrans (123) believes that this method is useful in research work but will not be generally used as a routine method for determining the relative stability of fats and oils.

IV. Influence of Heat Treatment Upon Oxidation.

Butter Oil: Extensive investigations have been carried out to determine the influence of a high temperature heat treatment upon the oxidation of numerous fats and oils (43, 63, 96, 103, 109).

Greenbank and Holm (43) found that heating butter fat to 100° C. reduced its keeping quality; the reduction in keeping quality being proportional to the length of time the fat was exposed to the high temperature. Schulz and Storck (109) heated butter oil to 90° C. and found that the oil thus treated kept well in storage for two years. Kochling and Taufel (63) and Ritter and Nussbaumer (96, 98) observed that the heating of butter fat from 150° to 250° C. reduced its induction period. Ritter and Nussbaumer (96, 98) report a decrease in initial peroxide value of stored fat when heated to 195° to 250° C., whereas, storage of the heated fat caused marked increases in peroxide value. They suggest that high temperatures destroy the antioxidant properties of the fat, thereby reducing the length of the induction period. Ritter and Nussbaumer (103) revealed that butter oil filtered at 100° C. oxidized less readily than the same oil filtered at 42° C.

Butter: The influence of heating butter upon its keeping quality in storage has been investigated by Patil and Hammer (88) and Ritter and Nussbaumer (101).

Patil and Hammer (88) worked with ghee, a product made by heating unsalted butter over a low fire until most of the water is evaporated from the butter, followed by filtration to remove the curd. These workers observed that ghee made using temperatures of 130° to 140° C. kept much better in storage than other lots heated at 110° to 120° C.

Ritter and Nussbaumer (101) found that at 104° C., butter oil oxidized to a greater extent in 8 hours than did butter in 24 hours. This difference was not due to water since the results were not materially affected by the addition of water. Butter fat from properly cooked butter showed slower increases of peroxide number than butter fat from non-heat treated butter. The authors believe this difference to be associated with the phospholipid content of the butter serum which was shown by Ritter (94) to be concentrated in the serum of the butter.

Milk and Cream: At present, pasteurization temperatures from 62.9° C. for 30 minutes up to as high as 148.8° C. flash are used in commercial dairy plants to pasteurize cream for buttermaking. Numerous workers have discussed the influence of heat upon milk and cream (12, 13, 16, 30, 38, 42, 48, 58, 81, 90, 93, 95, 105, 125, 128, 129). Hunziker (55) gives a complete discussion of the different types of pasteurizers used for commercial pasteurization of cream.

Mortensen (81) and Guthrie and co-workers (48) observed that cream pasteurized at 73.9° and 76.6° C. resulted in butter of superior keeping quality to lots of the same cream pasteurized at 62.9° C. Wiley (125), in an extensive study of the influence of pasteurization temperature upon the oxidation of stored butter, observed that heat treatment does have a definite influence upon the rate of oxidation of butter in storage. Data taken from Wiley's work (125) are presented below:

Influence of pasteurization temperature
of cream on keeping quality of butter.

Pasteurizing temp. ° F.*	Fat aldehyde value	Grade before storage	Grade after storage	Acid value before storage	Acid value after stor.
			Unsalted		
150	0.2	93.5	89.0	0.31	0.73
175	0.03	93.5	91.0	0.20	0.20
200	0.0	93.5	91.5	0.20	0.20
			Salted		
150	0.8	93.0	90.5	0.32	0.42
175	0.13	93.5	91.5	0.22	0.22
200	0.08	93.0	91.5	0.22	0.22

* All heat treatments were flash.

These results show that the oxidation of the butter in storage, as measured organoleptically and by the fat-aldehyde value, varied inversely with the temperature of pasteurization. The marked increase in acid value of butter made from cream pasteurized at 65.5° C. led Wiley (125) to suggest that the enzyme lipase may be responsible for the greater susceptibility of the fat toward oxidation when the lower pasteurizing temperature was used. Wilson and associates (128) later substantiated the findings of Wiley (125) in their studies with the Reid flash, Cooney flash, and vat pasteurizers. Platon and Olsson (90) observed that flash pasteurization at 90° to 96° C. gave butter with a higher keeping quality than pasteurizing at 80° to 83° C.

In a further study of the influence of pasteurization upon the oxidation of butter in storage, Wiley (125) compared the effect of pasteurizing sweet, ripened and acidified creams at different temperatures. The results of these experiments show that the higher temperature heat treatment reduced the rate of oxidation of the butter in storage.

Brown (12, 13), Fabricius and Bird (38), and Wilster (129) have made studies of a vacuum processed cream using a machine known as the "Vacreator." Generally observations have been that butter made from cream pasteurized by this process, in which the cream reaches a temperature from 88° to 96° C., kept better in storage than did butter from the same cream vat pasteurized at a lower temperature (38). Wilster (129) reports that at the end of one month storage, the butter from vacreated cream had depreciated 14.8 per cent less in score than butter from the same cream vat pasteurized at lower temperatures. Furthermore, after four months storage this same butter from vat pasteurized cream had depreciated 37.0 per cent more in score than that from cream processed at higher temperatures in the vacreator. A summary of the data presented by Fabricius and Bird (38) shows a significant difference in flavor score of fresh butter from vacuum pasteurized cream over the vat pasteurized cream. This same difference existed after storage at -17.70° C. for six months.

Roberts, Coulter, and Combs (105), using a laboratory high temperature pasteurization apparatus, pasteurized cream at 126.6° C. flash and in a vat at 71.1° C. for 30 minutes, and observed no appreciable difference in the keeping quality of the butter.

Several workers have noted that high temperature pasteurization of milk prevents the formation of oxidized flavor in milk (16, 30, 42, 58, 95). To enable a more complete understanding of the increased stability of milk toward oxidation Gould and Sommer (42) and Josephson and Doan (58) have carried on extensive investigations of sulphide production. In their extensive study of the effect of heat upon the cooked flavor of milk, Gould and Sommer (42) revealed that sulphide liberation takes place in cream held for three minutes at 66° to 68° C. It was found that as the temperature of milk was increased above 80° C. the tendency toward development of oxidized flavor was less pronounced while heating to temperatures above 84° C. almost entirely prevented the development of oxidized flavor. Cooked flavor did occur at these higher temperatures, the intensity of which depended upon the temperature of heat treatment.

V. Influence of Certain Metals With and Without Heat upon the Oxidation of Butter Fat.

That metal ions in milk, cream, or butter will catalyze chemical deterioration of the products with the production of fishy or oxidized flavors has been long realized (56, 107). The exact reaction involved in this acceleration of oxidation is unknown, although one general suggestion given by Hunziker (55) is that metals may act as oxygen-carriers forming metallic oxides which come into contact with the unsaturated fat giving up oxygen.

Metals in milk and cream: Davies (34, 35), Dahlberg and Carpenter (29), Krauss and Washburn (65), and Rice and Miscall (92) have investigated the metal content of various dairy products. Davies (35) found that normal milk contained about 0.5 ppm. of copper whereas pasteurized milk (34) varied from 0.4 to 4.0 ppm. copper, with most of the samples examined ranging from 0.6 to 1.3 ppm. of copper. The iron content of milk varied from 1.7 to 3.8 ppm. Dahlberg and Carpenter (29) found normal milk to contain 0.131 ppm. copper whereas the iron content was 0.379 ppm. Krauss and Washburn (65) studied the seasonal variation of copper and iron finding from 0.14 to 0.17 ppm. of copper and from 0.34

to 0.53 ppm. of iron. The variations between the figures presented by different workers may be partly due to different analytical methods as well as to different sources of the products examined.

Rice and Miscall (92), present data to show that the metals do not go with the fat itself, although Davies (35) noted that with special methods of separation there were higher concentrations of metals in the cream. Davies (35) attributes this to the adsorption of metals by the complex proteinates at the fat-globule surface.

Effect of heat and metals on milk and cream: Barnicoat and Palmer (4), Brown, Thurston, and Dustman (14), Gould and Sommer (42), Hunziker (55), and McFarland and Burgwald (76) have studied the influence of heat treatment of milk and cream upon metallic-induced oxidation.

Brown and associates (14) and Gould and Sommer (42) have found that copper added after pasteurization is more effective in producing an oxidized flavor in milk than adding the copper before pasteurization. Gould and Sommer (42) found that when milk containing 1.4 ppm. copper was heated it was necessary to use temperatures of at least 84° to 86° C. momentarily in order to have a marked retardation of oxidized flavor development, whereas normal milk showed the same influence at 76° to 78° C. Brown et al (14) are of the opinion that the copper combines with the proteins and in this way is inactivated as a catalyst. Gould and Sommer (42) suggest either that the copper may combine with the protein that liberates the heat-labile sulfur preventing the liberation of the sulphides at the temperature of sulphide liberation in normal milk, or that possibly the copper may combine with liberated sulphides preventing the cooked flavor from being noticeable until greater amounts of sulphides are liberated. Working with frozen cream, McFarland and Burgwald (76) found they could prevent the development of oxidized flavor during storage of frozen cream, even in the presence of copper, by heating to 77.7° C. for 5 minutes.

Metal content of butter: Several workers (35, 46, 54, 59, 127) have studied the metal content of butter.

Davies (35) found that butter made in the laboratory contained about 0.5 ppm. copper and 1.0 ppm. iron. It was found by Davies (35) that the amount of metals entering butter from cream is dependent upon the curd-nitrogen content of the butter. Keilling (59) determined the copper content of commercial pasteurized butter and found it to range from 0.76 to 7.0 ppm. The copper content of first grade New Zealand butter was found by Williams (127) to vary from 0.2 to 0.25 ppm. with the iron content ranging from 0.5 to 1.0 ppm. Horrall and Epple (54) found commerical butter to range from 0.14 to 4.0 ppm. copper, depending upon exposed copper equipment.

Effect of heat and metals on butter and butter oil: Barnicoat and Palmer (4) and Ritter and Nussbaumer (98) have investigated the influence of metals upon the oxidation of butter. Barnicoat and Palmer (4) observed that the addition of metals (0.05 to 0.25 ppm. Cu) to butter granules before working caused no significant increase in oxidation of the butter. These workers are of the opinion that it is the oxidation of the lecithoprotein membrane surrounding the fat globule that causes the accelerated oxidation in the presence of metals. The addition of copper to oxidized butter was found by Ritter and Nussbaumer (98) to inhibit further peroxide formation especially in the presence of heat and light.

Considerable attention has been given the influence of metals upon the oxidation of butter oil. Barnicoat and Palmer (4), Ritter and Nussbaumer (97), and Briggs (9) have made studies of the influence of metals upon oxidation. In other studies Battie et al (5) and Smedley-MacLean and associates (110) found catalytic influences when investigating the effect of cupric salts upon the oxidation of certain fatty acids. Battie and co-workers (5) observed that the action of cupric salts are only effective when the substance to be oxidized is an acid. In an alkaline or neutral solution of succinic acid little oxidation takes place.

Briggs (9) found that metallic ions hastened oxidation of butter fat at 100° C. In their extensive study of butter, Ritter and Nussbaumer (97) observed that butter containing 1 ppm. copper showed greater peroxide formation than the same butter with higher concentrations of copper when heated for eight hours at 104° C. Presumably, the copper not only hastens the formation of peroxides, but also accelerates the rearrangement of peroxides into acids, aldehydes and other substances. The addition of more copper to the oxidized fat further decreased the peroxide value. In addition, these workers (97) observed that copper accelerated the oxidation of butter fat stored in the dark whereas, the same copper-contaminated fat in the light, showed slower peroxide formation than the control fat. Later (97), it was observed that the peroxide content of the copper contaminated fat decreased in spite of light, whereas the peroxide value of the control fat continued to increase.

VI. Antioxidants.

Different fats vary in their stability toward oxidation, the variation being partly due to natural impurities present in the fat which tend to inhibit oxidation. These catalytic inhibitors are generally referred to as antioxidants.

Theories of antioxidant action: Maureu and Dufraisse (82) and Christiansen (20) were instrumental in developing explanations of the action of antioxidants. The theory of Maureu and Dufraisse (82) involves the formation of peroxides both from the peroxides of the oxidized fat and from the antioxidant. During the same reaction the oxidized fat is converted into a lower oxide. The antioxidant peroxide and the fat oxide then reacts to give the original unsaturated fat, the antioxidant, and oxygen. According to this theory, the antioxidant should act as a true catalyst, never being used up in the reaction.

Christiansen (20) presents a theory in which the antioxidant is used up or destroyed during the reaction. French, Olcott, and Mattill (39) also indicate that the antioxidant is destroyed during the induction period or at least it is not effective during the period of rapid oxidation. In line with this theory, Christiansen (20) believes there is a progressive chain reaction with the oxidizable fat reacting with the active oxygen forming a peroxide. Energy is liberated which is then passed on causing progressive reaction until thousands of molecules may become involved. In case an antioxidant is present, the energy liberated from the formation of the peroxide is absorbed by the antioxidant. Likely, the antioxidant is itself oxidized due to its increased energy level. Since the oxidation reaction of the antioxidant does not liberate energy, the energy chain reaction ceases. This explanation would account for the extended induction period in the case of fats containing antioxidants. Christiansen's (20) theory indicates that an antioxidant must be a substance easily oxidized.

Evans (37) suggests that antioxidants may combine with some of the oxidation catalysts present such as metals, thus preventing their accelerating effect upon oxidation. French et al (39) believe that all antioxidants act in the same manner since none are active after the end of the induction period.

Acids: Numerous acids have been found by Bird (8), Dagneaux (28), Greenbank and Holm (47), and Lea (69) to show inhibitive effects toward the oxidation of fats and oils.

Extensive investigations by Bird (8) revealed that hydrochloric acid as well as dry hydrogen chloride gas greatly reduced the induction period of halibut liver oil. Phosphoric acid was found to reduce the induction period slightly whereas acetic acid had no effect. Other acids studies were maleic, citric, tartaric, furmaroic, succinic, sulfuric, and ascorbic. All these showed considerable inhibitive effect upon the oxidation of halibut liver oils, while benzoic acid showed little influence. Greenbank and Holm (47) have shown that some unsaturated polybasic aliphatic acids, especially maleic, are excellent antioxidants. Dagneaux (28) found maleic acid to be an effective antioxidant in powdered milk. In studies with lard, Lea (69) found lactic, glycollic, and maleic acids to be moderate antioxidants whereas the polybasic hydroxy-acids, such as tartaric and citric, were powerful antioxidants. Malonic and phosphoric acids were also found to be effective.

Salts: One would expect the action of salts to be closely allied to the catalytic effect of the metal ion of the salt. Bird (8) and Lea (69) have studied the influence of salts upon the oxidation of fats.

The work of Bird (8) with fish viscera oil indicates salts cannot be used as antioxidants, since all salts, except sodium sulfate, showed destructive effects on the induction period. The degree of reduction of the induction period depends upon the metal ion of the salt, with cobalt, copper, calcium, magnesium, tin, and iron being the most destructive. Lea (69) found that sodium citrate and sodium malonate acted as powerful antioxidants in lard.

Bases: Alkalies are generally believed to cause a decreased stability of fats. Bird (8) found that an aqueous solution of alkalies materially reduced the induction period of halibut liver oil, but that dry alkalies had no destructive effect. This author believes that dry alkalies do not destroy the natural antioxidants present in oil, thus indicating that the destruction of the antioxidant substances is associated with the aqueous phase.

Proteins and amino acids: Briggs (9) and Lea (69) have noted that proteins exert antioxygenic influences upon the oxidation of fats. Lea (69) has demonstrated that some of the aliphatic amino-acids (glycine, asparatic and glutamic acids, and asparagine) are powerful antioxidants. Corbett and Tracy (25) observed that when condensed skim milk was added to cream to make a milk of four per cent fat, the tendency to develop an oxidized flavor was reduced. These workers suggested that some antioxidant was liberated from the milk protein during the condensing process which acted to prevent oxidation. Later Corbett and Tracy (26) found tyrosine and the more soluble esters of tyrosine to be effective in preventing oxidized flavor in milk in concentrations of 0.02 to 0.04 per cent. Normal amyl ester of leucine was also an effective antioxidant whereas glutamic acid gave no protection. Both of these compounds produced objectionable off flavors.

Phospholipids: Dagneaux (28), Dahle and Nelson (33), and Ritter and Nussbaumer (99, 101, 103) have observed that certain phospholipids exert a retarding influence upon the oxidation of fats.

Lecithin was originally believed by Evans (37) to have an antioxygenic effect. Later, it was found by Ritter and Nussbaumer (101) that lecithin had little or no retarding effect upon oxidation; but that cephalin, an impurity in most commercial lecithin, showed strong antioxidizing action. Ritter and Nussbaumer (99) observed that butter phosphatides from buttermilk powder retarded oxidation of butter oil. Further investigations by these workers (103) revealed that filtering butter at 100° C. gave butter oil with higher keeping qualities than filtering at 42° C. This difference was thought to be due to the regulation of the amount of phospholipids in the butter oil carried by the water content of the filtered butter oil.

Thurston et al (121) believe lecithin to be responsible for the oxidized flavor of milk rather than the butter fat, and Swanson and Sommer (119) found the phospholipids to be oxidized when the milk possessed an oxidized flavor.

In an attempt to determine the active fraction of oat and soy bean flour Dahle and Nelson (33) found that the phospholipid fraction showed considerable antioxygenic properties.

Carotene: Considerable discussion has been given the influence of carotene and vitamin A upon the oxidation of fats and oils. Bird (8), Dagneaux (28), Monagahn and Schmitt (79), and Olcovich and Mattill (86) have made studies of the influence of carotene and vitamin A upon the oxidation of fats and oils.

Monagahn and Schmitt (79) found that carotene inhibited the oxygen uptake of linoleic acid during the induction period, but after oxidation began, the oxygen uptake was accelerated. Dagneaux (28) states that carotene acts as a slight inhibitor toward oxidation. Contrary to these findings, Olcovich and Mattill (86) found carotene to act as a pro-oxidant and Bird (8) observed that vitamin A acts as an oxidation catalyst in fish oils, the accelerating effect depending upon the concentration of the vitamin A.

Enzymes: Recently, enzymes have been used as a means of preventing oxidation of milk. Anderson (1), Corbett and Tracy (26), Doan and Miller (36), and Nelson and Dahle (85) report the use of trypsin to prevent the development of oxidized flavor in milk. Doan and Miller (36) suggest that the enzyme acts upon the milk proteins liberating reducing substances. Contrary to this opinion, Nelson and Dahle (85) believe the protective action comes from the action of the enzyme upon the fatty materials rather than upon the milk proteins.

Avenex: More emphasis has been placed upon the use of Avenex, an oat flour product, than upon any other antioxidant used to retard the oxidation of fats and oils. Investigations by Peters and Musher (89) indicate that the oxidation of lard, cottonseed oil, castor oil, corn oil, and soybean oil can be retarded by Avenex. Conn and Asnis (23) show that it is possible to inhibit oxidation of the fat on potato chips by dusting them with five per cent of their weight with Avenex. Lowen and co-workers (72) observed oxidation of fish fats to be retarded by oat flour, but not to as great an extent as in the case of lard or vegetable oils. During their work with frozen cream, Maack and Tracy (73) observed that an oat flour content of 1.5 per cent protected frozen cream from developing an oxidized flavor.

Corbett and Tracy (24, 26), Corbett et al (27), and Dahle and Josephson (32) have studied the application of Avenex to cream to prevent development of oxidation in butter. These workers found that oat flour preparations retarded the onset of oxidation, but did not prevent it. Corbett, Tracy, and Hansen (27) observed that adding Avenex concentrate to sweet cream at the rate of 0.15 to 0.3 per cent of the fat retarded the development of oxidized flavor, but did not prevent it entirely. They noted further, that the addition of Avenex concentrate to second grade cream was of more advantage in improving the score than adding it to first grade cream.

Combs, Coulter, and Whitman (22), Dahle and Josephson (31), and Koenig (64) have compared the use of avenized parchment with standard parchment wrappers for protection of butter prints from oxidation. These workers found that the avenized parchment exhibit protective action against oxidative changes especially at the surface of the butter.

Dahle and Nelson (33), Musher (83), Ritter and Nussbaumer (99), and Schulz and Storck (109) found that oat flour preparations added to butter oil retarded oxidation of butter oil in storage. The following data are taken from Musher's work (83):

Effect of Avenex in preventing oxidation of butter oil.*

Treatment	Stability (hours)
Control butter oil	5
Plus 1% Avenex	9
Plus 2% Avenex	10.5
Plus 5% Avenex	14.0

* As determined by fat stability apparatus.

The butter oil used in the above trial shows an abnormally short induction period as measured by the method used. However, the same general trend may be expected with normal butter oil.

SCOPE OF INVESTIGATION

Because of the need of further information regarding factors that influence the oxidation of butter fat, this study was conducted with the following intentions:

1. To investigate the methods of determining the stability of fats.
2. To study the influence of heat treatment of (a) cream, (b) butter, and (c) butter oil upon the stability of the fat to oxidation.
3. To determine the effect of metals with and without heat in (a) cream and (b) butter oil upon the ability of the fat to withstand oxidation.
4. To ascertain the influence of the antioxidant, Avenex, when added to cream and to butter oil.
5. To study the destruction of carotene with oxidative changes.

Since there is little available information concerning the reliability of the fat stability and hot air oven methods, extensive studies were made to determine the accuracy of the methods as well as a comparison of the two methods. Also, extensive investigations were carried out upon the influence of various heat treatments upon oxidation while only minor studies were made of Avenex and carotene.

EXPERIMENTAL PROCEDURE

Since many of the laboratory studies were of factors influencing the oxidation of butter oil, it was necessary to standardize methods of preparation in order to eliminate, in so far as possible, all variable influences upon oxidation.

Source and general treatment of milk and cream: Raw milk used for this work was obtained either from the College Creamery or directly from the College Farm. In instances where raw milk was separated, the milk was secured from the College Farm with particular care being taken to prevent metal contamination. The raw milk was centrifugally separated into cream testing from 30 to 40 per cent fat. This cream was either immediately used for oxidation trials or stored at about 3° C. until used. Where pasteurized cream was desired and slight metal contamination was of no significance, sweet pasteurized cream separated from mixed-herd milk and pasteurized at the College Creamery was obtained. All raw cream used in any trial was pasteurized prior to churning at temperatures of at least 62.8° C. for 30 minutes.

Methods of securing and purifying the butter oil: Cream was churned in motor-driven, two-gallon Daisy churns. These churns were well tinned, and agitation was supplied by means of a wooden paddle. After the buttermilk was drained, the granules were washed either with cold tap water or with distilled water depending upon the significance of metal contamination.

When butter was desired, the granules were worked by hand with a wooden paddle into a homogeneous mass and placed into pyrex glass beakers. However, if only the butter oil was desired, the granules were melted in a water bath at 50° C., the water and curd were siphoned off, and the butter oil centrifuged and filtered. Filtering was attained in an incubator held between 40° and 50° C. In case the oil was not to be studied immediately it was refrigerated at 3° C.

Heat treatment of cream, butter, and butter oil: Laboratory pasteurization of cream to temperatures below that of boiling water was accomplished in a round-bottomed glass flask suspended in a water bath, and using a mechanical glass agitator to stir the cream. To attain temperatures above 100° C., cream in 2 liter Erlenmeyer flasks, stoppered with cotton plugs (600 ml. cream per flask), was heated in the autoclave for 30 minutes at the desired steam pressure after allowing 15 minutes for the autoclave to reach the desired temperature and pressure. Cooling was then accomplished by submerging the samples in cold water. In every case the cream was aged in the same container in which it was heated for a specified number of hours before churning.

The heat treatment of the butter and butter oil to temperatures below 100° C. was also accomplished in a water bath. Special care was taken to protect the sample from light during the heating period. To secure temperature above 100° C., 200 ml. samples were placed into 250 ml. Erlenmeyer flasks stoppered with cotton plugs and heated in the autoclave at the desired pressure for 30 minutes. After heating, the samples were either immediately cooled to the storage temperature or, in certain cases where butter was involved, the butter was centrifuged and the butter oil filtered.

Treatment and care of glassware; Laug (66) and Stebnitz and Sommer (115) have shown the necessity of using special care in cleaning glassware used in fat oxidation work. For this purpose these workers used chromic acid cleaning solution ($H_2SO_4.K_2Cr_2O_7$). Hot water was found by

Laug (66) to be effective in removing the cleaning solution from the glass. Stebnitz and Sommer (115), in their work with the fat stability apparatus, indicate that the best results could be obtained by washing the tubes and then soaking them in cleaning solution over night, followed by several consecutive leachings in distilled water for a period of three or four days. Laug (66) showed that several successive leachings with distilled water were necessary to remove all of the chromic acid from the glass.

Special precautions were taken of the glassware used for all determinations in these studies. The glassware was first washed in a strong solution of tri-sodium phosphate, rinsed several times with hot tap water, and then held in chromic acid cleaning solution and placed into large glass jars and rinsed six or more times with hot tap water. After this, the glassware was rinsed with six or more changes of distilled water, each lot of water being allowed to remain over the glassware for 12 hours or more. Before use, the glassware was thoroughly dried in a hot air oven. It was also found necessary to treat the rubber stoppers used in the oxidation tubes of the fat stability apparatus with a strong alkali followed by thorough soaking and rinsing in distilled water.

Methods of analysis: The peroxide determination was used to measure the degree of oxidation of fats. The method used was essentially that outlined by Wheeler (124). To five grams of liquid butter oil in a 250 ml. beaker was added 50 ml. of chloroform-acetic acid solution. This fat solution was allowed to react with 2 ml. of 50 per cent KI solution for one minute, after which time 100 ml. of distilled water was added. The iodine liberated from the KI was then titrated with standard 0.01 N. sodium thiosulfate solution using a soluble 2 per cent starch solution as an indicator. The results are expressed as moles of peroxides per 1000 grams of butter oil.

The free fatty acids content of the butter fat was determined according to the method outlined by The Association of Official Agricultural Chemists (2). Ten grams of butter oil are heated to boiling in 25 ml. of neutral ethyl alcohol containing phenolphthalein. This hot mixture is then titrated with standard 0.05 N. NaOH solution until a pink color persisted for at least 30 seconds. The results obtained are expressed as acid degrees (ml. of 1N. NaOH per 100 grams of butter oil), a value easily obtained by dividing the ml. of NaOH required by 10 grams of butter by two.

Carotene determinations were made by a modification of the method devised by Moore (80). To 10 grams of liquid butter oil ~~was~~ were added 10 ml. of ethyl alcohol (95 per cent) and 10 ml. of benzine (distilled at 20° to 21.1° C.). After thorough mixing, the samples were placed into cold water for 10 minutes, being shaken three times during this period. Distilled water (10 ml.) was then added to cause inversion of the phases and the samples were centrifuged for 4 minutes at 1900 r.p.m. The color of the benzine-fat protion was measured photometrically, using a one ml. sample in an Evelyn photometer with a 440 filter. The carotene content was expressed as micrograms per gram of fat.

Methods of accelerating fat oxidation: An accelerated method of oxidation was used to enable rapid determination of the stability of fats. In these studies two such methods have been compared and used, namely: (a) Swift's fat stability apparatus (an aeration method) and (b) hot air oven.

The Swift's fat stability apparatus was devised by King, Roschen, and Irwin (62). In this method, the fat to be oxidized was placed in test tubes and heated to the temperature of boiling water in an oil bath. A constant flow of air was maintained through each tube of fat being oxidized. As the fat reached the end of the induction period samples were periodically taken from the apparatus and tested quantitatively to determine the degree of oxidation.

Another acceleration method used was a hot air oven. In this method definite amounts of fat in 100 ml. beakers were placed in a thermostatically controlled hot air oven having a mechanical air agitator. As the fat oxidized, samples were taken from the oven at definite time intervals and the degree of oxidation determined.

RESULTS

Studies of the Peroxide Method

The peroxide method in one or more of its modifications has been accepted as a standardized procedure for determining the degree of oxidation for a considerable time. However, since the quantity of chloroform, acetic acid, and potassium iodide required for the many determinations in this study was large and also relatively expensive, studies on the possibility of reducing the amount of solvent and potassium iodide seemed especially appropriated. Furthermore, the preparation of a fresh saturated potassium iodide solution for each group of titrations as well as maintaining a solution of constant strength during the determinations were other problems which seemed to indicate the desirability of using a weaker solution of this reagent if it were found feasible.

Variation in solvent: To study the influence of varying the quantity of solvent used in the peroxide determination four samples of butter oil were oxidized in the hot air oven to different peroxide contents. Duplicate peroxide determinations were then made upon these four samples by three procedures. In one, the standard method for the peroxide determination was used. This involved the use of 50 ml. of solvent. In the other two, lesser amounts of solvent were utilized; in one 40 ml., and in the other 30 ml. The results obtained are given in table 1.

Table 1. Influence of volume of solvent upon peroxide values.

Volume of solvent (ml.)			Deviation (per cent)	
50	40	30	(2) from (1)	(3) from (1)
(1)	(2)	(3)	(4)	(5)
4.6	4.2	3.1	8.7	32.6
8.5	7.8	6.0	8.3	30.0
13.0	11.9	8.8	8.5	32.4
24.4	20.7	17.1	15.0	29.9

From the above data it is apparent that decreasing the amount of solvent 20 per cent causes a decrease in peroxide value of 8.3 to 15.0 per cent whereas a decrease in solvent of 40 per cent resulted in about 30 per cent decreases in peroxide values. From these results it is evident that changing the volume of solvent definitely decreases the peroxide values obtained.

<u>Variations in concentration of potassium iodide solution</u>: In order to determine the possibility of using a weaker potassium iodide solution than the recommended saturated solution, fat was examined having widely different peroxide values, using a saturated, a 50, and a 33 per cent potassium iodide solution. The results of these investigations are given in table 2.

Table 2. Influence of concentration of KI solution upon peroxide values.

Trial 1

Saturated solution (1ml.)	Thirty-three per cent solution			
	1 ml.	2 ml.	Deviation (per cent) (2) from (1)	(3) from (1)
(1)	(2)	(3)	(4)	(5)
5.8	4.5	4.6	22.4	20.7
11.1	8.5	8.5	23.4	23.4
17.0	13.0	13.0	23.5	23.5
31.9	24.2	24.4	24.1	23.5

(contd.)

Table 2. (contd)

Trial 2

Saturated solution (1 ml.)	Fifty per cent solution			
	1 ml.	2 ml.	Deviation (per cent)	
			(2) from (1)	(3) from (1)
(1)	(2)	(3)	(4)	(5)
5.8	5.7	5.7	1.7	1.7
11.1	10.5	10.9	5.4	1.8
17.0	16.3	16.6	4.1	2.4
31.9	29.4	29.9	7.8	6.3

When two milliliter of 50 per cent potassium iodide solution were used, the per cent decrease from the control sample varied from one to two per cent at peroxide values ordinarily determined in oxidative studies. The decrease was slightly greater if one milliliter of the 50 per cent solution was used. In the case of the 33 per cent solution, the percentage deviation from the saturated solution was from 20 to 25 per cent. From these results it appears that 50 per cent potassium iodide solution can be used without introducing excessive errors into the results. For this reason, the peroxide determinations in all the following studies were made using two milliliters of 50 per cent potassium iodide solution in place of the saturated solution that is commonly recommended.

Comparison of sensitivity of peroxide value and acid degree in detecting end of induction period: In conducting these studies on fat oxidation data were secured which permitted a comparison of two methods of measuring the degree of oxidation in the fat, namely; (a) the peroxide value and (b) the acid degree. It appeared desirable to tabulate and analyse these data the results of which are presented in table 3.

Table 3. Comparison of peroxide value and acid degree in detection of end of induction period.

Peroxide value range	Number of samples	Range in acid degree	Average acid degree
0 - 2	19	0.16 - 0.34	0.25
2 - 5	13	0.16 - 0.34	0.22
5 - 10	6	0.19 - 0.34	0.24
10 - 20	3	0.21 - 0.31	0.25
20 - 45	8	0.34 - 0.45	0.45

These results indicate, generally, no close dependable correlation between oxidation as determined by peroxide formation and by titration of free fatty acids. Samples showing excessive peroxide formation had considerable higher acid values, but often samples having a peroxide value of 5 to 10 showed normal acid degrees. Consequently, titrations for the free fatty acids do not appear to be a satisfactory method of detecting the end of the induction period.

Acceleration Methods

Two methods of accelerating fat oxidation were used in these studies. At the beginning of the experiments, an aeration method, developed in Swift's laboratories and commonly referred to in literature as the "Swift's Fat Stability Apparatus," was used. However, because of the complexity, this method and the limitation of the number of samples that could be oxidized at one time, this procedure was later replaced by a hot air oven method.

Aeration Method:

Oxidation is accelerated in this method by air, the flow of which is measured by a Sargent wet test meter. After passing through the meter, the air is washed with distilled water and then passed through a solution of potassium permanganate to remove any reducing substances present. After this treatment, the air is then measured from central chambers through small capillaries into the fat. From the fat, the air passes out into the atmosphere through an atmospheric condenser that returns the condesible vapors to the fat. The fat (25 milliliters) is contained in 2 x 20 centimeter test tubes suspended in an oil bath which is heated by a surrounding boiling water bath.

In studying the aeration method as a means of oxidation acceleration, trials were conducted to determine the influence of certain factors and techniques on irregularities in the results secured as well as to determine the precision of the method in detecting the end of the induction period.

Temperature fluctuations of aeration method: Since variations in peroxide values had been observed between samples taken from different positions of the oil bath at the same time there was some question as to the uniformity of the temperature of the oil bath. Consequently, one point considered was the possibility of errors in speed of oxidation of the fat due to temperature fluctuations in the oil bath. To ascertain the extent of differences that might exist, the apparatus was allowed to heat for several hours and the temperature taken at different positions in the oil bath. Results of these investigations are presented in table 4.

Table 4. Temperature of oil bath of fat stability apparatus at different locations.*

Tube number	1	4	6	9	12	15	18
Temperature °C.	97.5	97.5	97.5	97.8	97.8	97.7	97.7

* Tubes numbered from left to right.

These results show that the center of the oil bath is slightly higher in temperature than either end which is likely due to the effect of the heater that is located in the center of the bath; however, such slight differences in temperature would doubtless have no marked influence upon the accuracy or reliability of the aeration apparatus.

Reliability of aeration method based on peroxide value of individual samples: In studies dealing with the aeration method of rapid oxidation, variation between duplicate samples was ascertained by determining the peroxide values of duplicate samples taken from the apparatus at the same time. These values were then arranged at different peroxide values according to the deviation and are presented in table 5.

Of the 163 duplicate determinations, 6.7 per cent showed no variation, a little less than one third (30.7 per cent) had a difference between duplicate samples of from 0.1 to 0.5 peroxide units, whereas 28.2 per cent of the determinations showed variations between 0.6 and 2.0 peroxide units. About one-fifth of the comparisons varied by a peroxide value of 2.1 to 5.0. In all, 65.6 per cent of the duplicate determinations varied not over 2.0 peroxide units. However, consideration must be given to the fact that 65 per cent of the samples had a peroxide value ranging below 10.0 and there appears to be a tendency for the deviation between duplicate samples to increase directly with the peroxide value of the samples.

In order to determine if the peroxide numbers agree more closely when a relatively short induction period is obtained for the fat, the data in table 1 of the appendix were rearranged placing all duplicate

Table 5. Deviation of peroxide value between duplicate samples taken from the aeration apperatus at the same time.*

Peroxide value	Deviation of duplicate samples							
	0	1-5	0.6-2.0	2.1-5.0	5.1-10.0	10.1-20.0	20.1 and above	% of total
0-2.0	9	32	4	1	1	1		29.4
2.1-5.0	1	7	11	7	1	1		17.2
5.1-10.0	1	4	14	9	2			18.4
10.1-20.0		6	15	8	4			21.0
20.1 and above		1	2	9	9	2	1	14.7
% of total	6.7	30.7	28.2	20.9	10.4	2.5	0.6	100.0

*Total of 163 duplicate determinations. Complete data presented in Table I, Appendix.

Table 6. Deviation of peroxide value between duplicate samples taken from aeration apparatus after oxidizing for less then 18 hours.*

Peroxide value	Deviation of duplicate samples							
	0	0.1-0.5	0.6-2.0	2.1-5.0	5.1-10.0	10.1-20.0	20.1 and above	% of total
0-2.0	5	28	4			1		49.3
2.1-5.0		3	5	1	1			13.0
5.1-10.0	2	3	3	2				13.0
10.1-20.0		1	8	2	2			16.9
20.1 and above		1	1	4				7.8
% of total	9.1	46.7	27.3	11.7	3.9	1.3	0.0	100.0

* Total of 77 duplicate determinations. Complete data presented in Table I, Appendix.

Table 7. Deviation of peroxide value between duplicate samples taken from aeration apperatus after oxidizing 18 hours or more.*

Peroxide value	Deviation of duplicate samples							
	0	0.1-0.5	0.6-2.0	2.1-5.0	5.1-10.0	10.1-20.0	20.1 and above	% of total
0- 2.0	3	5		1	1			11.6
2.1- 5.0	1	4	6	6		1		21.0
5.1-10.0		1	11	7	2			24.4
10.1-20.0		5	7	7	2			24.4
20.1 and above			1	4	10	1		18.6
% of total	4.7	17.4	29.1	29.1	17.4	2.3	0.0	100.0

* Total of 86 duplicate determinations. Complete data presented in Table I, Appendix.

taken from the apparatus before 13 hours in one group and those taken from the apparatus after 18 hours in another group. The deviation between duplicate samples in each group were then tabulated at various peroxide values. These results are presented in tables 6 and 7.

These two tables show that 73.1 per cent of the duplicate samples taken from the apparatus before 18 hours varied from each other by not more than 2.0 peroxide units, whereas only 51.2 per cent of these samples taken from the apparatus after 18 hours oxidation showed variations under 2.0 peroxide units.

However, because the samples taken from the apparatus after 18 hours oxidation had, on the average, oxidized to a greater extent than those samples taken before 18 hours, it is possible that the above data would be more fairly represented by expressing them as percentage deviation.

Reliability of aeration method based on induction period: Additional studies were made to determine the precision of the deviation method in ascertaining the end of the induction period.

For this purpose, a sample of butter oil was oxidized in the aeration apparatus and the end of the induction period determined in triplicate. Through interpolation from the oxidation-time curves, the induction period of each triplicate determination was secured. The results are shown in table 8.

Table 8. Hours induction period of triplicate determinations of the same fat as measured by the aeration method.*

Trial number	Triplicates a	b	c	Maximum** error (per cent)
1	5.5	5.3	5.4	3.8
2	10.0	9.3	9.0	11.1
3	11.0	10.7	11.2	4.7
4	21.7	21.9	21.0	4.3
5	22.4	21.1	22.6	7.1

* Peroxide value of 5 was used as end of induction period.
** Per cent error calculated from minimum induction period.
Complete data presented in table II, Appendix.

These trials were made with fat having an induction period range from 5.4 to 22.3 hours in length, consequently, covering any induction period normally encountered with butter fat. These data present a maximum error for the five trials ranging from 3.8 to 11.1 per cent, with an average maximum error of 6.2 per cent. From the average maximum error obtained, one may expect an average variation in induction period of 0.3, 0.6, and 1.2 hours in fat having induction periods of 5, 10, and 20 hours respectively.

<u>Hot air oven method:</u>

Different workers have made use of hot air ovens as a means of accelerating fat oxidation. In many cases however, consideration has not been given to the possibility that the accuracy of a hot air oven method may be greatly affected by various factors. Consequently, it appeared desirable to ascertain the influence of certain factors upon the rate of oxidation of fat in the hot air oven so that the results secured would be accurate, uniform, and reliable.

For these studies use was made of a Cenco-DeKhotinsky triple wall, thermostatically controlled oven having temperature control to within

± 1° C. The heating elements are shielded with ducts leading to the sides of the oven where the hot air enters at about six and 12 inches from the bottom of the oven. Two shelves were used, the bottom shelf being six inches and the top shelf ten inches from the floor of the oven. A centrigrade thermometer graduated to 1° C. was inserted through the top, the bulb of which extended to the top shelf of the oven. To insure positive air agitation, a Cenco air agitator was installed in the top of the oven.

Preliminary studies on temperature fluctuations: To determine how temperature influences may effect oxidation of fat, three samples of fat (50 ml. fat per beaker) were placed upon the floor, the bottom shelf, and upon the top shelf respectively. The oven was then regulated at 100° C. without the air agitator running. At the end of 24 hours oxidation time the temperature of all nine samples was taken and the peroxide content determined. The results of these preliminary investigations are given in table 9.

Table 9. Preliminary trials with oven method to determine the temperature and corresponding peroxide values at different positions in the oven.*

Position in oven	Sample number	Temperature (° C.)	Peroxide number
	1	83	1.3
Floor	2	105	39.8
	3	126	49.6
	1	78	1.6
Bottom shelf	2	86	6.4
	3	91	10.3
	1	73	1.5
Top shelf	2	77	1.7
	3	80	1.8

* No air agitation

These results show that the temperature of the oven varies widely and, consequently, influences the rate of oxidation at different positions in the oven. The floor of the oven is considerably higher in temperature and varies to a greater extent than any other position in the oven. Also, the bottom shelf is higher in temperature than the shelf four inches above it. Even individual samples on each shelf show several degrees variation in temperature between each other. This prelininary study indicates that if a hot air oven method is to be used some consideration should be given to temperature control.

Wide distribution of samples on shelf: After finding such wide temperature variations in the oven, consideration was given to the arrangement of samples on the shelves to obtain maximum temperature control.

In the first series of trials, beakers of fat were spaced equal distances apart on the bottom shelf, the outside rows being against the walls of the oven as is illustrated in figure 1, diagram I. Thermometers were placed into the fat and arranged so as to permit reading of the temperature through the glass window of the inside door. Because of lack of space, thermometers could not be placed into beakers of fat on the top shelf. However, empty beakers were placed on the top shelf in order to simulate normal operating conditions. After allowing several hours for an equilibrium temperature to be established, at least three temperature readings were taken at two hour intervals. Trials were conducted with and without air agitation. The average of these readings are presented in table 10.

Table 10. Temperature of fat samples distributed widely over the bottom shelf of the hot air oven.

Beaker number	No air agitation (° C.)	Air agitation (° C.)
1	99.1	97.8
2	102.5	99.1
3	105.4	100.3
4	105.2	99.1
5	100.3	97.9
6	103.5	99.2
7	105.0	99.5
8	104.4	99.0
9	100.0	97.6
10	102.3	98.6
11	103.8	99.3
12	103.9	99.3
13	99.0	99.3
14	101.8	99.8
15	101.7	99.9
16	100.5	99.0

When there was no air agitation a maximum temperature difference of 6.4° C. was observed. Samples having the highest temperatures were beside the left side wall near the heating vent and beside the left side of the back wall, whereas the lowest temperatures were found near the door and along the front part of the right wall. Those temperatures taken while the air agitator was running indicate much closer temperature control. In these determinations, a maximum temperature variation of 3° C. was obtained as compared to over twice that value in the trials with no air circulation. Again, as before, the highest temperatures were obtained along the left and back walls whereas the lower temperatures were observed along the front and right walls.

Close distribution of samples on shelf: Since the preceding study showed the samples placed around the sides of the oven varied appreciably in temperature, additional trials were conducted in which the samples were grouped compactly in the center of the shelf as is shown in figure 1, diagram II. Furthermore, since there was some question as to the influence of the presence of beakers on the top shelf upon the temperature of samples on the bottom shelf, this angle was also given consideration. Consequently, as before, the temperature was taken with and without agitation and, in addition, with and without empty beakers on the top shelf. The results of these trials are presented in table 11.

Table 11. Temperature of samples grouped compactly in the center of the bottom shelf.

	Top shelf empty		Beakers on top shelf	
Beaker number	No air agitation (° C.)	Air agitation (° C.)	No air agitation (° C.)	Air agitation (° C.)
1	100.5	100.2	101.7	99.0
2	104.1	100.9	105.2	99.1
3	105.6	101.7	107.2	100.2
4	105.7	102.1	106.8	100.2
5	101.2	100.0	102.1	100.2
6	104.2	101.2	105.4	99.7
7	105.6	101.4	107.0	100.6
8	106.0	101.8	107.1	99.9
9	102.4	100.3	102.8	98.7
10	104.9	101.5	105.8	99.8
11	105.7	101.7	107.2	100.6
12	104.9	101.5	105.9	99.2
13	100.3	99.7	101.7	98.7
14	102.9	99.2	103.7	98.8
15	104.2	100.8	104.6	99.5
16	103.9	101.2	104.5	99.8

When the beakers are placed in the center of the shelf with no beakers on the top shelf, a maximum temperature variation of 5.7° C. was observed without air agitation. This value is 0.7° C. less than was observed when the samples were distributed widely on the shelf. Again, however, the samples with the highest temperatures were located around the left side and back, whereas the lowest temperatures were found along the front and right side.

Under the same conditions with air agitation, a 2° C. maximum variation was obtained. This is 1° C. less variation than was secured when the samples were widely dispersed over the entire shelf.

When beakers were on the top shelf there was an increase in temperature of approximately 1° C. throughout, both with and without air agitation. However, the temperature variation was not appreciably influenced as there was 5.5° C. maximum variation with neither beakers nor agitation as compared to 5.7° without the beakers. When the air was agitated, a 1.9° C. maximum variation in temperature was found when beakers were present as compared to 2.0° C. variation when there were no beakers on the top shelf. The slight tendency toward a lower variation in each instance is insignificant.

From these studies it was concluded that having the beakers in the center of the shelf as in diagram II gave the most accurate results. Consequently, all studies made with the oven method utilized this arrangement.

Temperature of samples on the top and bottom shelves: All temperature measurements presented so far were taken from the bottom shelf, because of lack of space between the top shelf and the oven ceiling. However, a brief study was made of the top shelf temperatures by placing samples of fat into petri dishes and then inserting the thermometers at an angle. Samples were spaced on both the top and bottom shelves as shown in figure 1, diagram III. The temperature relationship found in these studies is presented in table 12.

Table 12. Temperature of fat samples in petri dishes on top and bottom shelves of hot air oven.

Petri dish number	No air agitation	Air agitation
	Bottom shelf	
	(° C.)	(° C.)
1	103.7	99.6
2	105.8	99.9
3	102.5	99.9
4	105.3	99.8
5	103.3	99.5
6	103.1	99.2
	Top shelf	
	(° C.)	(° C.)
7	98.7	98.3
8	101.4	99.2
9	97.7	97.6
10	101.3	99.5
11	98.1	97.9
12	98.5	98.2

This data shows conclusively that the bottom shelf is normally higher in temperature than the top shelf. With the air agitator running the bottom shelf averaged 1.2° C. higher in temperature than the top shelf. The same relationship existed when there was no air agitation excepting there was 4.7° C. difference in temperature.

These additional data indicate, therefore, that when the hot air oven is to be used, consideration should be given to the fact that a temperature difference may exist between shelves which may appreciably affect the results.

Difference in rate of peroxide formation on different shelves: Additional trials were conducted to find the relation that exists between the rate of peroxide formation on the different shelves and the corresponding temperature. In this study, 25 mls. of fat in 100 ml. beakers were oxidized on both shelves. After definite oxidation had taken place peroxide determinations were made on all samples.

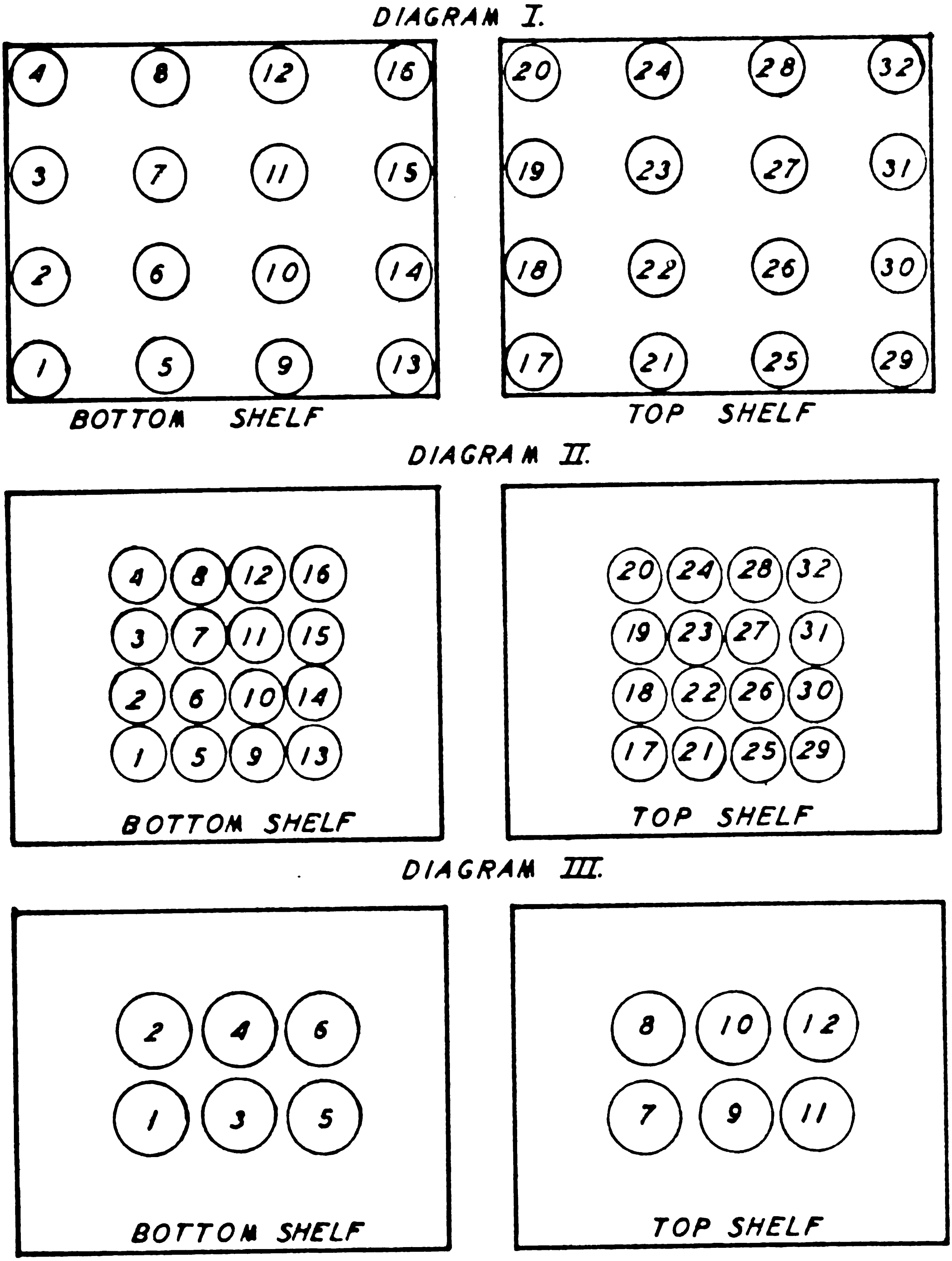


Figure 1. Diagrammatic arrangement of beakers and petri dishes in hot air oven.

Because the temperature determinations of the fat samples on the top shelf were made in petri dishes and the oxidation of the fat was carried out in 100 ml. beakers, the temperatures of the samples are comparably but not exactly accurate. This results since the petri dishes were not at exactly the same position as the beakers of fat. The results of these investigations are presented in table 13.

Table 13. Peroxide formation and relative temperatures at different locations in the hot air oven.

Bottom shelf			Top shelf		
Beaker number	Temperature (° C.)	Peroxide value	Beaker number	Temperature (° C.)	Peroxide value
1	99.6	5.5	17	98.3	7.8
2	99.6	7.4	18	98.3	4.7
3	99.9	4.4	19	99.2	5.0
4	99.9	6.1	20	99.2	3.5
5	99.9	4.9	21	97.6	3.6
6	99.9	3.6	22	97.6	2.0
7	99.8	4.6	23	99.5	2.5
8	99.8	5.3	24	99.5	3.0
9	99.9	4.8	25	97.6	3.3
10	99.9	3.8	26	97.6	2.7
11	99.8	4.5	27	99.5	6.0
12	99.8	9.8	28	99.5	7.1
13	99.5	3.4	29	97.9	2.7
14	99.5	4.4	30	97.9	3.1
15	99.2	5.0	31	98.2	3.5
16	99.2	6.9	32	98.2	9.4
Average	99.6	5.27		98.9	4.37

The average of these relative temperatures shows the bottom shelf to be 0.7° C. higher in temperature than the top shelf. Corresponding to this temperature difference, there is an average difference of 0.9 peroxide units: the bottom shelf has an average peroxide value of 5.27, whereas that of the top shelf is 4.37.

That there is a difference between the top and bottom shelves is further substantiated by the data presented in table III of the appendix. These data constitute those secured on 784 samples in which one lot was oxidized on the top and the other on the botton shelf of the hot air oven.

The results show the average peroxide value of all samples taken from the bottom shelf to be 8.10 whereas that of the top shelf is 7.16, an average difference of 0.94 peroxide value. Further treatment of these data shows 66.5 per cent of the samples from the bottom shelf oxidized sooner than those from the top shelf, 27.3 per cent of the samples from the top shelf oxidized before those from the bottom, whereas 6.2 per cent of the samples oxidized equally fast on either shelf.

Length of induction period of top and bottom shelves: Further studies of the results secured with the top and bottom shelves of the hot air oven were carried out by utilizing the induction periods involved. From the induction period curve, assuming a peroxide value of five as the end of the induction period, the induction periods of the samples of fat on each shelf were determined. A summary of these data is presented in table 14.

Table 14. Hours difference in induction period of the same fat when oxidized on the top and bottom shelves of the oven.*

Induction period (hours)	Number of trials	Hours difference in induction period between top and bottom shelves		
		0 - 1.0	1.1 - 2.0	2.1 - 3.75
10.0-15.0	10	6	3	1
15.1-17.5	17	8	9	0
17.6-20.0	21	9	9	2
20.1-22.5	11	5	4	2
22.6-above	7	4	3	0
Percent		50.0	42.5	7.5

* Complete data presented in table IV, Appendix.

These data show that 50.0 per cent of the trials on different shelves varied from each other by not more than 1 hour induction period time, 42.5 per cent from 1.1 to 2.0 hours, and 7.5 per cent had an induction period variation greater than two hours. Further analysis of data from the original 66 trials, shows that 85.0 per cent of the trials from the bottom shelf had the shortest induction period whereas only 10.5 per cent of the trials from the top shelf oxidized first. Thus, there were 4.5 per cent of the trials oxidizing with equal rapidity irrespective of shelf. The average length of the induction period of the top shelf was 19.07 hours whereas the bottom shelf had an induction period of 18.06 hours, an average difference of 1.01 hours.

The normal difference in induction period between the two shelves is about 5.2 per cent. Even though the error between the shelves is not great, it may materially influence the results in oxidative studies.

Influence of temperature upon rate and extent of oxidation: Little consideration has been given the importance of temperature in the formation and destruction of peroxides. Possibly some temperature exists at which destruction of the peroxides will be at a minimum and formation at a maximum, thus increasing the sensitivity of rapid oxidation methods. To determine the relative accuracy of accelerating oxidation at different temperatures, two identical Cenco-Dekhotinsky thermostatically controlled hot air ovens were obtained, each having a mechanical air agitator. The same sample of butter oil was oxidized in quadruplicate, first at 70° and 100° C., and then at 100° and 130° C. A summary of the data obtained is presented in table 15.

Table 15. Influence of temperature of hot air oven upon the rate of oxidation and upon the uniformity of results.*

Oven temperature	Average induction period*	Maximum induction period*	Minimum induction period*	Deviation between minimum and maximum	
				Hours	Per cent
70	142.0	152.0	138.0	14.0	9.9
100	26.5	28.0	25.5	2.5	9.4
130	3.5	3.7	3.4	0.3	11.6

*Peroxide value of 5 considered to be the end of induction period. Complete data presented in table V, Appendix.

As would be expected, the lower temperature considerably extended the length of the induction period. However, samples oxidized at 130° C. showed a somewhat greater percentage error (about 1.5 per cent more) than those oxidized at lower temperatures. However, the percentage deviations of induction period of the quadruplicate determinations at 70° and 100° C. are about the same, indicating that these lower temperatures of oxidation had little influence upon the precision of the method in detecting the end of the induction period.

Inasmuch as the lower temperatures used have no great influence upon the accuracy of the oven method, a temperature should be used within this range that is best adaptable to the laboratory work. However, too low a temperature extends the induction period to considerable length, consequently the purpose of the rapid oxidation method would be invalidated if too low a temperature is used. Therefore, a temperature sufficiently high to bring about rapid oxidation should be used. Consequently, 100° C. was considered best suited for the purpose of oxidizing butter fat because at this temperature oxidation normally occurs within 12 to 24 hours. Therefore, all studies with this method were made at 100°C.

Effect of sample size upon rate of oxidation of butter oil in the hot air oven: Studies of the rate of oxidation of samples of different sizes were deemed desirable to determine the consideration that should be given this factor when using the hot air acceleration method.

For this purpose, 10, 20, 30, and 40 ml. samples of butter oil were placed into 100 ml. beakers and oxidized in the hot air oven. Samples were taken from the oven at definite time intervals and the peroxide content determined. Two trials were conducted with duplicate samples being used in each trial. The results are presented in table 16 and are shown graphically in figure 2.

Table 16. Influence of sample size upon rate of fat oxidation in hot air oven.*

Hours oxidation	Size of sample (ml.)			
	10	20	30	40
14	6.0	1.7	2.0	1.7
16	7.5	5.6	3.6	2.9
17	13.4	4.4	3.8	2.9
18	18.0	7.6	6.1	3.7
19	22.6	11.6	11.5	6.0
20	26.3	11.9	9.2	9.6
Average	16.1	7.1	6.0	4.5

* Results are reported in peroxide values.
** Values in peroxide number.

These results show that the size of the sample definitely influences the rate of oxidation, with the smaller samples oxidizing more rapidly than the larger ones. Obviously therefore, the size of sample must be considered and maintained uniform in any oxidation work with the hot air oven. Consequently, in all studies to follow, 25ml. samples of fat were used.

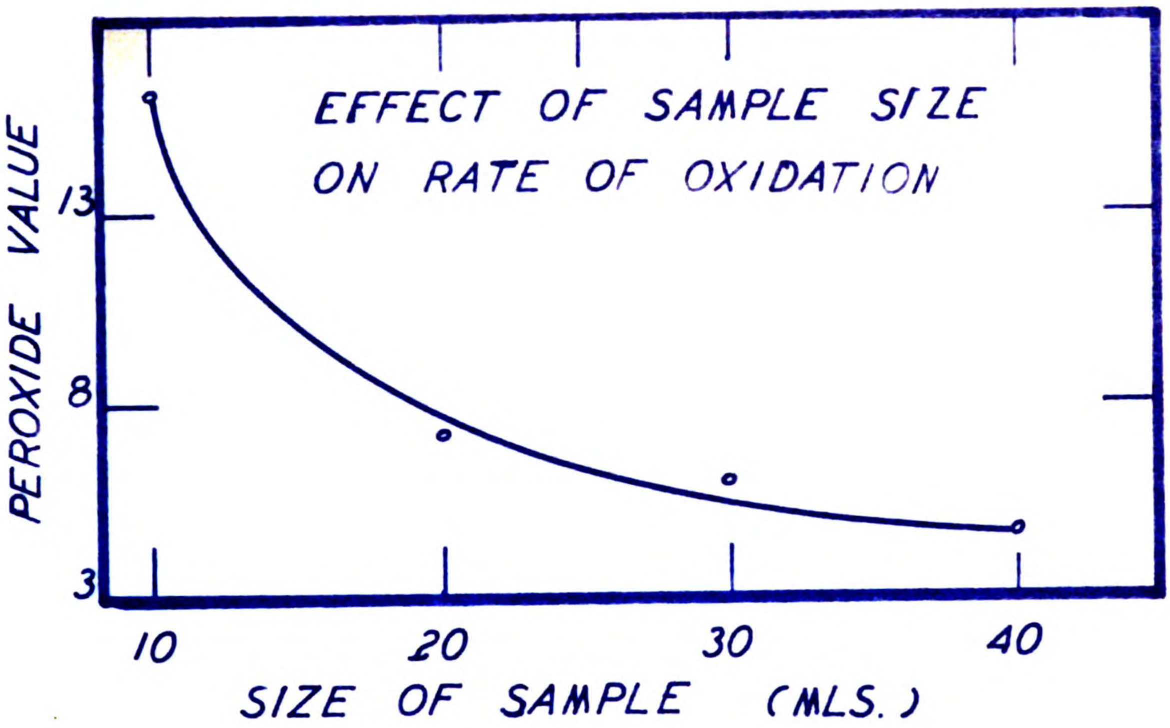
Effect of surface area upon rate of oxidation in hot air oven: Another factor, the surface area, was given consideration as a possibility of influencing oxidation. To determine the effect of surface area upon the rate of oxidation of butter oil in the hot air oven, 25 ml. samples of butter oil were placed into glass containers having 62.5, 33.0, 23.7, 18.1, and 11.3 square centimeters surface area respectively. These samples were oxidized in the hot air oven at 100°C. and the end of the induction period determined. The average results of two trials are given in table 17 and are presented graphically on figure 2.

Table 17. Relationship of surface area to the rate of oxidation in the hot air oven. *

Hours oxidation	Surface area in square centimeters				
	62.5	33.0	23.7	18.1	11.3
13	3.3**	1.5	1.7	1.4	1.5
14	7.4	1.6	2.0	1.9	1.7
15	14.4	3.6	4.0	1.6	2.1
16	18.5	8.1	7.3	4.6	5.0
17	25.0	18.4	5.7	3.0	3.9
18	28.8	18.4	6.8	8.5	6.3
Average	16.2	8.6	4.6	3.5	3.4

* Results are reported in peroxide values.
** Values in peroxide number.

As may be observed from these data, the rate of oxidation is definitely dependent upon the surface area: the greater the surface area exposed, the more rapid the oxidation of the fat. From this, it may be concluded that the surface area must be kept constant when oxidizing fat in hot air ovens if uniform results are to be obtained. Therefore, in the studies to follow, 100 ml. beakers were used exclusively.



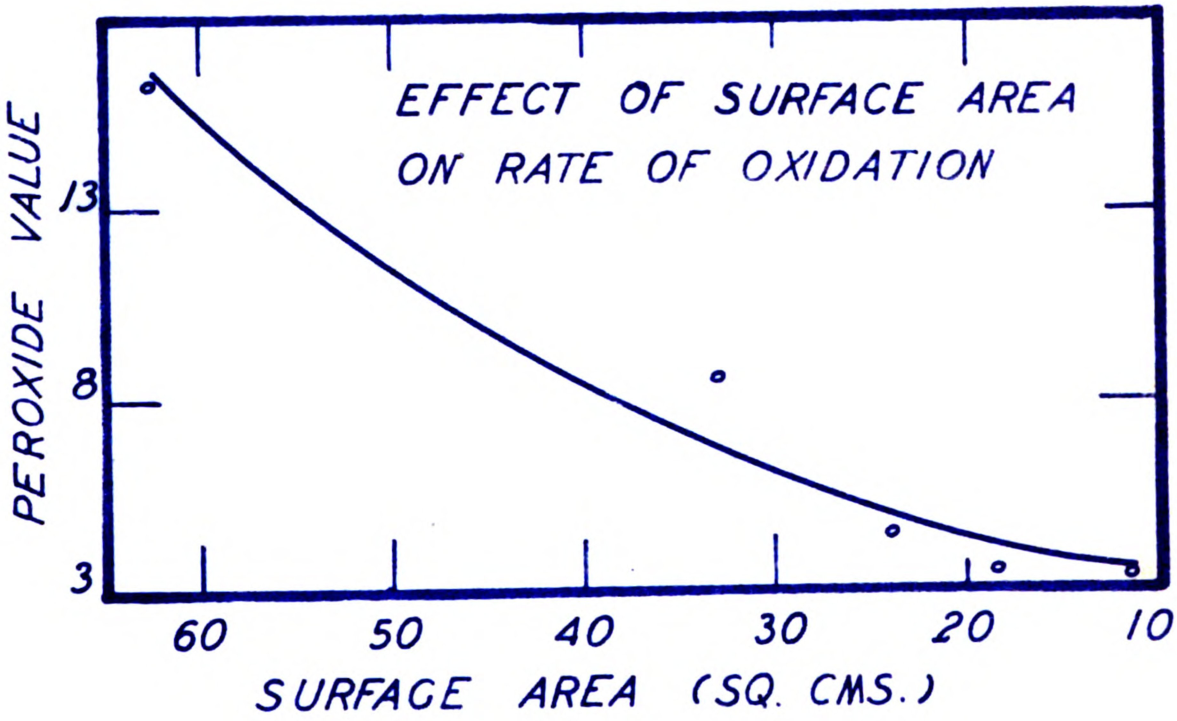


Figure 2. Effect of sample size and surface area on rate of oxidation of butter oil.

Reliability of oven method on basis of peroxide value of individual samples: Studies were made utilizing the oven method to ascertain the degree of accuracy which may be expected under carefully controlled conditions. To determine the accuracy of the method based on the peroxide number, duplicate determinations of the same fat taken from the same shelf were made. The results were recorded and the deviations between the duplicates secured. The data are shown in table 18.

Of the 328 duplicate samples studied 79.5 per cent had a peroxide value below ten. Of this group, 80.8 per cent showed a deviation between duplicates of less than 2.0 peroxide units, 16.1 per cent varied between 2.1 and 5.0 peroxide units, and 3.1 per cent of the samples showed a variation between 5.1 and 10.0 peroxide units.

Sixty seven of the samples, or 20.4 per cent, had a peroxide value of 10.1 or above. Of these, 52.3 per cent had a variation between duplicates of less than 2.0 peroxide units.

Of the total of all comparisons, 75.1 per cent showed a deviation of less than 2.0 peroxide units, 18.0 per cent varied between 2.1 and 5.0 units, 4.9 per cent varied between 5.1 and 10.0 units, and 2.1 per cent varied to a greater extent than 10.0 peroxide units.

Table 18. Deviation of peroxide values between duplicate samples taken from same shelf of hot air oven.*

Peroxide value	Deviation of duplicate samples**							
	0	1-5	0.6-2.0	2.1-5.0	5.1-10.0	10.1-20.0	20.1 and above	% of total
0- 2.0	10	36	3	1				15.2
2.1- 5.0	2	23	52	10				26.5
5.1-10.0	4	15	66	31	8			37.8
10.1-20.0	1	3	29	14	4	2		16.2
20.1 and above			2	3	4	4	1	4.3
% of total	5.2	23.5	46.4	18.0	4.9	1.8	0.3	100.0

* Total of 328 duplicate determinations. Complete data presented in Table VI, Appendix.
**Values in peroxide numbers.

<u>Reliability of oven method on basis of induction period</u>: Further information was secured dealing with the hot air oven method in detecting the end of the induction period. In this connection, several trials were run in which the induction period of a butter fat was determined in quadruplicate by placing four samples upon each the bottom and top shelves of the hot air oven. By assuming a peroxide value of five as the end of the induction period, the induction periods of all determinations were interpolated from the oxidation-time curves and are presented in table 19.

Table 19. Hours induction period of quadruplicate determinations of the same fat as measured by the hot air oven method.*

Trial number	Hours induction period				Maximum difference	
	a	b	c	d	Hours	Per cent
1	16.75	18.00	17.75	17.25	1.25	7.2
2	24.50	24.00	25.50	25.50	1.50	6.0
3	34.00	32.50	31.00	30.75	3.25	10.1
4	33.00	33.50	33.50	31.00	2.50	7.6
5	28.25	27.75	30.50	31.00	3.25	11.1
6	30.50	30.00	31.00	30.50	1.00	3.3
7	26.00	25.25	27.25	27.25	2.00	7.5
8	27.25	27.75	28.00	26.25	1.75	6.4
9	25.50	26.50	27.00	25.25	1.75	6.7
10	27.00	27.50	29.25	25.25	4.00	14.7
11	27.75	26.50	27.50	27.00	1.25	4.6
12	25.25	26.00	27.50	26.50	2.25	8.5
Average					2.15	7.8

* Complete data in Table VII, Appendix

These data show a maximum difference between quadruplicate induction periods ranging from 3.3 to 14.7 per cent with an average maximum difference of 7.8 per cent. In terms of hours as based on the average results of this experiment, this method could be expected to vary 0.78, 1.56, and 2.34 hours respectively for trials having induction periods of 10, 20, and 30 hours. However, certain trials did show greater variation than this: for instance, trials, 3, 5, and 10 showed a maximum difference of 10.1, 11.1, and 14. 7 per cent respectively.

Comparison of aeration and hot air oven methods:

Because the studies so far have involved the aeration and hot air oven methods individually at different times and with different fats, it seemed important that a direct comparison of the two methods be made to permit the drawing of more definite conclusions. Consequently, the following experiments were conducted with this in view.

Rate and extent of oxidation of fat in hot air oven and aeration apparatus at 100° C.: In order to determine the relative speed with which fat is oxidized by the hot air oven and aeration methods at the same temperature, samples of butter fat were oxidized by the two methods using the regular standardized procedures. The averages are presented in table 20.

Table 20. Relative rate of oxidation of fat by hot air oven and aeration methods. *

Trial number	Hours induction period** Oven method (1)	Aeration method (2)	Difference (1) from (2) Hours	Per cent
1	19.50	22.00	2.50	12.0
2	18.00	18.00	0.00	0.0
3	22.00	23.00	1.00	4.4
4	11.25	16.00	4.75	34.8
5	10.75	15.00	4.25	33.0
6	15.75	21.00	5.25	28.6
7	14.00	17.00	3.00	19.4
8	13.75	21.00	7.25	41.7
9	11.75	18.25	6.50	43.3
10	17.50	24.25	6.75	32.3
11	13.75	19.25	5.50	33.3

* The average of columns (1) and (2) was used to calculate the per cent difference.

** Peroxide value of 5 used to determine end of induction period Complete data in Table VIII, Appendix.

These data show the induction period of fat in the hot air oven to be an average of 4.25 hours shorter than that of the same fat oxidized by the aeration method, an average of 25.7 per cent more oxidation. As may be observed, this increased rate of oxidation in the case of the hot air oven is not constant and ranged from 0.0 to 43.3 per cent. However, seven of the eleven trials gave from 19.4 to 43.3 per cent faster oxidation in favor of the hot air oven.

One reason for the aeration method having a longer induction period may be due to the use of saturated air to aspirate the fat. To study this possibility, trials were conducted in which a portion of the fat-containing tubes in the apparatus was aspirated with air previously dried by passing it through sulfuric acid. The remaining tubes were aerated in the usual fashion by the use of saturated air. The average results of a typical trial (trial 1) are presented in table 21.

Table 21. Influence of drying the air upon the rate of oxidation of fat by aeration method. *

	Peroxide values	
Hours oxidation	Saturated air	Dry air
12.0	1.1	1.3
16.5	1.5	2.8
17.0	1.7	8.1
17.5	---	14.2
19.5	5.8	---

* Complete data presented in table IX, Appendix

These results indicate that the rate of oxidation is accelerated from 10 to 15 per cent by drying the air used for aspirating the fat. One additional observation made was that the drying of the air possibly may present a means of obtaining greater precision with this method. More data are needed, however, before definite conclusions may be drawn in this connection.

To study further the rate as well as the extent of oxidation, a sample of fat was oxidized both by the oven and aeration methods, samples being taken at definite time intervals to determine the degree of oxidation. The results of two trials are presented in table X of the Appendix, and data of a typical trial (trial 1) are protrayed in figure 3.

These curves show, again that fat in the hot air oven oxidizes before that in the aeration apparatus. However, once the oxidation starts in the aeration apparatus it proceeds rapidly while the oxidation in the hot air oven shows a gradual progressive rate of peroxide formation. At the end of 48 hours there were no more samples in the hot air oven so the extent of oxidation in this case could not be studied further. However, in the case of the aeration method samples were taken for a period of 144 hours. In this case the peroxide content increased to a maximum at about 70 to 90 hours and then, upon further oxidation, showed marked decreases.

Precision of hot air oven and aeration method in measuring the end of the induction period: In order to obtain more definite information as to the precision of each method in detecting the end of the induction period, curves were plotted of the induction periods of 11 duplicate determinations obtained with each method. From these curves, the length of the induction periods of each duplicate determination, to the nearest half hour, was secured. The results are presented in table 22.

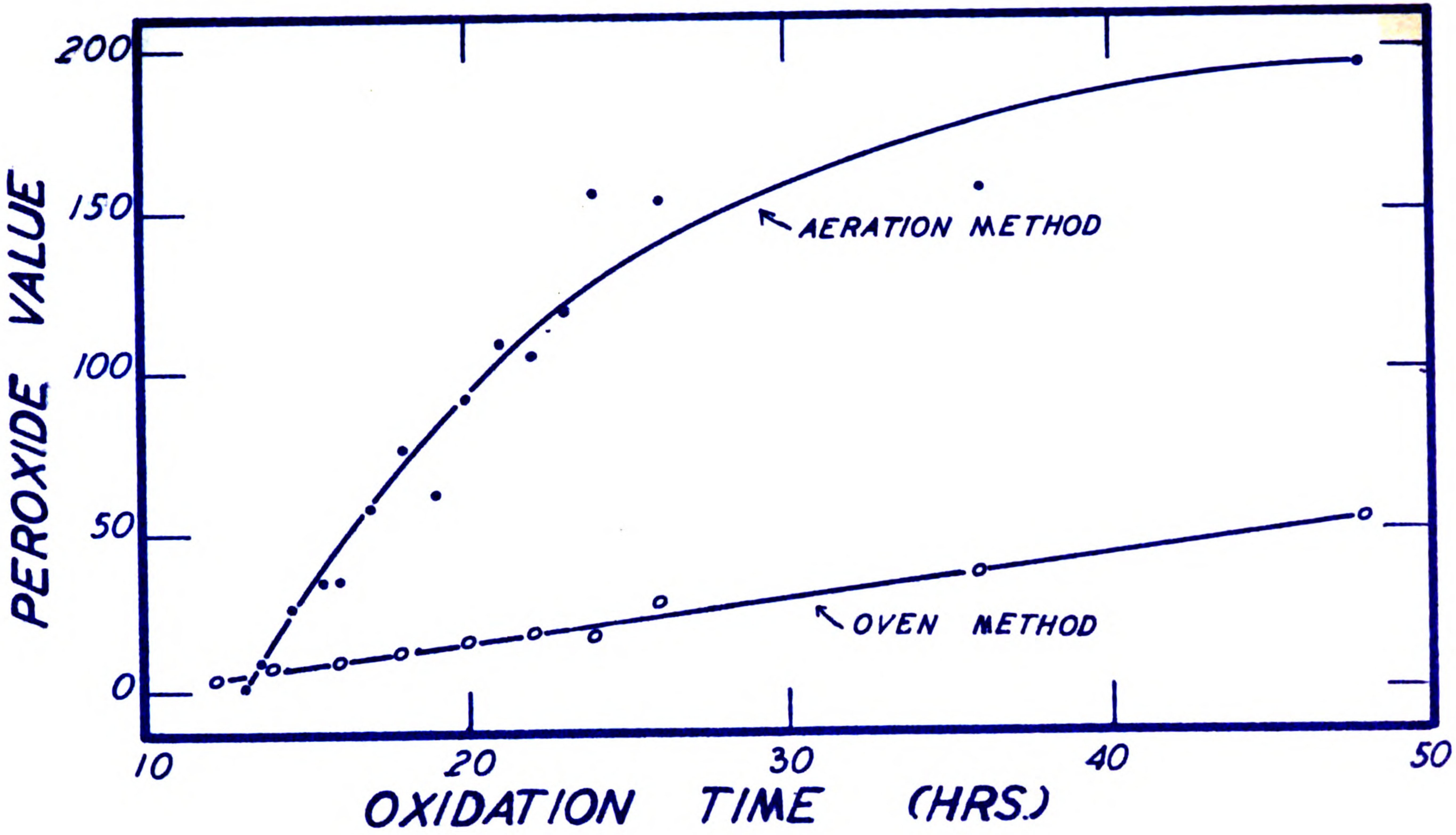


Figure 3. Rate and extent of oxidation of butter oil by aeration and oven methods.

Table 22. Induction periods of duplicate samples of fat oxidized by hot air oven and aeration methods. *

Trial number	Hot air oven Duplicates a	b	Difference	Aeration method Duplicates a	b	Difference
1	19.5	19.5	0.0	21.5	22.5	1.0
2	17.5	18.5	1.0	18.5	17.5	1.0
3	22.5	21.5	1.0	23.0	23.0	0.0
4	11.5	11.0	0.5	16.0	16.0	0.0
5	11.0	10.5	0.5	14.5	15.5	1.0
6	16.0	15.5	0.5	20.5	21.5	1.0
7	14.0	14.0	0.0	17.0	17.0	0.0
8	13.5	14.0	0.5	21.0	21.0	0.0
9	12.0	11.5	0.5	18.0	18.5	0.5
10	17.0	18.0	1.0	25.0	23.5	1.5
11	13.5	14.0	0.5	19.5	19.0	0.5
Average	15.27	15.27	0.55	19.50	19.54	0.59

* Complete data presented in table VIII, Appendix.

The above data shows that in the trials run, the hot air oven showed a maximum variation of 1.0 hours induction period as compared to 1.5 hours maximum variation for the aeration method. On the basis of percentage variation, the hot air oven varied 5.6 per cent whereas the aeration method varied 6.4 per cent. However, the average percentage difference between the duplicates is 3.59 per cent in the case of the hot air oven as compared to 3.02 per cent for the aeration method, an insignificant difference.

Heat Influence on Butter and Butter Oil

Influence of heat treatment upon the oxidation of butter oil: To determine the effect of high temperature heat treatment of butter oil upon its stability toward oxidation, trials were conducted in which oil was heated in the autoclave at six and 22 pounrs pressure for 30 minutes. Four trials were conducted in the case of the six pounds steam pressure 109.8° C. and three trials in the case of the 22 pounds pressure 127.0°C. Averages of these trials are given in table 23 and are presented graphically in figure 4.

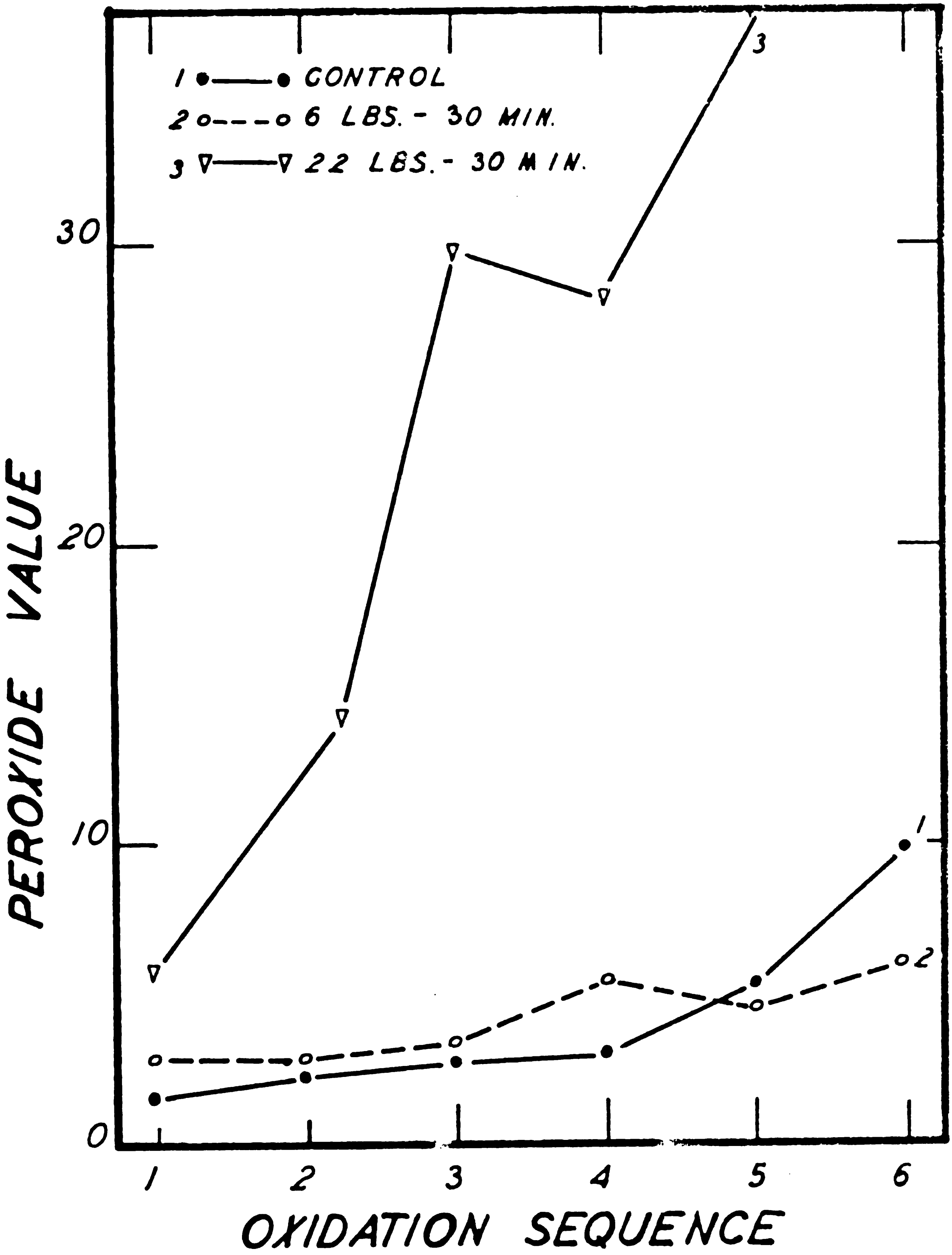


Figure 4. Influence of heating butter oil upon its stability toward oxidation.

Since the different fat samples had different length induction periods, the trials could not be averaged according to hours oxidation. Consequently, the trials were combined into one by averaging all samples taken first, second, third, etc. These averages are tabulated according to oxidation sequence; therefore, they represent the order of taking the samples as well as a definite oxidation time.

Table 23. Influence of heat treatment of butter oil upon its stability toward oxidation. *

Oxidation sequence	Peroxide value		
	Control	109.8°C. - 30 min.	127°C. - 30 min.
1	1.5	2.9	5.8
2	2.3	2.9	14.3
3	2.6	3.5	29.6
4	3.0	5.5	28.0
5	5.2	4.5	56.1
6	9.7	6.0	58.8

* Complete data presented in table XI, Appendix.

These data show, generally, that heat treatment of butter oil at the temperatures used in these experiments decreases its stability toward oxidation. In the trials involving the six pounds steam pressure, oxidation began more rapidly than in the control samples, ususally by about one hour; however, the extent of oxidation was somewhat less at the close of the experiment. Those samples heated to 127° C. (262.0° F.) oxidized about five hours before the control samples and, also, the oxidation proceeded rapidly to a much greater degree.

Influence of heat treatment of butter upon the oxidation of butter oil: To study the influence of heating butter upon the keeping quality of the butter oil, a sample of butter was divided into three lots. Lot 1 was filtered and the butter oil used as the control; Lot 2 was filtered and the oil extracted and heated to 127.0° C. (262.0° F.) for 30 minutes; and Lot 3 was heated as butter at 127.0° C. (262.0° F.) for 30 minutes and then separated into butter oil. In trials 1 and 2 the butter oil was filtered from the butter soon after it was taken from the autoclave at a temperature around 80.0° C.; whereas, in trial three, the heated butter was cooled to 45.0° C. before filtering. The data of these determinations are presented in table 25.

Table 25. Influence of heat treatment of butter and butter oil upon the oxidation of the butter oil.

Trial number	Hours oxidation	Peroxide Value: Control	Peroxide Value: Oil 127°C. - 30 min.	Peroxide Value: Butter 127°C. - 30 min.
1*	10	0.9	1.1	1.2
	12	1.0	1.5	1.3
	14	1.1	1.5	1.4
	16	1.5	2.3	3.7
	18	1.7	3.2	5.7
	21	3.2	9.6	16.3
	25	10.8	20.5	22.5
2*	11	1.0	1.3	1.3
	13	1.2	1.4	1.5
	15	1.3	2.2	2.5
	17	1.8	7.5	3.7
	19	1.9	6.2	7.4
	20	4.9	11.6	10.5
	21	3.9	10.1	14.9
3**	17	1.1	6.2	0.9
	19		8.6	
	22		15.7	
	24		12.8	
	26		19.3	
	29	3.0		2.0
	31	4.8		3.1
	33	5.3		4.6
	35	11.0		5.7

* Trials 1 and 2 filtered while hot.
** Trial 3 filtered at 45° C.

In all cases, as was shown before, the heating of the butter oil to high temperatures lessened its stability toward oxidation. However, in those trials involving the heating of the butter, somewhat variable results were secured. In the first two trials, in which the butter was filtered at a relatively high temperature, the butter oil was definitely less stable than the control butter oil, oxidizing about five hours sooner. In these trials also, the butter showed about the same resistance toward oxidation as did the heated butter oil. However, in trial 3, in which the heat treated butter was cooled to a lower temperature before filtering, the butter oil exhibited slightly more stability toward oxidation than the control butter oil. Whether or not the difference observed in the butter trials are due to the temperature of filtering would need to be determined by more complete studies.

Influence of Heating Cream on Oxidation

Influence of heat treatment of cream upon oxidation of butter oil: Since recent trends have been toward high temperature pasteurization of cream, studies were conducted to ascertain the influence of different heat treatments upon oxidation of the butter oil. In this connection, two factors influencing oxidation were studies, first, the influence of temperature and secondly, the influence of holding time.

In the first phase of this study, cream was heated to the following temperatures: 62.8° C. (145.0° F.), 85.0° C. (185.0° F.), 90.6° C. (195.0° F.), six pounds pressure (229.6° F.) and 22 pounds pressure (262.0° F.). After cooling to churning temperature, the cream was aged for 12 hours and churned. The butter oil was oxidized in the hot air oven to determine its oxidative stability. The averages of five comparisons are presented in table 26. The rates of oxidation of three temperatures, 62.8° C. (145.0° F.) for 30 minutes, and 90.6° C. (195.0° C.) flash, and 109.8° C. (229.6° F.) for 15 minutes are presented graphically in figure 5.

Table 26. Influence of temperature of pasteurization of cream upon the oxidation of the butter oil. *

Number of trials	Oxidation sequence	Peroxide value 62.8°C. - 30 min.	
			85.0°C. - 5 min.
	1	1.8	1.8
	2	3.1	3.1
6	3	3.6	4.5
	4	5.8	7.9
	5	7.0	9.8
	6	9.9	11.3
			90.6°C.flash
	1	0.8	0.8
	2	1.1	1.0
6	3	1.1	1.0
	4	1.7	1.6
	5	3.7	3.9
	6	6.6	7.5
			109.8°C.-15 min.
	1	0.9	1.2
	2	1.2	1.3
3	3	1.3	2.0
	4	1.7	4.1
	5	3.2	7.7
	6	5.4	15.2
			127.0°C.-15 min.
	1	0.8	3.8
	2	1.4	8.6
2	3	1.3	9.5
	4	1.7	9.9
	5	2.5	18.7
	6	7.8	19.2
			127.0°C.-30 min.
	1	1.7	7.3
	2	2.9	13.9
7	3	3.7	14.1
	4	6.8	14.7
	5	7.9	18.5
	6	10.6	23.1

* Complete data presented in table XII, Appendix

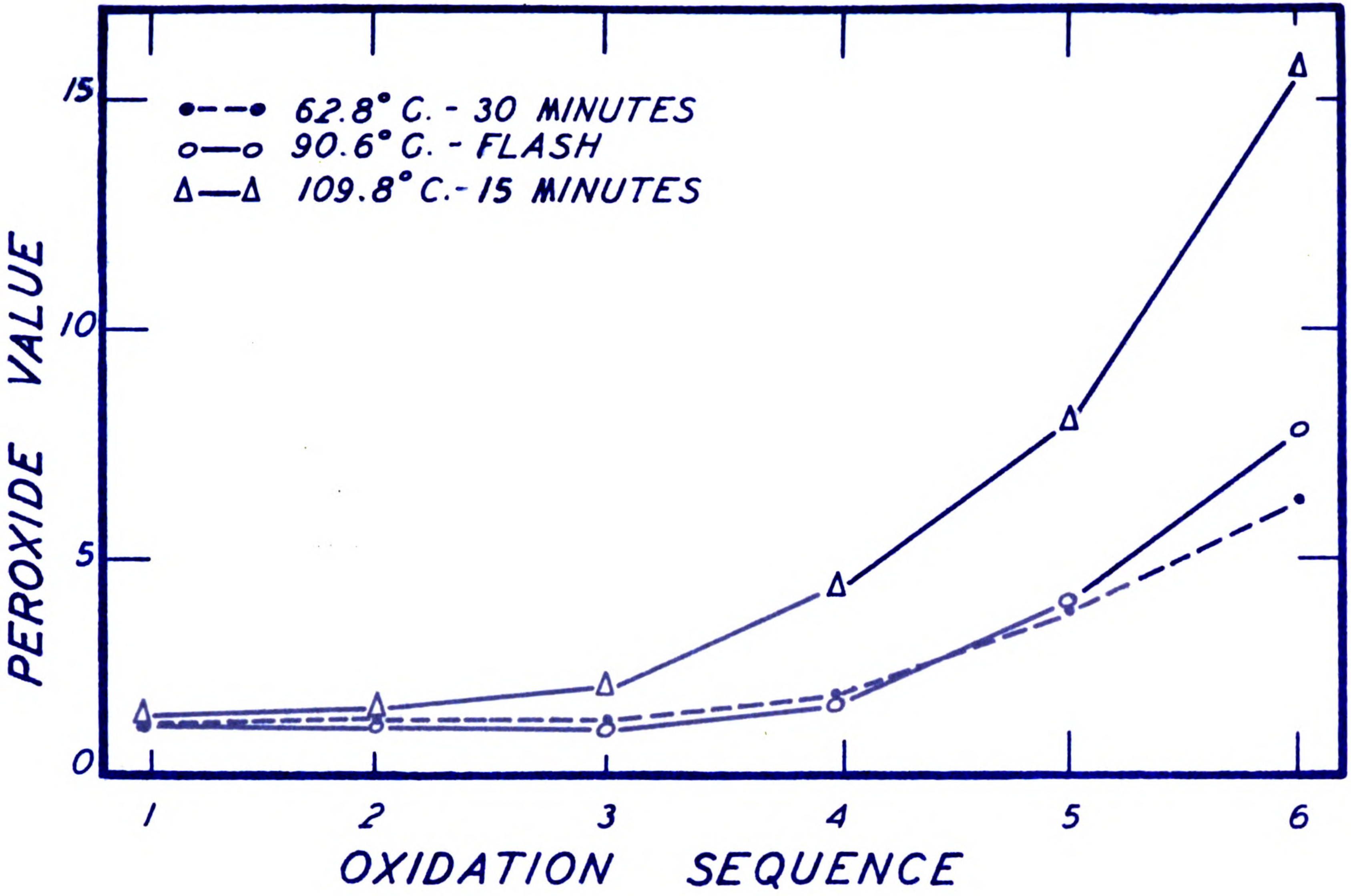


Figure 5. Influence of temperature of pasteurization of cream upon oxidation of butter oil.

These average values show that heating the cream either to 85.0° C. (185.0°F.) for five minutes or to 90.6° C. (195.0°F.) flash had no significant influence upon the rate of oxidation as compared to 62.8° C. (145.0° F.) for 30 minutes. Higher temperatures such as were secured by the use of six and 22 pounds pressure, increased the oxidative tendency of the fat, there being a direct relationship between susceptibility of the fat to oxidation and the temperature to which the cream was subjected. An examination of the data secured from each trial shows the same general trend observable in table 26. In the case of the higher heat treatment all the individual trials showed a tendency for the cream with the high temperature heat treatment to oxidize much sooner than that which was pasteurized at 62.8°C. (145.0° F.) for 30 minutes. In this connection, the heating of cream to 109.8° C. (229.6°F.) for 30 minutes caused an average of three hours shorter induction period, whereas heating the 127.8° C. (262.0°F.) for 30 minutes resulted in an average induction period difference of about five hours.

In the second phase of this study, steps were taken to determine the influence of the holding period upon the oxidation of butter oil. In this study, raw cream was divided into four lots which were heated to 90.6° C. (195.0°F.) and held at this temperature for the following periods: 0, 5, 15, and 30 minutes. The induction period of the respective fats was then determined by the oven method. Average results for three trials run are given in table 27 and are presented graphically in figure 6.

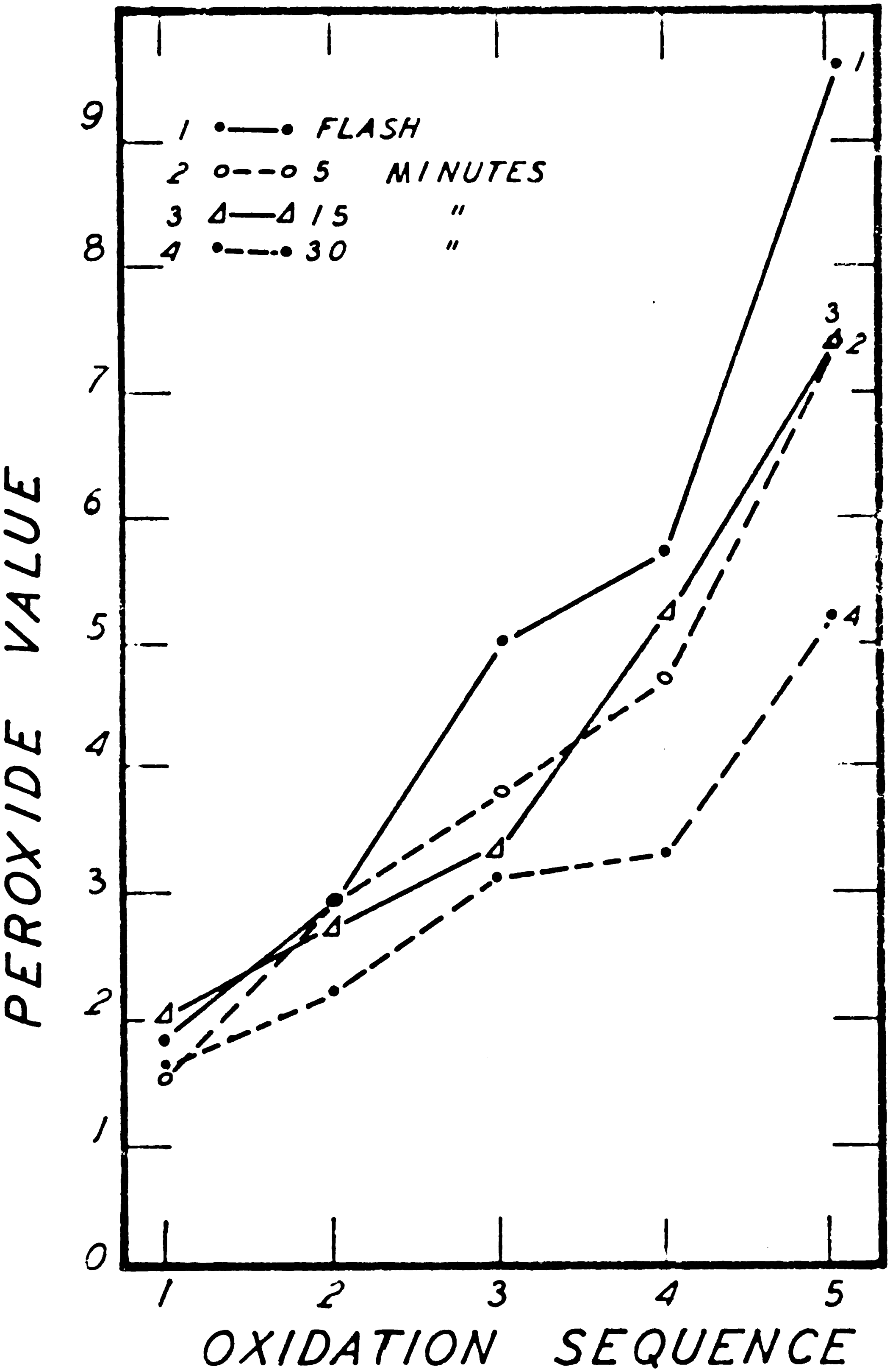


Figure 6. Influence of length of heating period of cream at 90.0°C. upon oxidation of the butter oil.

Table 27. Influence of length of heating period of cream upon the oxidation of the butter oil. *

Oxidation sequence	Peroxide value Flash	5 min.	15 min.	30 min.
1	1.8	1.5	2.0	1.6
2	2.9	2.9	2.7	2.2
3	5.0	3.8	3.3	3.1
4	5.7	4.7	5.3	3.3
5	9.6	7.4	7.4	5.2

* Complete data presented in table XIII, Appendix.

The data show on the average, a tendency for the samples having the longer holding periods to have a greater stability toward oxidation than the cream that was flash treated. This tendency for the longer holding periods to stabilize the fat against oxidation was also observed in each individual trial. The samples heated for the five and 15 minute holding periods showed little difference in stability, whereas the lots held for 30 minutes showed distinctly greater stability than any of the other lots.

Effect of heat treatment upon metallic induced oxidation: A study was made of the influence of metals upon the oxidation of butter oil when the catalyst was added before and after pasteurization of the cream at different temperatures.

In the first series of experiments, metal contamination was prior to heat treatment. The cream was divided into four lots. The first lot was pasteurized at 62.8°C. (145.0°F.) for 30 minutes and served as the control. To the remaining three lots, five ppm. of copper were added in the form of copper sulfate solution. One of these copper-contaminated lots was pasteurized at 62.8°C. (145.0°F.) for 30 minutes another at 85.0°C. (185.0°F.) flash, and the other at 90.6°C. (195.0°F.)

flash. The stability of the butter oil of each of these lots of cream toward oxidation was determined in the hot air oven. Average results of two trials are presented in table 28 and are shown graphically in figure 7.

Table 28. Influence of heat treatment of cream upon metallic induced oxidation when metal contamination occurs before pasteurization. *

Oxidation sequence	Peroxide value			
	Control	5 ppm. copper		
	62.8°-30min	62.8°-30min	85.0°-flash	90.6°-flash
1	2.6	8.4	1.8	1.7
2	3.1	12.2	3.2	2.2
3	7.6	12.0	4.5	3.7
4	8.3	15.9	5.4	5.4
5	9.7	19.7	6.3	7.1

* Complete data presented in table XIV, Appendix.

These results show the expected marked increase in the rate of oxidation of the copper contaminated samples pasteurized at 62.8°C. (145.0°F.). Fat from the metal containing cream heated to 85.0°C. (185.0°F.) showed slightly more stability toward oxidation than the control cream whereas that cream containing metals heated to 90.6°C. (195.0°F.) showed considerably greater stability toward oxidation than the control lot. In earlier studies, the results of which are presented in table 26, it was observed that such temperatures showed no appreciable influence upon the stability of the oil as compared to those samples pasteurized at 62.8°C. (145.0°F.). No explanation is offered as to why samples heated to 85.0° and 90.6° C. in the presence of metals show less oxidation than when no metal was present.

The influence of adding metals after pasteurization upon the oxidation of the butter oil was likewise studied by somewhat the same procedure. In this experiment, the pasteurized cream was cooled to

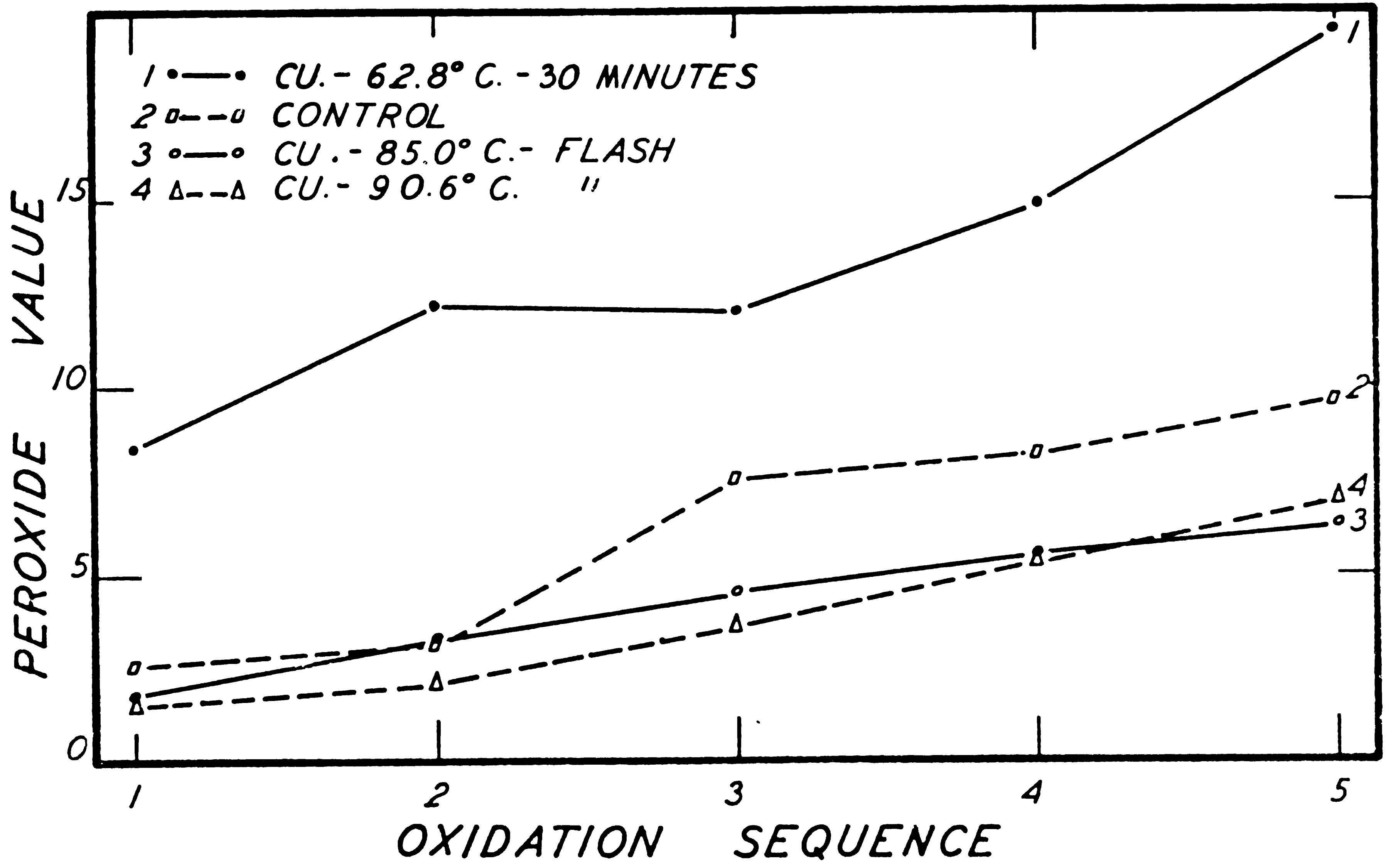


Figure 7. Influence of heat treatment of cream upon oxidation of butter oil when metal contamination occurred before pasteurization.

holding temperature and the copper was added and thoroughly mixed with the cream. The cream was then aged for 12 hours before churning. The average values of four trials are given in table 29, and are illustrated by figure 8.

Table 29. Influence of heat treatment of cream upon metallic induced oxidation when metal contamination occurs after pasteurization.

Oxidation sequence	Peroxide value			
	Control	5 ppm. copper		
	62.8°-30min.	62.8°-30min.	85° - flash	90.6°-flash
1	2.8	7.3	3.4	3.2
2	3.1	9.0	4.2	3.8
3	4.9	9.9	7.3	5.7
4	6.9	13.0	8.7	7.9
5	12.7	21.3	10.6	9.9

* Complete data presented in table XV, Appendix.

These data show the addition of metals to cream after pasteurization to be effective in accelerating oxidative changes regardless of the temperature used for pasteurization. However, the cream heated to 85.0°C. (185.0°F.) and 90.6°C. (195.0°F.) show greater stability against metallic induced oxidation than the samples of cream heated only to 62.8°C. (145.0°F.). Nevertheless, none of the samples having metal contamination showed as much stability as the control samples. The samples pasteurized at 90.6° C. (195.0°F.) showed the greatest stability of those samples having metal contamination and was only slightly less resistant to oxidation than the fat from the control lot.

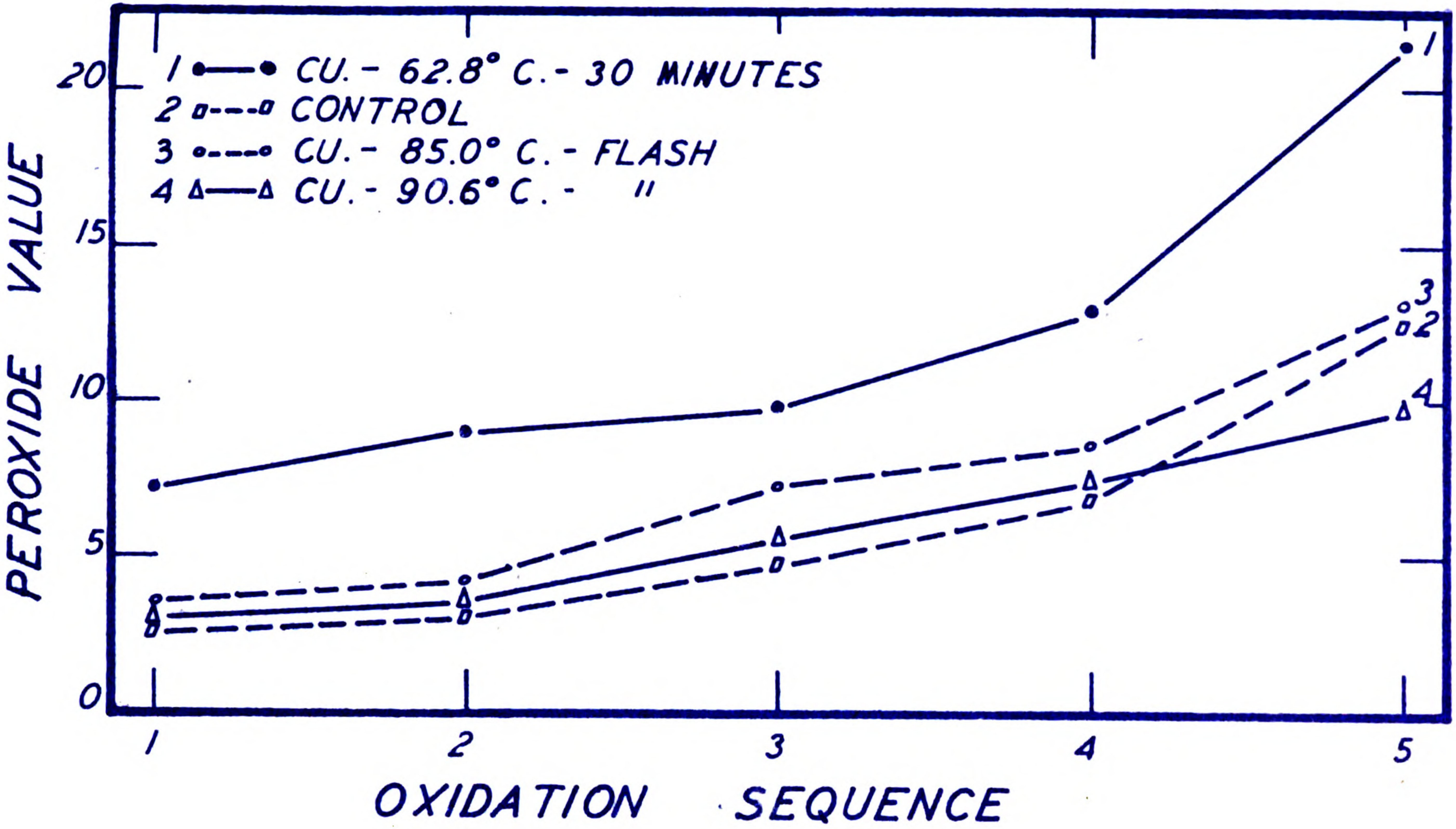


Figure 8. Influence of heat treatment of cream upon oxidation of butter oil when metal contamination occurred after pasteurization.

Studies on Avenex Concentrate

The antioxidant power of Avenex Concentrate was studied when the Concentrate was added to filtered butter oil and to the cream.

In studies dealing with butter oil, cream from raw milk was pasteurized, cooled, and aged six hours before churning. After filtering the butter oil from the butter, five ppm. copper in the form of copper chloride solution was added to half of the butter oil. Both the control and metal containing samples of butter oils were divided into equal portions and 0.015 per cent Avenex Concentrate was added to one portion of each. To be certain of thorough mixing, 300 ml. of all samples were placed into 500 ml Erlenmeyer flasks and shaken in a mechanical shaker for 15 minutes. The butter oil was then stored for 12 hours after which time it was oxidized in the hot air oven. Average results of three trials are presented in table 30. Since there was a difference of several hours between the induction periods of the normal and copper containing samples, such a comparison cannot be made from the average data in table 30 but will have to be made from individual trials in table XVI of the Appendix.

Table 30. Influence upon oxidation of adding Avenex Concentrate to butter oil. *

Oxidation	Normal butter oil		5 ppm. copper	
sequence	No Avenex	Avenex	No Avenex	Avenex
1	4.2	3.1	4.2	4.2
2	4.0	4.5	4.6	5.1
3	6.0	4.6	7.1	5.3
4	7.7	6.4	7.7	6.6
5	11.7	9.7	10.8	8.3

*Complete date presented in table XVI, Appendix.
**Values in peroxide number.

These averages, as well as the individual trials, indicate a slight retarding influence on oxidation by Avenex Concentrate when added to both normal and copper containing butter oil. However, this inhibitive influence is not too definite, the samples containing concentrate having about one hour shorter induction period than those with no antioxidant.

Further studies were conducted in which 0.05 per cent Avenex Concentrate was added to normal cream and to cream containing five ppm. added cooper. A control sample was included in each trial. The cream was aged 12 hours after adding the copper and antioxidant, then churned, and the oil filtered from the butter. The induction periods of these samples of butter oil were determined and the average of three trials are given in table 31.

Table 31. Influence upon oxidation of adding "Avenex Concentrate" to cream after pasteurization.*

Oxidation sequence	Normal cream		5 ppm. copper	
	No Avenex	Avenex	No Avenex	Avenex
1	2.0**	1.7	4.9	3.3
2	3.6	3.4	6.2	3.9
3	4.6	4.7	8.6	5.8
4	6.4	5.3	9.4	6.1
5	6.8	5.4	11.6	8.6

* Complete data presented in table XVII, Appendix.
** Values in peroxide numbers.

These results show no appreciable influence of Avenex Concentrate upon oxidation when added to normal cream. However, in the presence of a metallic catalyst, the Concentrate did show an appreciable antioxidant action. This tendency was uniform in all three individual trials, with the Avenex-containing samples showing about two hours longer induction period than similar samples without the antioxidant.

Carotene Destruction as Related to Peroxide Formation

Since the destruction of vitamin A in fish oils has been shown to coincide closely to the peroxide formation, the possibility exists of a relationship between carotene destruction in butter fat and peroxide formation. If a close relationship does exist, determination of the carotene content could possibly be used as a method of determining the end of the induction period. Studies of the carotene content of butter oil oxidized to different peroxide contents at different temperatures in the hot air oven were made. The results of these studies are given in table 32 an average of which is shown graphically in figure 9.

Table 32. Comparison of carotene destruction and increases in peroxide content of butter fat.*

70° C.		100° C.		130° C.	
Peroxide value	carotene	Peroxide value	carotene	Peroxide value	carotene
0.0	8.14	0.0	8.14	0.0	8.14
1.1	4.42	0.8	5.11	3.1	2.36
1.9	3.37	1.4	4.14	4.1	1.32
2.5	2.52	2.0	3.99	5.3	0.90
3.5	1.81	2.9	3.16	6.5	1.26
4.5	2.11	5.0	1.81	7.5	1.22
7.8	0.72	7.3	1.09	8.5	0.86
19.6	1.05	10.0	0.63	9.5	0.63
37.3	0.55	13.4	0.74	12.0	0.48

* Carotene expressed as micrograms per gm. fat.

These figures and the curve show a close correlation between the destruction of carotene and the formation of peroxides, especially at the lower peroxide values. The most rapid changes in carotene content resulted before a peroxide value of three was reached. Beyond this peroxide value the carotene content decreased steadily but slowly.

No significant difference was observed between the samples oxidized at various oven temperatures.

From the data obtained, it seems entirely feasible to use the carotene determination as an index for quantitatively measuring oxidative changes of butter fat. The carotene determination is especially sensitive in detecting initial changes of oxidation; for example, about 50 per cent of the carotene had been destroyed before a peroxide value of 1.5 had been reached. The oxidation, as measured by the peroxide value, takes place slowly up to a peroxide value of 1.5. Above this value,the peroxide formation increases rapidly. Therefore, the carotene determination offers a more sensitive index of initial oxidative changes of butter oil oxidized in the hot air oven than does the peroxide value. However, this same relationship between initial oxidative changes and carotene destruction may not exist if the butter fat is allowed to oxidize under different conditions, such as the oxidation of cream or butter in storage.

In all trials, the carotene content of the fat did not drop below 0.48 micro-grams per gram of fat even in extreme cases of oxidation. This discrepancy may be due to the presence of small amounts of unoxidized carotene or possibly other substances that may interfere with the photometric measurement.

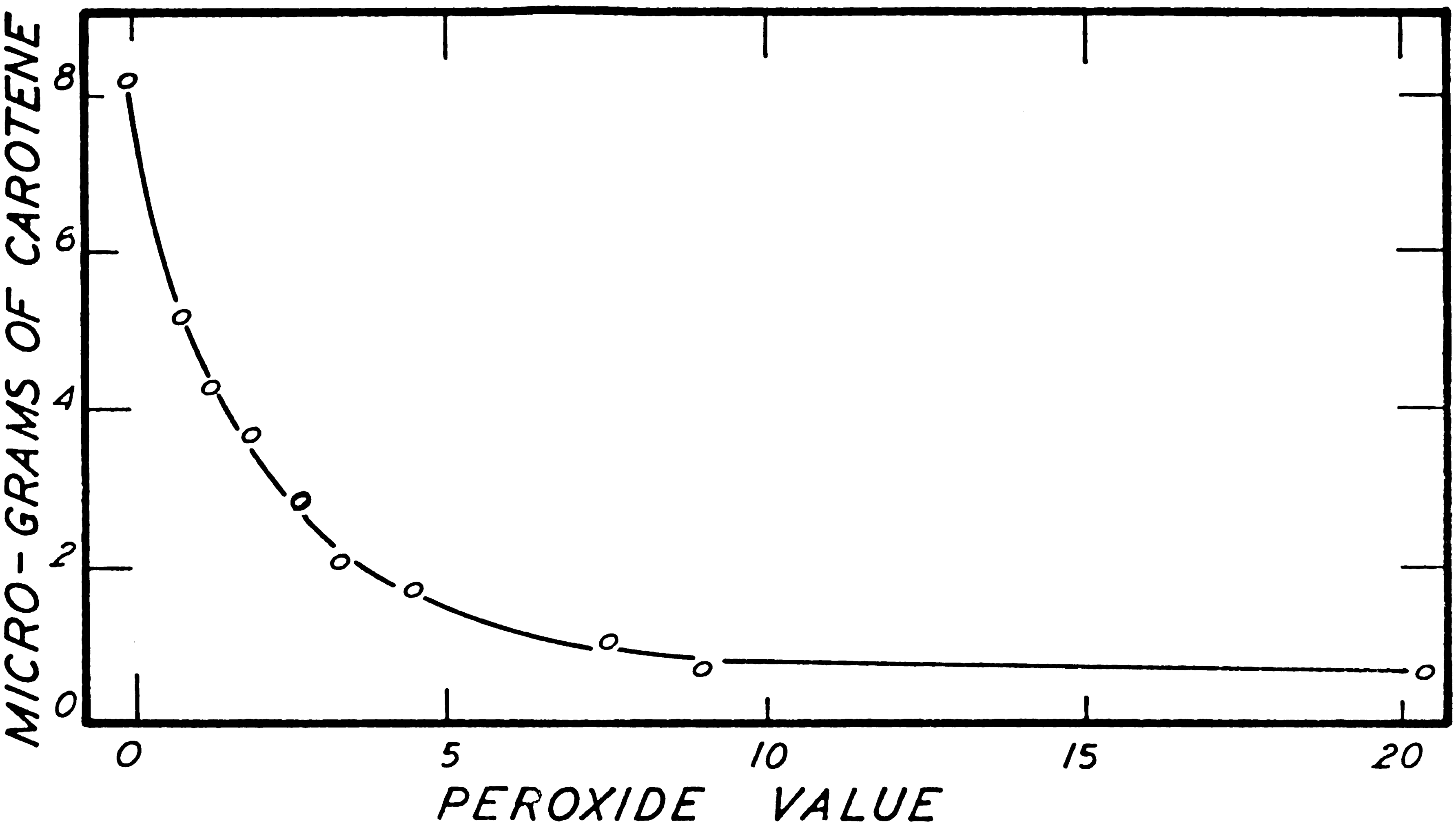


Figure 9. Destruction of carotene with peroxide formation.

DISCUSSION

Studies of the aeration method of accelerating oxidation of butter fat indicate that if proper technique is exercised this method will give reliable results. Triplicate determinations of the induction period of five fats having induction periods ranging from 5.4 to 22.3 hours in length revealed maximum errors in induction periods from 3.8 to 11.1 per cent. These results show even greater accuracy than reported by Freyer (40) who found that by allowing 17 per cent error in induction period, some of 11 different laboratories reported results outside this liberal range.

The oven method, when variable factors were controlled, also permitted accurate determination of the induction period. Studies involving quadruplicate determinations of the same fat showed a maximum difference in induction periods from 3.3 to 14.7 per cent, results comparable to those secured with the aeration method. Additional data obtained by making a direct comparison of the two methods, indicate no significant difference in their accuracy.

The oven method used in this study gave much closer agreement between duplicate samples than the method used by Dahle and Nelson (33). These workers oxidized butter fat in an oven at 60° C. in 50 ml. stoppered test tubes. The samples were allowed to oxidize for 15 days and samples were taken at definite intervals for peroxide determinations. The induction period of identical tubes of the same fat varied as much as four days; a 26.7 per cent variation.

Control of factors which may contribute to uneven oxidation is necessary if an oven method is to be satisfactorily used. The factor of temperature control is especially important. The position of the samples in the oven, and the use of positive air circulation are other necessary considerations as shown by the results secured in this study.

The temperature at which fat should be oxidized in an acceleration method is a question of importance. Too low an oxidation temperature will prolong the induction period, thereby invalidating the advantage of the rapid oxidation method. Although 100° C. was found to be a desirable temperature to oxidize butter fat, different temperatures would necessarily be used with other fats having greater or less stability toward oxidation than butter fat. Furthermore, it may be advantageous to vary this temperature in the case of butter fat for different size samples or for differences in surface area of the fat.

Since the rate of oxidation of fat in the hot air oven is influenced by the surface area per volume of fat, the splashing of fat on the sides of the beakers must be carefully avoided. Such a practice increases the surface area thereby increasing the speed of oxidation.

Even though the oven method is not perfect in many respects, it does offer certain advantages over the aeration method. The oven method appears to be as accurate as the aeration method and in certain commercial laboratories would likely be even more accurate. It requires fewer pieces of apparatus to cause contamination and the operation procedure is more simple. Furthermore, the apparatus necessary for the oven method is relatively inexpensive and may be used in other laboratory operations. However, before adopting an oven method, preliminary studies should be made by each technician to determine the control measures necessary to insure accurate results under his own conditions and with the apparatus available. Individual ovens may vary greatly as to air agitation and temperature uniformity, and consideration should be given to the possibility of such variations.

One point that should be mentioned in connection with any method used for the acceleration of oxidation is that of cleaning and rinsing the glassware coming into contact with the fat. The test tubes, air tubes, and condenser tubes of the aeration apparatus, and the beakers or other fat containers used in the hot air oven, should be given a thorough treatment so as to be certain that every trace of impurity is removed. That the treatment of glassware used in oxidation studies must be thorough and rigorous is indicated by the work of Laug (66) and Stebnitz and Sommer (115) who found that several rinsings with distilled water were necessary to eliminate cleaning compounds from the glass.

The fact that extremely high temperatures may effect destruction of the natural antioxidant properties of butter fat or possibly produce slight oxidation, is indicated by the results of the studies on heat treatment of butter oil. This may be an important consideration in the preservation of butter oil by heat sterilization.

Heating the cream to temperatures above the temperature of boiling water caused considerable decrease in stability of the resulting butter fat. Practical application of these findings may be made in the case of the cream pasteurization processes involving extremely high temperatures. However, in practice, when temperatures above boiling are used, the exposure is instantaneous. Thus, the actual treatment would be less drastic than in these trials in which the steam autoclave was used and the samples were held at the designated temperature for 30 minutes.

The studies of the effect of heat treatment of cream upon metallic induced oxidation were particulerly interesting. In this connection temperatures of 85.0° C. (185.0° F.) and 90.6° C. (195.0° F.) were of advantage in reducing oxidative changes as compared to 62.8° C. (145.0° F.) both when the metal was added before and after pasteurization. These observations are in line with the findings of Ritter (93) who found that cream pasteurized at 90°C. did not develop fishy flavors whereas that processed at lower temperatures was conducive to this flavor change.

From the findings of Gould and Sommer (42) and Josephson and Doan (58) the preservation action of temperatures of 85.0° C. (185.0°F.) and 90.6° C. (195.0°F.) may be expected. These investigators found that heating milk momentarily to 76.0° to 78.0°C. caused a formation of highly-reducing sulphur-compounds resulting in a lower oxidation-reduction potential and a prevention of oxidized flavor development. Therefore, pasteurization of cream to temperatures from 85.0° C. (185.0° F.) to 90.6° C. (195.0° F.) should form sulphides, some of which would remain with the butter after churning causing the butter and butter oil to be more stable toward oxidative changes during storage.

The sulphur compounds also would prevent oxidation by inactivation of metallic catalysts which may be present. This fact is especially pertinent to many processing plants practicing high temperature pasteurization of cream. The results in this study show that temperature of 85.0° C. (185.0° F.) and 90.6° C. (195.0° F.) have an inhibiting influence upon metallic induced oxidation, especially when metal contamination occurs before pasteurization. The findings indicate the extreme necessity of preventing metallic contamination after pasteurization in order to enable maximum stability of butter against oxidation during storage.

Results secured with Avenex Concentrate agree with those obtained by Dahle and Nelson (33) but do not agree in all phases with those secured by Musher (83). The data herein presented show that adding Avenex Concentrate to butter oil with and without metal contamination created no great increase in stability of the oil toward oxidation. Contrary to this, Musher (83) found the addition of from one to five per cent Avenex to butter oil increased its stability against oxidation from 180 to 280 per cent. The reason for this difference may have been (a) Musher's use of extremely unstable butter oil, (b) Avenex was used by Musher whereas Avenex Concentrate was used in these studies, and (c) Musher used much higher concentration of the oat flour product than was used in these investigations.

SUMMARY AND CONCLUSIONS

Preliminary studies were conducted of the peroxide method to ascertain the possibility of decreasing the amount of solvent and potassium iodide used. Data which were secured indicate that the amount of solvent cannot be reduced appreciably without decreasing considerably the maximum peroxide value obtained. However, the concentration of the potassium iodide solution can be altered from the recommended saturated solution to a 50 per cent solution with only a slight decrease in peroxide value.

Comparison of the acid degree and peroxide value indicates that the acid degree is not sufficiently sensitive to be a desirable method for detecting initial oxidative changes in butter oil.

Investigations were made to compare the aeration and hot air oven methods of accelerating fat oxidation at 100° C. Results showed these two methods to be equally reliable when careful technique was exercised and consideration was given the influence of certain variable factors. Under carefully controlled conditions, the differences in the induction periods of the same fat should agree within 6.4 per cent. This would amount to 0.64, 1.28, and 1.92 hours difference at 10, 20, and 30 hours induction periods respectively.

Air agitation, position of the samples in the oven, size of samples, and the surface area of the samples are factors influencing the accuracy of the oven method. In the oven used, it was found necessary to use positive air circulation to enable minimum temperature variation between different samples on the same shelf. Also grouping of samples in the middle of the shelf reduced the maximum temperature variation. The bottom shelf of the oven studies had a consistently higher temperature than the top shelf.

The induction period of butter fat as determined in the oven method is influenced by the surface area and the size of sample. The rate of oxidation increases directly with the surface area and inversely with the size of sample.

Studies were made of the oxidation of butter fat in the hot air oven at different temperatures by using two identical ovens regulated at 70.0° and 100.0° C. and then at 100.0° and 130.0° C. The temperature of oxidation was found to have little influence upon the accuracy of the method, the results showing 9,9, and 9.4, and 11.6 per cent maximum deviation respectively between 70.0°, 100.0°, and 130.0° C. Consequently, the temperature which should be used, in one which permits rapid acceleration of oxidation without undue destruction of peroxides. At the same time, the rate of oxidation should be so governed that samples may be removed for examination at intervals convenient to the laboratory operator.

The heating of butter oil to 127.0° C. (262.0° F.) for 30 minutes in the autoclave markedly decreased its stability, whereas heating to 109.8° C. (229.6° F.) for 30 minutes did not appreciably influence its rate of oxidation as compared to non-heated butter oil. Butter, as such, appears to be much more stable against oxidative changes than the filtered butter oil.

Pasteurization temperatures of the cream up to and including 90.6° C. (195.0° F.) did not decrease the stability of the resulting butter oil. In fact, longer exposures of the cream to 90.6° C. (195.0° F.) appeared to increase the stability of the butter oil slightly, although the data were somewhat variable.

The addition of five ppm. copper to cream before pasteurization did not increase the rate of oxidation of the butter oil when pasteurization temperatures of 85.0° C. (185.0°F.) and 90.6° C. (195.0° F.) were used. However, the fat from similarly treated cream that was pasteurized at 62.8° C. (145.0° F.) oxidized considerably sooner than the control.

When metal contamination occurred after pasteurization, the copper containing samples oxidized more rapidly than the control at all temperatures studied. However, in the case of samples heated to 85.0° C. (185.0° F.) and 90.6° C. (195.0° F.) this increase was only slight whereas at 62.8° C. (145.0° F.) the increase was considerable.

Although heating cream to 85.0° C. (185.0° F.) and 90.6° C. (195.0° F.) had no harmful influence on the stability of the butter oil, temperatures of 109.8° C. (229.6° F.) and 127.0° C. (262.0°F.) decreased the stability of the butter oil. The higher temperature was especially effective in this connection.

On the basis of the results of this study, pasteurization temperatures for cream of approximately 85.0°C. (185.0° F.) to 90.6° C. (195.0° F.) should be used from the standpoint of reducing the catalytic influence of metallic contamination.

The addition of 0.015 per cent Avenex Concentrate to butter oil retarded only slightly the rate of oxidation of the butter oil. When 0.05 per cent Avenex Concentrate was added to cream after pasteurization, the retarding influence on oxidation was only slight when normal cream was used. However, when metal contaminated cream was used, the influence of the antioxidant was more pronounced.

Comparisons were made of the carotene destruction and peroxide formation by determining the carotene content of butter fat at various peroxide values. Average results showed that by the time the peroxide value had increased to 2.0, the carotene content decreased from 8.1 to 3.4 micro-grams per gram of fat, a decrease of 58.0 per cent of the total carotene. This indicates the possibility that the carotene determination may be a more sensitive index of the initial oxidation of butter oil than the peroxide value.

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APPENDIX TABLES

Table I. Variation between oxidation of duplicate samples by aeration method.

Hours oxidation	Peroxide value a	Peroxide value b	Difference between a and b	:	Hours oxidation	Peroxide value a	Peroxide value b	Difference between a and b
4	3.3	3.9	0.6	:	5	7.9	8.4	0.5
6	14.7	13.8	0.9	:	7	22.2	18.1	4.1
8	24.6	25.0	0.4	:	9	27.6	30.0	2.4
4	3.3	3.6	0.3	:	5	7.9	8.6	0.7
6	14.7	13.4	1.3	:	7	22.2	18.0	4.2
8	24.6	25.3	0.7	:	9	27.6	31.6	4.0
6	0.6	0.9	0.3	:	9	3.7	4.2	0.5
10	4.9	11.0	6.1	:	11	7.7	7.4	0.3
12	16.3	16.5	0.2	:	13	14.0	19.5	4.5
6	0.6	0.7	0.1	:	9	3.7	5.1	1.4
10	4.9	8.4	3.5	:	11	7.7	12.1	4.4
12	16.3	14.6	1.7	:	13	14.0	14.6	0.6
7	1.0	1.0	0.0	:	8	1.2	1.1	0.1
9	1.5	1.3	0.2	:	10	4.4	3.3	1.1
11	5.1	6.0	0.9	:	12	8.6	8.5	0.1
7	1.0	1.2	0.2	:	8	1.2	1.2	0.0
9	1.5	1.3	0.2	:	10	4.4	2.6	1.8
11	5.1	4.5	0.6	:	12	8.6	8.6	0.0
8	0.7	0.8	0.1	:	11	1.2	1.3	0.1
12	1.5	1.4	0.1	:	13	1.5	1.3	0.2
15	1.8	1.7	0.1	:	18	1.6	2.1	0.5
8	0.7	1.0	0.3	:	11	1.2	1.1	0.1
12	1.5	1.3	0.2	:	13	1.5	1.6	0.1
15	1.8	1.7	0.1	:	18	1.6	1.5	0.1
23	5.0	8.1	3.1	:	24	8.5	9.7	1.2
25	12.9	11.0	1.9	:	26	20.8	12.2	8.6
27	28.5	19.3	9.2	:	28	32.7	25.5	7.2
23	5.0	10.0	5.0	:	24	8.5	9.8	1.3
25	12.9	21.2	8.3	:	26	20.8	10.8	10.0
27	28.5	34.0	5.5	:	28	32.7	28.3	4.4
20	3.0	4.8	1.8	:	21	3.2	2.8	0.4
22	6.3	5.3	1.0	:	23	14.5	15.5	1.0
24	25.2	45.2	20.0	:	25	28.5	22.0	6.5
20	3.0	3.1	0.1	:	21	3.2	4.9	1.7
22	6.3	9.2	2.9	:	23	14.5	17.8	3.3
24	25.2	26.4	1.2	:	25	28.5	33.7	5.2
19	1.3	1.3	0.0	:	20	4.1	8.3	4.2
21	7.2	11.2	4.0	:	22	4.1	3.1	1.0
23	7.2	13.6	6.4	:	19	5.3	6.7	1.4
20	11.8	11.6	0.2	:	21	21.2	18.6	2.6
20	11.8	15.7	3.9	:	21	21.2	15.9	5.3
12	1.2	1.1	0.1	:	16.5	1.6	1.5	0.1
12	1.2	1.2	0.0	:	16.5	1.9	3.8	1.9
17	12.7	3.5	9.2	:	12	1.4	1.3	0.1
17.5	15.0	13.5	1.5	:	19	1.3	1.3	0.0

Table I. (continued.)

Hours oxidation	Peroxide value a	Peroxide value b	Difference between a and b	Hours oxidation	Peroxide value a	Peroxide value b	Difference between a and b
22	4.1	8.3	4.2	23	7.2	11.2	4.0
22	4.1	3.1	1.0	23	7.2	13.6	6.4
19	5.3	6.7	1.4	20	11.8	11.6	0.2
21	21.2	18.6	2.6	20	11.8	15.7	3.9
21	21.2	16.0	5.2	22	1.6	1.9	0.3
24	8.1	9.0	0.9	22	1.6	1.8	0.2
24	8.1	7.6	1.5	24	8.1	9.5	1.4
24	18.6	18.4	0.2	22	10.6	9.9	0.7
24	18.6	17.3	1.3	22	10.6	12.3	1.7
24	18.6	18.4	0.2	18	1.7	1.7	0.0
20	7.4	10.6	3.2	21	12.9	16.5	3.6
18	1.7	1.6	0.1	20	7.4	6.4	1.0
21	12.9	14.9	2.0	18	11.3	13.2	1.9
19	16.8	18.1	1.3	20	36.8	28.7	8.1
18	11.3	5.7	5.6	19	16.8	21.1	4.4
20	36.8	31.8	5.0	16	2.0	1.8	0.2
18	11.8	13.7	1.9	16	2.0	3.2	1.2
13	1.7	1.0	0.7	16	14.6	19.1	4.5
13	1.7	.50	1.2	15	6.3	2.5	3.8
16	14.6	16.1	1.5	17	1.5	1.6	0.1
19	2.7	2.5	0.2	21	1.6	8.6	7.0
17	1.5	1.5	0.0	19	2.7	1.4	1.3
21	1.6	4.3	2.7	15	1.4	1.7	0.3
16	1.6	1.4	0.2	17	6.2	6.2	0.0
15	1.4	1.6	0.2	19	1.5	1.8	0.3
20.5	2.4	2.4	0.0	21	8.4	10.1	1.7
19	1.5	1.9	0.4	20.5	2.4	4.1	1.7
21	8.4	11.7	3.3	18	4.8	3.6	1.2
18.5	5.7	5.5	0.2	19	16.4	18.7	2.3
18	4.8	4.7	0.1	18.5	5.7	7.4	1.7
19	16.4	14.1	2.3	20	6.0	1.6	4.4
22	1.9	1.7	0.2	24	3.7	7.3	3.6
20	6.0	1.6	4.4	22	1.9	1.9	0.0
24	3.7	4.1	0.4	18	1.7	1.8	0.1
19	10.4	4.3	6.1	19.5	4.8	18.1	13.3
18	1.7	12.8	11.1	19	10.4	10.0	0.4
19.5	4.8	7.1	2.3				

Table II. Preliminary trials with aeration method.

Hours oxidation	Peroxide values of duplicates a	b	c
	Trial 1		
4	3.3	3.9	3.6
5	7.9	8.4	8.6
6	14.7	13.8	13.4
7	22.2	18.1	18.0
8	24.6	25.0	25.3
9	27.6	30.0	31.6
	Trial 2		
6	0.6	0.9	0.7
9	3.7	4.2	5.1
10	4.9	11.0	8.4
11	7.7	7.4	12.1
12	16.3	16.5	14.6
13	14.0	19.5	14.6
	Trial 3		
7	1.0	1.0	1.2
8	1.2	1.1	1.2
9	1.5	1.3	1.3
10	4.4	3.3	2.6
11	5.1	6.0	4.5
12	8.6	8.5	8.6
	Trial 4		
20	3.0	4.7	3.1
21	3.2	2.8	4.9
22	6.3	5.3	9.2
23	14.5	15.5	17.8
24	25.2	45.2	26.4
25	28.5	22.0	33.7
	Trial 5		
19	1.3	1.3	
22	4.1	8.3	3.1
23	7.2	11.2	13.6

Table III. Variation in peroxide value of duplicate samples taken from top and bottom shelves of hot air oven at same time.

Bottom shelf	Top shelf	Difference	Bottom shelf	Top shelf	Difference
8.0	9.4	-1.4	4.1	1.9	2.2
8.3	6.8	1.5	1.6	1.5	0.1
19.4	16.7	2.7	2.0	2.3	-0.3
28.6	24.0	4.6	5.1	2.7	2.4
1.8	1.8	0.0	9.6	5.1	4.5
5.7	5.4	0.3	19.6	17.3	2.3
8.3	6.9	1.4	32.4	25.2	7.2
13.3	10.6	2.7	2.2	1.1	1.1
1.6	3.5	-1.9	10.8	5.4	5.4
4.1	3.2	0.9	13.2	23.5	-10.3
5.1	7.0	-2.9	2.2	1.7	0.5
10.1	8.3	1.8	8.5	6.1	2.4
2.1	1.8	0.3	7.9	5.8	2.1
3.3	2.6	0.7	2.0	1.8	0.2
3.5	3.9	-0.3	5.8	3.4	2.4
8.6	10.5	-1.9	8.3	8.6	-0.3
1.7	4.8	-3.1	1.8	1.6	0.2
20.3	8.5	11.8	4.1	1.9	2.2
23.2	26.8	-3.6	6.0	6.5	-0.5
1.4	1.6	-0.2	3.9	4.6	-0.7
3.7	3.6	0.1	6.7	3.7	3.0
12.1	24.7	-12.6	7.9	13.4	-5.5
1.7	1.6	0.1	7.9	5.7	2.2
3.1	4.9	-1.8	8.9	8.2	0.7
6.3	5.0	1.3	10.4	9.7	0.7
1.4	1.4	0.0	11.8	8.7	3.1
1.7	1.5	0.2	9.4	11.5	-2.1
11.1	15.7	-4.6	50.5	38.1	12.4
13.0	14.3	-1.3	55.7	40.9	14.8
14.3	23.8	-9.5	0.9	0.9	0.0
17.3	18.0	-0.7	1.0	1.0	0.0
15.3	15.0	0.3	1.2	1.1	0.1
14.4	25.2	-10.8	1.6	1.4	0.2
19.4	35.8	-16.4	1.6	1.8	-0.2
19.3	43.4	-24.1	3.7	2.7	1.0
35.4	29.1	6.3	17.4	4.1	13.3
40.4	49.1	-8.7	1.1	1.2	-0.1
46.2	76.8	-29.6	1.5	1.4	0.1
49.6	39.3	10.3	1.6	1.5	0.1
1.2	1.5	-0.3	2.8	1.8	1.0

(continued)

Table III. (continued)

Bottom shelf	Top shelf	Difference	Bottom shelf	Top shelf	Difference
1.3	1.6	-0.3	2.6	3.9	-1.3
1.9	1.6	0.3	9.2	10.0	-0.8
1.8	2.0	-0.2	24.8	15.1	9.7
4.6	3.9	0.7	1.2	1.2	0.0
3.8	3.2	0.6	1.4	1.3	0.1
12.2	13.2	-1.0	1.4	1.5	-0.1
7.7	8.5	-0.8	4.9	2.5	2.4
11.6	10.3	1.3	7.7	3.8	3.9
10.0	11.9	-1.9	14.8	17.6	-2.8
17.2	17.5	-0.3	20.5	24.6	-4.1
18.5	21.5	-3.0	1.0	1.1	-0.1
22.5	25.1	-2.6	1.2	1.1	0.1
23.5	34.3	-10.8	1.3	1.3	0.0
26.9	40.8	-13.9	1.9	1.7	0.2
39.1	39.2	-0.1	2.3	1.5	0.8
40.8	49.0	-8.2	7.3	1.5	5.8
53.1	34.3	18.8	6.2	1.6	4.6
1.3	1.4	-0.1	18.8	26.2	-7.4
1.3	1.4	-0.1	1.0	1.0	0.0
2.6	1.8	0.8	1.4	1.5	-0.1
7.8	7.2	0.6	4.9	1.9	3.0
6.8	5.0	1.8	7.9	4.4	3.5
11.5	11.7	-0.2	7.2	13.3	-6.1
13.8	6.3	7.5	16.2	16.6	-0.4
1.3	1.2	0.1	21.1	14.5	6.6
1.4	1.5	-0.1	4.8	7.1	-2.3
3.0	2.0	1.0	12.3	10.7	1.6
2.5	4.8	-2.3	9.4	11.0	-1.6
8.1	6.8	1.3	13.6	16.1	-2.5
14.3	6.7	7.6	13.2	16.7	-3.5
18.4	11.3	7.1	15.2	21.3	-6.1
0.7	1.0	-0.3	20.3	26.9	-6.6
1.5	1.4	0.1	4.4	4.8	-0.4
3.6	1.8	1.8	7.8	7.3	0.5
3.9	8.3	-4.6	8.2	9.0	-0.8
12.7	9.2	3.5	10.4	17.0	-6.6
16.1	9.3	6.8	10.7	15.0	-4.3
15.6	9.6	6.0	14.3	11.8	2.5
1.3	1.3	0.0	18.1	12.2	5.9
1.8	1.4	0.4	5.6	6.7	-1.1

(continued)

Table III. (continued)

Bottom shelf	Top shelf	Difference	Bottom shelf	Top shelf	Difference
3.2	3.5	-0.3	17.1	19.2	-2.1
7.3	11.3	-4.0	7.5	16.9	-9.4
11.3	12.5	-1.2	9.2	18.6	-9.4
15.8	8.8	7.0	9.7	9.9	-1.2
14.1	11.5	2.6	1.8	1.3	0.5
21.2	14.0	7.2	4.3	1.8	2.5
1.7	1.5	0.2	7.7	2.0	5.7
2.1	1.9	0.2	1.0	1.0	0.0
3.6	3.5	0.1	1.1	1.1	0.0
7.6	3.4	4.2	1.4	1.3	0.1
8.3	4.9	3.4	1.6	1.4	0.2
10.6	6.0	4.6	1.5	1.8	-0.3
13.1	8.2	4.9	3.3	1.7	1.6
1.6	1.5	0.1	8.8	1.6	7.2
3.6	1.7	1.9	2.8	3.1	-0.3
2.1	1.1	1.0	11.1	8.2	2.9
5.9	4.1	1.8	14.1	10.2	3.9
8.0	4.2	3.8	12.4	10.8	1.6
9.1	6.1	3.0	14.1	22.2	-8.1
12.5	6.8	5.7	19.0	16.0	3.0
8.1	15.4	-7.3	27.6	25.9	2.3
18.9	12.5	6.4	0.9	1.0	-0.1
19.6	17.4	2.2	2.2	1.1	1.1
13.3	13.3	0.0	3.4	1.4	2.0
13.3	21.4	-8.1	8.8	3.8	5.0
15.7	27.5	-11.8	11.4	4.7	6.7
18.7	17.2	1.5	15.6	9.1	6.5
1.0	1.0	0.0	1.4	1.2	0.2
1.1	1.1	0.0	5.4	3.8	1.6
1.5	1.2	0.3	13.0	8.1	4.9
1.7	1.4	0.3	12.5	19.9	-7.4
24.6	19.6	5.0	28.3	18.5	9.8
28.1	19.7	8.4	0.9	0.9	0.0
6.5	5.6	0.9	1.4	1.5	-0.1
17.2	18.0	-0.8	1.9	1.5	0.4
21.6	16.3	4.3	4.5	2.1	2.4
18.5	16.2	2.3	14.8	7.8	7.0
24.1	27.5	-3.4	18.2	11.4	6.8
28.2	21.0	7.2	1.0	0.9	0.1
0.8	0.7	0.1	1.3	1.3	0.0

Table III. (continued)

Bottom shelf	Top shelf	Difference	Bottom shelf	Top shelf	Difference
0.9	0.9	0.0	1.5	1.3	0.2
1.3	1.2	0.1	1.8	1.3	0.5
1.4	1.3	0.1	7.7	2.1	5.6
2.2	1.8	0.4	9.0	6.6	2.4
7.9	1.6	6.3	19.4	13.4	6.0
11.0	1.6	9.4	27.8	16.7	11.1
0.9	1.0	-0.1	23.0	43.7	-20.7
1.1	0.9	0.2	33.7	30.2	3.5
1.2	1.3	-0.1	26.6	31.4	-4.8
1.8	1.5	0.3	23.6	30.9	-7.3
6.3	2.0	4.3	33.7	56.7	-23.0
11.3	5.9	5.4	48.9	40.2	8.7
15.2	11.0	4.2	1.0	0.8	0.2
1.3	1.2	0.1	2.8	1.0	1.8
2.4	1.5	0.9	1.2	1.2	0.0
6.7	6.1	0.6	2.1	1.7	0.4
11.4	11.6	-0.2	3.8	2.2	1.6
17.3	24.3	-7.0	19.8	4.9	14.9
19.4	19.2	0.2	12.4	6.4	6.0
0.9	0.7	0.2	16.1	8.4	7.7
1.1	1.1	0.0	0.8	1.1	-0.3
1.5	1.3	0.2	1.1	1.0	0.1
3.5	1.6	1.8	1.6	1.3	0.3
9.7	5.2	3.5	1.8	1.7	0.1
17.8	6.2	11.6	6.0	1.9	4.1
26.1	16.2	10.1	6.9	3.5	3.4
7.2	6.1	1.1	14.6	10.1	4.5
21.8	10.1	11.7	1.1	1.0	0.1
18.0	17.2	0.8	1.3	1.1	0.2
20.3	15.8	4.5	1.2	1.2	0.0
26.3	40.5	-14.2	1.6	1.5	0.1
27.1	39.0	-11.9	2.0	1.8	0.2
45.6	43.1	2.5	5.7	2.0	3.7
0.7	0.6	0.1	10.5	9.3	1.2
1.1	0.8	0.3	1.1	0.9	0.2
1.1	1.1	0.0	1.0	1.2	-0.2
1.6	1.5	0.1	1.1	1.1	0.0
2.2	1.5	0.7	1.4	1.6	-0.2
5.0	1.7	3.3	1.6	1.6	0.0
13.1	1.9	11.2	3.7	1.7	2.0

(continued)

Table III. (continued)

Bottom shelf	Top shelf	Difference	Bottom shelf	Top shelf	Difference
1.2	1.0	0.2	8.1	8.2	-0.1
1.2	1.2	0.0	1.2	1.3	-0.1
1.6	1.7	-0.1	1.5	1.4	0.1
3.0	1.8	1.2	1.6	2.8	-1.2
8.8	3.5	5.3	7.4	3.9	3.5
8.6	6.2	2.4	2.4	1.6	0.8
12.3	19.1	-6.8	7.3	2.9	4.4
21.6	19.6	2.0	12.7	3.3	9.4
0.8	1.0	-0.2	16.5	12.7	3.8
1.1	1.1	0.0	1.0	1.0	0.0
1.2	1.2	0.0	1.2	1.1	0.1
1.8	1.5	0.3	1.6	1.3	0.3
3.3	1.7	1.6	1.9	1.7	0.2
6.7	1.8	4.9	7.7	3.7	4.0
14.2	5.8	8.4	11.8	12.7	-0.9
1.0	1.0	0.0	20.0	12.4	7.6
0.9	1.1	-0.2	1.1	1.2	-0.1
1.3	1.2	0.1	1.4	1.4	0.0
1.7	1.5	0.2	1.7	1.6	0.1
2.2	1.6	0.6	4.7	3.4	1.3
10.6	5.7	4.9	10.3	7.4	2.9
15.2	4.4	10.8	13.0	17.2	-4.2
0.9	1.1	-0.2	23.4	21.4	2.0
1.1	1.2	-0.1	1.1	1.1	0.0
1.5	2.6	-1.1	1.4	1.4	0.0
3.2	1.7	1.5	1.9	1.4	0.5
8.6	5.0	3.6	7.7	2.6	5.1
12.8	16.7	3.1	15.7	16.6	-0.9
23.1	14.1	9.0	1.0	1.0	0.0
1.0	0.9	0.1	1.4	1.1	0.3
1.1	1.3	-0.2	1.5	1.4	0.1
1.4	1.4	0.0	2.4	2.2	0.2
12.6	6.9	5.7	2.2	3.2	-1.0
0.9	0.9	0.0	3.3	2/6	0.7
1.6	1.1	0.5	5.1	2.4	2.7
1.2	1.1	0.1	6.4	2.7	3.7
1.6	1.3	0.3	2.5	2.3	0.2
2.4	1.6	0.8	5.5	2.9	2.6
18.3	7.5	10.8	6.6	2.8	3.6
8.4	8.5	-0.1	9.6	3.6	6.0

(continued)

Table III. (continued)

Bottom shelf	Top shelf	Difference	Bottom shelf	Top shelf	Difference
13.6	14.7	-1.1	13.3	10.9	2.4
20.5	6.8	13.7	2.3	2.4	0.1
30.6	15.2	15.4	5.1	2.3	2.8
1.8	1.7	0.1	5.8	4.0	1.8
3.7	2.7	1.0	6.5	3.5	3.0
9.3	7.9	1.4	10.6	8.0	2.6
8.2	6.0	2.2	2.5	3.5	-1.0
8.9	16.0	-7.1	6.4	2.3	4.1
1.8	1.8	0.0	5.7	3.6	2.1
3.2	4.1	-0.9	5.5	7.9	2.4
6.6	3.3	3.3	13.4	9.6	3.8
7.8	6.5	1.3	2.1	2.2	-0.1
10.9	7.8	3.1	3.0	2.3	1.0
1.6	1.8	-0.2	5.3	2.4	2.9
2.3	2.0	0.3	5.7	2.6	3.1
3.9	3.2	0.7	9.1	5.7	3.4
10.9	3.4	7.5	1.3	1.0	0.3
9.1	4.7	4.4	1.8	1.4	0.4
1.5	1.6	-0.1	1.4	1.3	0.1
5.0	1.7	3.3	0.8	0.9	-0.1
6.4	1.9	4.5	1.3	1.4	-0.1
1.2	1.2	0.0	1.5	1.3	0.2
1.5	1.2	0.3	1.7	1.8	-0.1
1.6	1.4	0.2	16.0	10.0	6.0
2.6	1.7	0.9	0.8	0.8	0.0
5.4	1.8	3.6	1.3	1.3	0.0
1.3	1.2	0.1	1.7	1.4	0.3
1.6	1.4	0.2	3.0	1.7	1.3
1.8	1.4	0.2	6.8	7.5	-0.7
2.4	1.6	0.8	1.6	1.6	0.0
6.3	1.6	4.7	5.9	3.4	2.5
1.3	1.2	0.1	10.7	9.1	1.6
1.5	1.4	0.1	16.6	13.5	3.1
3.7	1.4	2.3	20.0	26.7	-6.7
4.6	1.7	2.9	1.3	1.5	-0.2
5.4	1.9	3.4	2.1	1.8	0.3
1.0	0.7	0.3	4.8	2.2	2.6
1.3	1.3	0.0	6.3	6.7	-0.4
1.5	1.2	0.3	12.5	8.3	4.2
2.0	1.7	0.3	1.5	1.3	0.2

(continued)

Table III. (continued)

Bottom shelf	:	Top shelf	:	Difference	*	Bottom shelf	:	Top shelf	:	Difference
7.2	:	16.6	:	-9.4	*	1.9	:	1.7	:	0.2
0.9	:	0.9	:	0.0	*	4.6	:	2.2	:	2.4
1/5	:	1.2	:	0.3	*	7.8	:	3.3	:	4.5
1.6	:	1.4	:	0.2	*	10.8	:	4.2	:	6.6
4.1	:	2.0	:	2.1	*	1.3	:	1.6	:	-0.3
12.4	:	20.5	:	-8.1	*	2.0	:	1.9	:	0.1
5.8	:	2.9	:	2.9	*	9.9	:	13.3	:	-3.4
7.9	:	3.9	:	4.0	*	5.7	:	4.7	:	1.0
12.0	:	6.0	:	6.0	*	8.6	:	6.8	:	1.8
1.7	:	1.8	:	-0.1	*	8.3	:	8.3	:	0.0
2.0	:	1.8	:	0;2	*	10.2	:	11.2	:	-1.0
5.4	:	3.4	:	2.0	*	15.0	:	20.1	:	-5.1
7.8	:	4.9	:	2.9	*	1.8	:	1.8	:	0.0
8.6	:	10.1	:	-1.5	*	3.6	:	2.0	:	1.6
3.7	:	3.2	:	0.5	*	5.4	:	5.5	:	-0.1
5.6	:	6.1	:	-0.5	*	11.5	:	4.5	:	7.0
6.9	:	5.7	:	1.2	*	10.4	:	6.7	:	3.7
9.5	:	8.7	:	0.8	*	1.8	:	1.8	:	0.0
13.9	:	13.2	:	0.7	*	3.6	:	3.2	:	0.4
1.8	:	1.7	:	0.1	*	5.9	:	4.1	:	1.8
4.1	:	2.1	:	2.0	*	6.8	:	4.3	:	2.5
6.8	:	4.4	:	2.4	*	13.2	:	7.0	:	6.2
9.2	:	6.7	:	2.5	*	2.1	:	2.1	:	0.0
12.4	:	5.7	:	6.7	*	2.1	:	2.2	:	-0.1
1.5	:	1.6	:	-0.1	*	4.5	:	2.4	:	2.1
2.3	:	1.8	:	0.5	*	7.1	:	2.6	:	4.5
4.9	:	3.4	:	1.5	*	10.5	:	11.0	:	-0.5
5.8	:	4.3	:	1.5	*	6.4	:	6.3	:	0.1
8.2	:	5.3	:	2.9	*	11.7	:	8.1	:	3.6
1.6	:	1.5	:	0.1	*	14.3	:	10.7	:	3.6
2.1	:	2.0	:	0.1	*	14.8	:	18.6	:	-3.8
5.1	:	2.6	:	2.5	*	22.8	:	24.9	:	-2.1
6.7	:	4.5	:	2.5	*	2.1	:	1.7	:	0.4
3.0	:	2.0	:	1.0	*	9.1	:	7.5	:	1.6
7.3	:	4.9	:	2.4	*	11.7	:	9.9	:	1.8
9.5	:	6.2	:	3.3	*	2.3	:	1.8	:	0.5
14.0	:	9.3	:	4.7	*	2.4	:	2.2	:	0.2
2.1	:	1.8	:	0.3	*	11.3	:	2.5	:	8.8
3.7	:	2.3	:	1.4	*	13.4	:	3.2	:	10.2
8.1	:	4.9	:	3.2	*	11.4	:	7.0	:	4.4

(continued)

Table III. (continued)

Bottom shelf	Top shelf	Difference	Bottom shelf	Top shelf	Difference
20.4	4.4	16.0	10.1	9.3	0.8
13.6	10.2	3.4	15.1	10.2	4.9
5.1	6.4	1.3	14.3	12.2	2.1
6.2	6.5	0.3	18.0	17.3	0.7
8.9	7.0	1.9	24.9	25.5	-0.6
9.9	11.8	-1.9	2.0	1.8	0.2
16.4	21.3	-4.9	3.7	2.3	1.4
16.2	12.4	3.8	6.8	3.3	3.5
13.2	11.8	1.4	6.9	4.2	2.7
12.3	12.4	-0.1	8.9	3.3	5.6
13.4	17.6	4.2	1.8	1.8	0.0
35.5	25.0	10.5	3.0	2.1	1.1
7.6	10.4	-2.8	5.0	2.9	2.1
8.7	7.8	0.9	9.0	2.4	6.6
13.4	10.6	2.8	15.3	2.2	13.1
12.7	9.8	2.9	1.7	4.4	-2.7
13.2	13.4	-0.2	4.7	3.2	1.5
4.7	10.0	-5.3	8.5	8.1	0.4
6.0	6.9	-0.9	11.3	5.3	6.0
8.0	5.8	2.2	13.0	7.2	5.8
6.9	7.3	-0.4	9.6	6.9	2.7
12.4	11.0	1.4	8.0	7.2	0.8
9.3	12.1	-2.8	15.8	16.9	-1.1
14.9	13.3	1.6	3.6	10.6	-7.0
17.3	11.1	6.2	7.8	4.4	3.4
1.7	1.5	0.2	5.4	4.2	1.2
4.8	1.7	3.1	6.6	5.0	1.6
5.2	2.7	2.5	11.6	10.0	1.6
7.2	3.2	4.0	1.8	1.6	0.2
7.7	5.3	2.4	6.4	1.9	4.5
1.6	1.6	0.0	6.1	2.3	3.8
2.0	1.8	0.2	3.4	2.8	0.6
4.8	1.9	2.9	9.5	6.3	3.2
6.4	3.5	2.9	1.8	1.7	0.1
6.8	3.9	2.9	4.1	3.1	1.0
11.3	7.7	3.6	7.6	1.9	5.7
4.5	6.9	-2.4	6.2	2.3	3.9
6.9	8.0	-1.1	8.4	2.5	5.9
12.6	12.7	-0.1	3.1	4.0	0.9
14.9	26.5	-11.6	5.5	2.2	3.3

(continued)

Table III. (continued)

Bottom shelf	Top shelf	Difference	Bottom shelf	Top shelf	Difference
16.9	15.0	1.9	8.1	6.8	1.3
15.5	13.4	2.1	7.5	5.1	2.4
12.4	17.2	-4.8	9.7	5.8	3.9
17.1	20.3	-3.2	2.8	1.9	0.9
22.7	29.3	-6.6	4.2	2.4	1.8
7.1	13.0	-5.9	6.7	5.3	1.4
4.8	4.8	0.0	5.9	3.5	2.4
10.7	4.4	6.3	2.5	2.0	0.5
1.8	2.9	-1.1	3.0	1.9	1.1
4.9	2.0	2.9	4.6	3.5	1.1
6.7	6.1	0.6	5.0	2.3	2.7
9.9	12.3	-2.4	5.3	10.0	-4.7
8.1	6.5	1.6	6.8	7.9	-1.1
1.6	2.0	-0.4	9.5	10.1	-0.6
5.7	3.1	2.6	11.8	9.8	2.0
6.1	7.8	-1.7	15.0	13.1	1.9
12.0	3.1	8.9	2.3	5.3	-3.0
9.7	4.6	5.1	3.8	5.3	-1.5
3.5	3.6	-0.1	6.1	4.4	1.7
7.2	7.2	0.0	6.9	3.8	3.1
9.0	8.3	0.7	9.4	6.7	2.7
10.3	11.1	-0.8	1.6	1.7	-0.1
13.6	13.3	0.3	3.8	2.3	1.5
1.8	3.6	-1.8	5.6	2.2	3.4
5.2	2.8	2.4	4.0	2.3	1.7
6.9	5.4	1.5	5.7	2.5	3.2
9.8	6.4	3.4	1.7	1.6	0.1
12.8	8.0	4.8	3.3	2.0	1.3
1.7	1.9	-0.2	2.5	2.1-	0.4
4.0	2.1	1.9	2.8	2.1	0.7
4.2	2.1	2.1	4.4	3.9	0.5
5.8	4.4	1.4	1.9	2.1	0.2
7.4	3.1	4.3	3.5	2.4	1.1
1.6	1.6	0.0	2.8	2.1	0.7
5.1	3.8	1.3	3.2	2.9	0.3
7.8	4.8	3.0	3.9	3.4	0.5
1.6	1.6	0.0	5.9	4.1	1.8
2.5	3.3	-0.8	7.2	6.2	1.0
2.1	1.8	0.3	9.6	14.1	-4.5
3.3	2.0	1.3	2.2	1.9	0.3

(continued)

Table III. (continued)

Bottom shelf	Top shelf	Difference	Bottom shelf	Top shelf	Difference
4.1	1.9	2.2	4.1	2.3	1.8
8.5	7.1	1.4	4.6	3.8	0.8
4.8	6.0	-1.2	6.2	6.7	-0.5
8.9	9.2	-0.3	9.6	10.4	-0.8
11.5	11.2	0.3	3.5	4.8	-1.3
16.4	18.0	-1.6	4.9	4.0	0.9
6.1	5.6	0.5	6.8	6.6	0.2
9.3	5.9	3.4	9.5	6.3	3.2
8.4	6.0	2.4	11.7	10.3	1.4
9.8	11.1	-1.3	2.9	6.9	-4.0
15.0	15.2	-0.2	3.9	5.5	-1.6
6.4	6.6	-0.2	6.0	4.4	1.6
8.7	5.5	3.2	7.2	6.0	1.2
13.4	10.9	2.5	8.6	9.1	-0.5
12.1	9.4	2.7	7.8	14.1	-6.3
16.9	13.4	3.5	8.1	12.6	-4.5
5.7	6.7	-1.0	4.9	9.8	-3.9
7.2	8.1	-0.9	8.0	4.6	3.4
9.8	7.5	2.3	5.6	14.2	-8.6
11.9	9.3	2.6	5.8	6.3	-0.5
14.4	11.6	2.8	7.0	3.0	4.0
7.0	5.5	1.5	7.2	7.9	-0.7
3.4	4.9	-1.5	5.5	6.1	-0.6
7.7	11.9	-4.2	4.5	5.3	-0.8
4.9	7.2	-2.3	5.3	5.7	-0.4
7.4	6.7	0.7	5.5	7.8	-2.3
7.4	4.7	2.7	4.4	5.0	-0.6
6.1	3.5	2.6	4.9	3.6	1.3
3.6	2.0	1.6	4.6	2.5	2.1
5.3	3.0	2.3	4.8	3.3	1.5
3.8	2.7	1.1	4.5	6.0	-1.5
9.8	7.1	2.7	3.4	2.7	0.7
4.4	3.1	1.3	5.0	3.5	1.5
6.9	9.4	-2.5			

Table IV. Relative length of hours induction periods of top and bottom shelves of hot air oven. *

Top shelf	Bottom shelf	Difference	Top shelf	Bottom shelf	Difference
18.0	17.0	1.0	17.0	15.5	1.5
17.0	17.5	-0.5	19.0	17.0	2.0
19.0	18.0	1.0	21.0	19.0	2.0
16.75	15.25	1.5	22.0	20.25	1.75
15.5	15.0	0.5	14.75	16.0	-1.25
15.25	14.75	0.5	22.75	19.0	3.75
15.25	14.50	0.75	20.75	19.5	1.25
16.5	15.25	1.25	18.75	17.25	1.5
15.5	15.25	0.25	18.5	16.5	2.0
16.5	15.0	1.50	18.0	17.25	0.75
13.25	13.0	0.25	16.25	16.0	0.25
13.75	12.75	1.0	16.75	15.75	1.0
23.0	19.50	3.5	17.0	16.75	0.25
14.5	12.0	2.5	20.25	19.75	0.5
11.5	11.0	0.5	19.50	20.0	-0.5
20.0	19.0	1.0	21.0	20.75	0.25
14.0	12.75	1.25	20.25	20.75	-0.5
16.0	14.0	2.0	25.0	21.5	3.5
18.0	16.25	1.75	18.5	18.0	0.5
17.5	16.0	1.5	22.0	19.0	3.0
17.0	15.25	1.75	22.0	20.0	2.0
20.5	19.0	1.5	20.0	19.0	1.0
19.0	20.75	-1.75	20.0	18.75	1.25
20.0	20.0	0.0	20.0	19.0	1.0
19.0	19.0	0.0	22.0	22.0	0.0
24.0	22.0	2.0	23.5	22.0	1.5
21.5	20.0	1.5	22.0	21.0	1.0
20.5	19.0	0.5	21.25	20.0	1.25
25.0	23.25	1.75	23.75	23.25	0.50
23.0	21.75	1.25	26.0	25.0	1.0
26.0	25.25	0.75	23.5	23.25	0.25
25.0	23.25	1.75	19.0	20.25	-1.25
20.5	20.75	-0.25			

* Peroxide value of 2 considered end induction period.

Table V. Influence of temperature of hot air oven upon the rate of oxidation and upon the uniformity of results.

Hours oxidation	Quadruplicate determinations*			
	a	b	c	d
		Trial 1 - 100° C.		
26	4.3	3.8	3.8	5.9
28	6.8	5.8	5.0	9.7
30	12.4	7.4	7.0	12.5
32	17.8	13.3	14.7	15.0
		70° C.		
100	1.2	1.2	1.2	1.3
150	9.5	5.7	2.2	14.8
155	20.6	7.5	5.7	13.2
160	42.8	15.7	19.0	36.9
		Trial 2 - 100° C.		
22	--	1.9	1.6	1.7
24	1.9	2.6	2.7	3.3
26	5.8	4.7	3.6	3.8
28	9.3	8.2	5.6	7.0
		130° C.		
3	3.8	4.4	4.4	4.1
4	5.8	6.9	6.2	7.3
5	8.4	8.7	8.4	8.3
6	11.7	10.4	11.2	12.4

*Values reported in peroxide number.

Table VI. Trials determining variation between oxidation of duplicate samples of fat taken from the same shelf of the hot air oven.

Hours oxidation	Peroxide value a	Peroxide value b	Difference between a and b	Hours oxidation	Peroxide value a	Peroxide value b	Difference between a and b
16	2.3	2.2	0.1	22	8.5	8.9	0.4
23	9.3	6.5	2.8	25	8.9	8.3	0.6
16	2.7	2.3	0.4	23	10.4	6.2	4.2
25	9.1	14.7	5.6	18	2.7	3.6	0.9
22	4.6	5.2	0.6	24	8.7	9.3	0.6
24	8.7	12.8	4.1	24	8.7	8.4	0.3
22	7.7	7.3	0.4	24	15.9	17.7	1.8
18	7.0	4.2	2.8	22	7.7	11.6	3.9
24	15.9	16.9	1.0	24	15.9	14.8	1.1
14	19.8	28.5	8.7	14	19.8	15.3	4.5
14	19.8	18.3	1.5	14	19.8	18.1	1.7
14	15.4	13.3	2.1	14	15.4	16.0	0.6
14	15.4	13.4	2.0	14	19.2	18.8	0.4
14	19.2	16.5	2.7	14	19.2	20.2	1.0
14	15.6	22.2	6.6	14	15.6	17.1	1.5
14	15.6	14.1	1.5	14	15.6	15.6	0.0
13	3.8	2.6	1.2	15	7.3	6.7	0.6
15	7.3	7.8	0.5	11	1.3	1.1	0.2
13	4.5	4.1	0.4	14	3.7	4.4	0.7
11	1.3	1.3	0.0	13	4.5	3.6	0.9
11	3.9	5.3	1.4	13	8.4	8.1	0.3
11	3.9	4.1	0.2	12	6.5	14.0	7.5
13	8.4	11.0	2.6	14	1.7	1.4	0.3
16	1.7	2.7	1.0	17	4.3	3.8	0.5
14	1.7	1.5	0.2	17	4.3	3.1	1.2
11	1.3	1.5	0.2	14	7.6	5.5	2.1
11	1.3	1.8	0.5	12	4.0	4.8	0.8
14	7.6	7.6	0.0	12	4.6	3.7	0.9
14	13.4	5.7	7.7	16	8.2	9.7	1.5
18	8.7	11.5	2.8	20	15.7	14.3	1.4
22	23.8	18.0	5.8	24	15.0	25.2	10.2
26	35.8	43.4	7.6	36	29.1	49.1	20.0
48	76.8	39.3	37.5	12	3.9	6.7	2.8
14	7.9	7.9	0.0	16	8.9	10.4	1.5
18	11.7	9.4	2.3	20	11.1	13.0	1.9
22	14.3	17.3	3.0	24	15.3	14.4	0.9
26	19.4	19.3	0.1	36	35.4	40.4	5.0
48	46.2	49.6	3.4	15	1.5	1.6	0.1
17	1.6	2.0	0.4	19	3.9	3.2	0.7
21.5	13.5	8.5	5.0	23	10.3	11.9	1.6
27	17.5	21.5	4.0	35	25.1	34.3	9.2
39	40.8	39.2	1.6	43	49.0	34.3	14.7
48	38.1	40.9	2.8	15	1.2	1.3	0.1
17	1.9	1.8	0.1	19	4.6	3.9	0.7
21.5	12.2	7.7	4.5	23	11.6	10.0	1.6

(continued)

Table VI. (continued)

Hours oxidation	Peroxide value a	Peroxide value b	Difference between a and b	:	Hours oxidation	Peroxide value a	Peroxide value b	Difference between a and b
27	17.2	18.5	1.3	:	35	22.5	23.5	1.0
39	26.9	39.1	12.2	:	43	40.8	53.1	12.3
48	50.5	55.7	5.2	:	10	1.2	0.7	0.5
11	1.6	1.4	0.2	:	12	3.6	1.8	1.8
11	1.6	1.8	0.2	:	12	3.6	3.6	0.0
12	3.6	1.7	1.9	:	10	3.9	4.4	0.5
11	4.4	5.8	1.4	:	12	7.6	5.6	2.0
11	4.4	7.6	3.2	:	12	7.6	6.3	1.3
12	7.6	6.3	1.3	:	13	1.6	1.6	0.0
15	2.5	3.4	0.9	:	16	5.7	7.3	1.6
15	2.5	2.3	0.2	:	16	5.7	3.3	2.4
16	5.7	3.7	2.0	:	24	6.3	7.8	1.5
24	6.3	8.1	1.8	:	24	6.3	4.9	1.4
24	6.3	8.0	1.7	:	24	6.3	5.6	0.7
24	6.3	5.8	0.5	:	24	6.3	7.0	0.7
24	6.3	7.0	0.7	:	24	6.3	7.2	0.9
24	6.3	3.4	2.9	:	24	6.3	5.5	0.8
24	6.3	7;7	1.4	:	24	6.3	4.5	1.8
24	6.3	4.9	1.4	:	24	6.3	5.3	1.0
24	6.3	7.4	1.1	:	24	7.9	14.1	6.2
24	7.9	12.6	4.7	:	24	7.9	9.8	1.9
24	7.9	4.6	3.3	:	24	7.9	14.2	6.3
24	7.9	6.3	1.6	:	24	7.9	3.0	4.9
24	7.9	5.5	2.4	:	24	7.9	7.9	0.0
24	7.9	4.9	3.0	:	24	7.9	6.1	1.8
24	7.9	11.9	4.0	:	24	7.9	5.3	2.6
24	7.9	7.2	0.7	:	24	7.9	5.7	2.2
24	7.9	6.7	1.2	:	24	5.3	5.5	0.2
24	5.3	7.4	2.1	:	24	5.3	4.4	0.9
24	5.3	6.1	0.8	:	24	5.3	4.9	0.4
24	5.3	3.6	1.7	:	24	5.3	4.6	0.7
24	5.3	5.3	0.0	:	24	5.3	4.8	0.5
24	5.3	3.8	1.5	:	24	5.3	4.5	0.8
24	5.3	9.8	4.5	:	24	5.3	3.4	1.9
24	5.3	4.4	0.9	:	24	5.3	5.0	0.3
24	5.3	6.9	1.6	:	24	4.4	7.8	3.4
24	4.4	4.7	0.3	:	24	4.4	5.0	0.6
24	4.4	3.5	0.9	:	24	4.4	3.6	0.8
24	4.4	2.0	2.4	:	24	4.4	2.5	1.9
24	4.4	3.0	1.4	:	24	4.4	3.3	1.1
24	4.4	2.7	1.7	:	24	4.4	6.0	1.6
24	4.4	7.1	2.7	:	24	4.4	2.7	1.7
24	4.4	3.1	1.3	:	24	4.4	3.5	0.9
24	4.4	9.4	5.0	:	18	2.1	3.4	1.3
18	2.1	3.3	1.2	:	18	2.1	1.9	0.2

(continued)

Table VI. (continued)

Hours oxidation	Peroxide value a	Peroxide value b	Difference between a and b	:	Hours oxidation	Peroxide value a	Peroxide value b	Difference between a and b
18	2.1	1.8	0.3	:	18	1.5	1.4	0.1
18	1.5	1.7	0.2	:	18	1.5	1.5	0.0
18	1.5	1.5	0.0	:	16	2.6	2.4	0.2
16	2.6	2.7	0.1	:	16	2.6	2.9	0.3
18	8.7	6.1	2.6	:	18	8.7	5.1	3.6
18	8.7	10.8	2.1	:	20	12.9	14.2	1.3
20	12.9	11.4	1.5	:	20	12.9	9.7	3.2
22	11.9	10.5	1.4	:	22	11.9	10.3	1.6
22	11.9	20.0	8.1	:	16	1.4	1.0	0.4
16	1.4	1.2	0.2	:	16	1.4	1.5	0.1
18	1.5	1.4	0.1	:	18	1.5	1.7	0.2
18	1.5	1.6	0.1	:	22	2.3	3.2	0.9
22	2.3	1.8	0.5	:	22	2.3	2.1	0.2
25	6.6	8.0	1.4	:	25	6.6	3.9	2.7
25	6.6	3.9	2.7	:	26	1.9	3.4	1.5
26	1.9	1.4	0.5	:	26	1.9	1.5	0.4
29	2.8	1.9	0.9	:	29	2.8	3.0	0.2
29	2.8	3.7	0.9	:	31	4.1	2.4	1.7
31	4.1	5.2	1.1	:	31	4.1	5.6	1.5
34	6.8	5.1	1.7	:	34	6.8	11.6	4.8
34	6.8	11.9	5.1	:	26	1.4	1.4	0.0
26	1.4	1.3	0.1	:	26	1.4	1.4	0.0
29	1.6	1.5	0.1	:	29	1.6	1.6	0.0
29	1.6	1.8	0.2	:	31	2.0	1.8	0.2
31	2.0	1.9	0.1	:	31	2.0	5.0	3.0
34	7.1	6.0	1.1	:	34	7.1	6.2	0.9
34	7.1	12.8	5.7	:	26	2.0	1.9	0.1
26	2.0	2.2	0.2	:	26	2.0	2.1	0.1
28	4.5	5.4	0.9	:	28	4.5	1.7	2.8
28	4.5	1.7	2.8	:	30	6.3	8.2	1.9
30	6.3	8.3	2.0	:	30	6.3	4.4	1.9
32	10.3	8.1	2.2	:	32	10.3	9.0	1.3
32	10.3	11.5	1.2	:	26	1.7	1.7	0.0
26	1.7	1.5	0.2	:	26	1.7	1.7	0.0
28	2.1	2.2	0.1	:	28	2.1	1.8	0.3
28	2.1	1.8	0.3	:	30	3.7	4.3	0.6
30	3.7	4.9	0.2	:	30	3.7	2.3	1.4
32	7.2	5.5	1.7	:	32	7.2	7.5	0.3
32	7.2	9.3	2.1	:	24	2.1	3.9	1.8
24	2.1	1.7	0.4	:	24	2.1	1.8	0.3
26	3.8	5.0	1.2	:	26	3.8	5.8	2.0
26	3.8	2.9	0.9	:	28	9.7	8.0	1.7
28	9,7	10.8	1.1	:	28	9.7	7.9	1.8
30	10.7	10.3	0.4	:	30	10.7	11.5	0.8
30	10.7	11.5	0.8	:	24	1.9	1.6	0.3

(continued)

Table VI. (continued)

Hours oxidation	Peroxide value a	Peroxide value b	Difference between a and b	:	Hours oxidation	Peroxide value a	Peroxide value b	Difference between a and b
24	1.9	3.2	1.3	:	24	1.9	1.4	0.5
26	3.5	2.1	1.4	:	26	3.5	2.8	0.7
26	3.5	4.7	1.2	:	28	8.0	9.5	1.5
28	8.0	5.4	2.6	:	28	8.0	4.9	3.1
30	12.2	13.8	1.6	:	30	12.2	9.7	2.5
30	12.2	12.8	0.6	:	26	3.4	2.5	0.9
26	3.4	2.3	1.1	:	26	3.4	5.9	2.5
28	5.8	7.3	1.5	:	28	5.8	4.3	1.5
28	5.8	11.0	5.2	:	30	6.7	16.2	9.5
30	6.7	5.6	1.1	:	30	6.7	11.1	4.4
32	15.0	25.8	10.8	:	32	15.0	16.8	1.6
32	15.0	13.8	1.2	:	26	5.4	6.2	0.8
26	5.4	4.2	1.2	:	26	5.4	5.9	0.5
28	6.4	5.9	0.5	:	28	6.4	5.8	0.6
28	6.4	8.4	2.0	:	30	9.0	8.7	0.3
30	9.0	8.2	0.8	:	30	9.0	13.9	4.9
32	14.4	9.8	4.6	:	32	14.4	9.9	4.5
32	14.4	16.2	1.8	:	24	2.3	2.9	0.6
24	2.3	2.5	0.2	:	24	2.3	1.7	0.6
26	4.3	7.3	3.0	:	26	4.3	4.9	0.6
26	4.3	2.9	1.4	:	28	10.0	13.4	3.4
28	10.0	6.3	3.7	:	28	10.0	8.0	2.0
24	3.1	2.2	0.9	:	24	3.1	2.3	0.8
24	3.1	4.2	1.1	:	26	4.3	4.6	0.3
26	4.3	4.3	0.0	:	26	4.3	3.4	0.9
28	5.2	6.4	1.2	:	28	5.2	5.0	0.2
28	5.2	6.0	0.8	:	22	1.8	1.9	0.1
22	1.8	1.6	0.2	:	22	1.7	2.0	0.3
22	1.7	1.7	0.0	:				
Total samples 327								
Average	7.84	7.95						

Table VII. Accuracy of oven method in detecting the end of induction period.

Trial number	Hours oxidation	Quadruplicate*			
		a	b	c	d
1	16	2.9	2.7	2.4	2.6
	18	10.8	5.1	6.1	8.7
	20	14.2	12.9	11.4	9.7
	22	20.0	11.9	10.5	10.3
2	16	1.5	1.2	1.0	1.4
	18	1.7	1.6	1.5	1.4
	22	2.3	3.2	1.8	2.1
	25	6.6	8.0	3.9	3.9
3	26	1.4	3.4	1.9	1.5
	29	1.9	2.8	3.0	3.7
	31	2.4	4.1	5.2	5.6
	34	5.1	6.8	11.6	11.9
4	26	1.3	1.4	1.4	1.4
	29	1.5	1.6	1.6	1.8
	31	1.8	1.9	2.0	5.0
	34	7.1	6.0	6.2	12.8
5	26	2.0	1.9	2.2	2.1
	28	4.5	5.4	1.7	1.7
	30	8.2	8.3	4.4	6.3
	32	8.1	9.0	11.5	10.3
6	26	1.7	1.7	1.5	1.7
	28	2.1	2.2	1.8	1.8
	30	4.3	4.9	2.3	3.7
	32	7.2	5.5	7.5	9.3
7	24	2.1	3.9	1.7	1.8
	26	5.0	5.8	3.8	2.9
	28	8.0	10.8	7.9	9.7
	30	10.7	10.3	11.5	11.5
8	24	1.6	3.2	1.4	1.9
	26	3.5	2.1	2.8	4.7
	28	9.5	5.4	4.9	8.0
	30	13.8	9.7	12.2	12.8
9	26	6.2	5.4	4.2	5.9
	28	6.4	5.9	5.8	8.4
	30	8.7	8.2	9.0	13.9
	32	9.8	9.9	14.4	16.2
10	26	2.5	2.3	3.4	5.9
	28	7.3	5.8	4.3	11.0
	30	16.2	6.7	5.6	11.1
	32	25.8	16.8	15.0	13.8
11	22	--	2.0	1.7	1.7
	24	2.2	2.3	3.2	4.2
	26	4.3	4.6	4.3	3.4
	28	5.2	6.4	5.0	6.0
12	22	--	1.9	1.6	1.8
	24	1.7	2.9	2.3	2.5
	26	7.3	4.9	2.9	4.3
	28	13.4	10.0	6.3	8.0

* Values reported in peroxide numbers.

Table VIII. Precision of the oven and aeration methods of detecting the end of the induction period.

Oven method*			Aeration method*		
Hours oxidation	a	b	Hours oxidation	a	b
		Trial I			
11	0.8	---	19	1.3	1.3
16	2.3	2.2	22	4.1	8;3
22	8.5	8.9	23	7.2	11.2
23	9.3	6.5	---	---	---
25	8.9	8.3	---	---	---
		Trial II			
11	0.7	---	19	5.3	6.7
16	4.7	2.3	20	11.8	11.6
22	3.8	9.0	21	21.2	18.6
23	10.4	6.2	---	---	---
25	9.1	14.7	---	---	---
		Trial III			
18	2.7	3.6	22	1.6	1.9
22	4.6	5.2	24	8.1	9.0
24	8.7	9.3	---	---	---
		Trial IV			
10	1.2	---	16	2.0	1.8
11	1.6	1.7	17	4.5	---
12	3.6	3.6	18	11.8	13.7
		Trial V			
10	3.9	4.4	13	1.7	1.0
11	4.4	5.8	15	6.3	2.5
12	7.6	5.6	16	14.6	19.1
		Trial VI			
13	1.6	1.6	17	1.5	1.6
15	2.5	3.4	19	1.4	2.5
16	5.7	7.3	21	4.3	8.6
		Trial VII			
13	3.8	2.6	15	1.4	1.7
14	5.1	5.5	16	1.6	1.4
15	7.3	6.7	17	6.2	6.2
		Trial VIII			
11	1.3	1.1	19	1.5	1.8
12	4.5	4.1	20.5	2.4	2.4
13	3.7	4.4	21	8.4	10.1
		Trial IX			
11	3.9	5.3	18	4.8	3.6
12	5.3	6.5	18.5	5.7	5.5
13	8.4	8.1	19	16.4	18.7
		Trial X			
14	1.7	1.4	20	6.0	1.6
16	1.7	2.7	22	1.9	1.7
17	4.3	3.8	24	3.7	7.3
		Trial XI			
11	1.3	1.5	18	1.7	1.8
12	2.1	4.0	19	10.4	4.3
14	7.6	5.5	19.5	4.8	18.1

* Values reported in peroxide numbers.

Table IX. Influence of drying the air upon the rate of oxidation of fat by aeration method.

Hours oxidation	Control*		Air dried with H_2SO_4*			
	a	b	a	b	c	d
			Trial I			
12	1.2	1.1	1.2	1.2	1.4	1.3
16.5	1.6	1.5	1.9	3.8		
17	1.7		12.7	3.5		
17.5			15.0	13.5		
19.5	5.8					
			Trial II			
19.0	1.0		5.0		2.3	
19.5			1.3		1.8	
20.0			1.0		5.6	
20.5			1.0		4.7	
21.0			1.1		6.7	
22.0	1.3		34.1		5.9	
24.0	1.7					
26.0	1.3					
28.0	1.5					
30.0	4.4					
			Trial III			
20	1.0		1.2		1.1	
21			1.2		1.6	
23	1.3		1.4		1.6	
25			1.4		1.5	
27	1.5		2.2		2.3	
29	3.1		7.0		7.0	
31	2.1					

*Values reported in peroxide numbers.

Table X. Comparison of rate and extent of oxidation with hot air oven and aeration methods.

Hours oxidation	Trial I*		Trial II*	
	Oven	Aeration	Oven	Aeration
12.0	4.7	---	---	---
13.0	---	2.5	---	---
13.5	---	8.1	---	---
14.0	8.7	---	---	---
14.5	---	25.8	---	---
15.0	---	---	1.5	1.5
15.5	---	33.0	---	---
16.0	9.3	32.8	---	---
17.0	---	57.7	1.8	1.9
18.0	10.3	76.2	---	---
19.0	---	49.5	3.9	---
19.5	---	---	---	4.3
20.0	13.5	87.4	---	---
21.0	---	113.2	---	---
21.5	---	---	10.4	30.2
22.0	18.4	103.4	---	---
23.0	---	130.1	10.9	48.0
24.0	17.5	156.2	---	---
26.0	29.4	153.9	---	---
27.0	---	---	18.6	82.3
35.0	---	---	26.3	143.9
36.0	38.5	162.5	---	---
39.0	---	---	36.5	168.3
43.0	---	---	44.3	182.2
48.0	53.5	196.4	46.3	217.6
60.0	---	---	---	174.5
72.0	---	---	---	184.1
84.0	---	---	---	242.5
96.0	---	---	---	139.4
108.0	---	---	---	124.5
120.0	---	---	---	109.5
132.0	---	---	---	69.8
144.0	---	---	---	64.1

* Values reported in peroxide numbers.

Table XI. Influence of heat treatment of butter oil upon its stability toward oxidation.

Trial number	Hours oxidation	Peroxide value		
		Control	6#-30 min.	22#-30 min.
1	22	2.3	5.8	
	23	4.9	7.1	
	24	4.3	10.5	
	25	6.8	12.8	
	26	13.0	10.7	
2	26	1.3	1.4	7.9
	28	1.4	1.5	12.3
	30	6.7	42.3	39.5
	31	2.9	2.9	44.2
	32	2.2	2.9	55.8
	33	12.9	8.0	72.8
3	32	1.2	1.5	6.3
	33	1.5	1.7	14.6
	34	2.3	1.7	19.4
	35	2.1	4.8	20.8
	36	3.9	2.6	29.1
	37	14.4	7.9	36.5
4	26	1.3	---	3.2
	27	1.3	---	16.0
	28	1.3	12.7	30.0
	29	1.3	1.3	19.1
	30	1.2	1.4	---
	31	1.4	1.5	83.4
	32	1.7	1.8	---
	33	1.7	2.2	67.3

Table XII. Influence of high temperature pasteurization of cream upon oxidation of butter oil.

Trial number	Hours oxidation	Peroxide value 62.8°C.-30 min.	Peroxide value 85° C.-5 min.
1	12	0.9	1.3
	14	1.5	1.6
	16	2.7	3.3
	18	6.1	9.3
	20	10.9	11.9
	22	12.7	12.3
	24	12.6	22.5
2	13	6.0	4.6
	15	11.5	7.5
	16	10.2	8.6
	17	14.9	13.7
	18	15.0	12.8
	19	18.2	13.0
	20	23.6	15.1
3	12	1.0	1.0
	14	1.1	1.1
	16	1.3	1.3
	17	1.5	1.5
	19	1.6	1.7
	21	3.1	2.5
	23	4.8	5.2
4	10	0.9	1.3
	12	1.6	4.6
	14	2.4	10.6
	15	6.3	16.2
	16	8.0	22.1
	17	12.3	23.9
5	12	1.6	1.5
	13	2.0	2.6
	14	3.6	1.6
	15	5.5	5.0
	16	6.6	6.1
	17	8.3	7.6
	18	10.6	9.6
6	11	0.7	0.9
	13	0.9	1.0
	16	1.2	1.3
	18	1.3	1.6
	20	2.0	4.2
	22	4.8	8.6
	23	6.3	13.1

(continued)

Table XII (continued)

Trial number	Hours oxidation	Peroxide value 62.8°C.-30 min.	Peroxide value 90.6°C.-flash
1	10	0.9	0.9
	12	1.4	1.3
	14	1.7	1.4
	16	3.3	1.6
	18	11.3	4.9
	20	14.8	7.8
	22	31.9	16.4
2	10	0.9	0.8
	12	1.9	1.1
	14	1.2	1.4
	16	1.9	2.5
	18	3.0	7.4
	21	12.3	12.0
	23	19.3	21.1
3	10	0.7	1.1
	12	0.9	1.2
	14	1.1	1.6
	16	1.5	2.4
	18	1.9	6.1
	20	3.3	9.4
	22	7.5	12.2
4	11	1.0	1.0
	13	1.2	1.1
	15	1.2	1.1
	17	1.5	1.5
	19	1.9	1.6
	21	3.9	2.7
	23	9.9	8.1
5	11	0.9	1.0
	13	1.1	1.0
	16	1.2	1.2
	18	1.6	1.6
	20	2.5	1,9
	22	4.2	8.1
	24	10.0	9.8
6	11	0.9	1.0
	13	1.2	1.2
	15	1.4	1.5
	17	2.0	1.8
	19	5.1	5.7
	21	8.0	12.3
	23	12.6	16.2

(continued)

Table XII. (continued)

Trial number	Hours oxidation	Peroxide value	
		62.8°C.-30 min.	6# - 15 min.
1	11	1.0	1.3
	13	1.2	1.4
	15	1.2	2.2
	17	1.5	5.7
	19	1.9	7.4
	21	3.9	15.7
	23	9,9	20.6
2	11	0.9	1.0
	13	1.1	1.2
	16	1.2	2.0
	18	1.6	2.5
	20	2.5	6.8
	22	4.2	14.8
	24	10.0	18.6
3	11	0.9	1.2
	13	1.2	1.4
	15	1.4	1.7
	17	2.0	4.1
	19	5.1	8.8
	21	8.0	15.1
	23	12.6	21.4
			22# - 15 min.
1	10	0.9	6.7
	12	1.9	16.0
	14	1.2	17.6
	16	1.9	18.0
	18	3.0	33.4
	21	12.3	33.1
	23	19.3	44.4
2	10	0.7	0.9
	12	0.9	1.1
	14	1.1	1.4
	16	1.5	1.8
	18	1.9	3.9
	20	3.3	5.2
	22	7.5	12.4

(continued)

Table XII. (continued)

Trial number	Hours oxidation	Peroxide value	
		62.8°C.-30 min.	22#-30 min.
1	12	0.9	1.0
	14	1.5	1.4
	16	2.7	3.4
	18	6.1	6.2
	20	10.9	10.3
	22	12.7	16.4
	24	12.6	17.8
2	13	6.0	6.1
	15	11.5	18.2
	16	10.2	12.2
	17	14.9	13.9
	18	15.0	9.8
	19	18.2	12.8
	20	23.6	17.6
3	12	1.0	2.9
	14	1.1	9.6
	16	1.3	12.2
	17	1.5	11.6
	19	1.6	18.2
	21	3.1	17.5
	23	4.8	26.8
4	12	1.6	11.7
	13	2.0	15.7
	14	3.6	18.5
	15	5.5	13.3
	16	6.6	17.4
	17	8.3	21.6
	18	10.6	18.0
5	10	0.9	6.0
	12	1.6	17.6
	14	2.4	18.9
	15	6.3	17.3
	16	8.0	25.8
	17	12.3	24.6
6	11	0.7	1.2
	13	0.9	2.0
	16	1.2	6.4
	18	1.3	11.5
	20	2.0	20.8
	22	4.8	23.4
	23	6.3	21.1

(continued)

Table XII. (continued)

Trial number	Hours oxidation	Peroxide value	
		62.8°C.-30 min.	22#-30 min.
7	10	0.9	22.2
	12	1.4	33.3
	14	1.7	31.9
	16	3.3	29.0
	18	11.3	27.2
	20	14.8	45.2
	22	31.9	44.5

Table XIII. Influence of pasteurization holding time of cream on the oxidation of butter oil.

Hours oxidation	Peroxide value 90.6°C.-flash	90.6°C.-5 min.	90.6°C.-15 min.	90.6°C.-30 min.
		Trial I		
18	1.8	1.8	1.7	1.6
21	3.2	3.6	2.1	2.7
22	8.6	4.9	3.6	3.0
23	7.1	7.1	7.2	3.7
24	12.5	9.4	6.9	4.6
		Trial II		
24	2.4	2.4	3.0	2.1
25	4.2	3.7	4.4	2.5
26	4.7	4.9	4.6	3.8
27	6.6	5.0	6.7	4.1
28	12.1	9.3	11.5	7.4
		Trial III		
19	1.1	1.2	1.3	1.2
21	1.3	1.4	1.5	1.4
22	1.6	1.5	1.6	2.6
24	3.3	2.1	2.0	2.1
25	4.2	3.6	3.9	3.7

Table XIV. Influence of heat treatment of cream upon metallic induced oxidation. Metal added before pasteurization.

Hours oxidation	Peroxide value			
	Control 62.8°C.-30 min.	62.8°C.-30 min. 5 p.p.m. Cu.	85°C.-flash 5 p.p.m. Cu.	90.6°C.-flash 5 p.p.m. Cu.
		Trial I		
20	2.0	9.7	1.9	1.8
22	2.3	12.6	3.0	2.5
23	6.9	13.2	5.1	4.0
24	8.3	17.7	5.5	5.7
25	9.2	25.2	6.1	8.8
		Trial II		
18	3.1	7.1	1.6	1.6
20	3.9	11.7	3.3	1.8
21	8.3	10.7	3.9	3.3
22	8.3	14.1	5.2	5.0
23	10.1	14.2	6.5	5.3
		Trial III		
15	9.5	15.9	10.1	7.1
16	5.7	14.0	4.8	4.1
17	7.5	14.8	8.3	4.8
18	12.8	18.7	7.6	5.8
19	20.7	26.0	16.3	10.8

Table XV. Influence of heat treatment of cream upon metallic induced oxidation. Metal added after pasteurization.

Hours oxidation	Peroxide value: Control 62.8°C.-30 min.	62.8°C.-30 min.: 5 p.p.m. Cu.	85°C.-flash 5 p.p.m. Cu.	90.6°C.-flash 5 p.p.m. Cu.
		Trial I		
16	1.7	3.4	1.7	1.6
29	1.9	5.9	3.1	2.1
21	4.4	6.3	5.6	4.2
22	6.4	9.1	7.9	5.1
23	9.3	13.5	9.0	6.7
		Trial II		
17	1.5	5.2	1.8	1.8
20	2.1	7.7	2.8	3.4
22	3.9	8.3	5.5	5.1
23	5.6	10.7	8.0	5.6
24	11.6	17.5	8.6	10.1
		Trial III		
22	2.1	6.4	1.9	1.9
24	2.2	9.9	2.5	3.0
26	3.5	12.5	6.1	6.5
27	4.8	16.7	7.8	12.4
28	10.8	23.9	11.6	11.9
		Trial IV		
20	5.7	14.3	9.0	7.4
22	6.3	12.5	8.3	6.5
23	7.9	12.3	12.0	6.9
24	10.8	15.5	11.2	8.3
25	18.9	30.2	13.3	10.8

Table XVI. Influence upon oxidation of adding "Avenex Concentrate" to butter oil.*

Hours oxidation	Normal butter oil		5 ppm. copper	
	No Avenex	Avenex	No Avenex	Avenex
		Trial I		
19	1.7	1.6	2.0	1.6
21	---	---	2.4	2.9
22	---	---	2.5	2.0
24	---	---	4.4	2.7
25	3.1	2.6	6.4	3.0
26	3.9	2.3	---	---
27	3.2	2.4		
28	4.1	4.1		
		Trial II		
20	7.8	5.8	6.5	6.2
21	5.4	7.6	7.1	7.6
22	9.1	7.2	12.1	8.6
23	11.3	10.4	10.8	10.6
24	17.2	15.1	15.1	13.0
		Trial III		
22	3.1	2.0	4.2	4.9
23	3.6	3.2	4.4	4.7
24	5.0	4.2	6.7	5.2
25	6.7	6.5	7.9	6.6
26	11.9	10.0	11.0	8.8

* Values reported in peroxide numbers,

Table XVII. Influence upon oxidation of adding "Avenex Concentrate" to cream after pasteurization. *

Hours oxidation	Normal cream		5 ppm. copper	
	No Avenex	Avenex	No Avenex	Avenex
		Trial I		
22	1.7	1.7	3.5	2.4
23	---	---	3.9	3.3
24	---	---	7.4	6.0
25	4.2	3.6	6.8	4.7
26	4.2	4.7	7.8	7.5
27	3.1	4.3	---	---
28	7.9	5.4	---	---
		Trial II		
21	2.4	1.8	3.6	3.7
22	---	---	7.2	4.0
23	3.5	4.4	8.6	6.1
24	6.4	6.9	10.7	8.1
25	11.1	7.5	13.5	10.4
26	7.3	7,2	---	---
		Trial III		
22	1.8	1.6	7.7	3.8
23	---	---	7.4	4.5
24.5	---	---	9.8	5.2
25	---	---	10.8	5.4
26	3.0	2.2	13.5	8.0
27	3.2	2.4	---	---
28	5.1	4.1	---	---
29	5.3	3.6	---	---

* Values reported in peroxide numbers.