

RELATIONSHIP OF THE ORGANOLEPTIC TO CHEMICAL METHODS FOR
MEASURING INTENSITY OF OXIDIZED FLAVOR IN MILK FAT

by

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RELATIONSHIP OF THE ORGANOLEPTIC TO CHEMICAL METHODS FOR MEASURING INTENSITY OF OXIDIZED FLAVOR IN MILK FAT

INTRODUCTION

The lipids of dairy foods constitute from a fraction of a per cent to 100 per cent of the product, and like all food lipids, are subject to autoxidation. Quality impairment due to autoxidation is wide spread and manifests itself in many ways, depending upon the product involved. In certain dairy products, lipid autoxidation is a major deterrent to the development of an acceptable product.

The autoxidation reaction involves essentially the formation of lipid hydroperoxides which subsequently degrade to yield a multitude of compounds. It is the hydroperoxide fission products that are of major concern in quality deterioration of dairy products. Among the fission products are the family of carbonyl compounds and they have been shown repeatedly to be of major significance to oxidized flavor. Unfortunately, the concentration of these compounds is extremely low, even when the food has become unpalatable because of the flavor defect. This makes chemical detection of oxidized flavor very difficult. The general trend has been to develop a chemical procedure that measures some product of the oxidation reaction and then attempt to relate the test to

psychrometric methods. These tests are termed indirect measures of oxidized flavor since they do not measure the flavor per se. Many tests of this type have been evaluated, some have been satisfactory for specific food items, but most have been of limited value in evaluating dairy products. Some investigators have attempted to measure the flavor compounds but the approach, while feasible, has not been successful because information is unavailable on the variation of the carbonyl constituents of the flavor as affected by variation in milk lipids, stage of oxidation as well as other factors.

The purpose of this investigation was to quantitatively analyze the components of carbonyl mixtures obtained from different milk fats of different oxidative intensity, and to determine the relationship of the quantitative chemical data to sensory methods of flavor measurement. A number of the "indirect" chemical tests also were applied for comparative purposes.

REVIEW OF LITERATURE

Description of Oxidized Flavor

Oxidized flavor is one of several defects that may arise from lipid deterioration of dairy products. The defect is normally associated with the formation of secondary products of autoxidation. According to the literature, more than one type of oxidized flavor may occur, depending upon the product involved and the environmental conditions during the oxidation process. This may account for the many terms that have been used to describe the defect in dairy products. The flavor has been described as "mealy" (41, p. 1285-1286), "oily", "tallowy", "metallic", "oxidized", and "emery" (78, p. 1). The oxidized flavor of milk, in addition to the above terms, has been described as "cardboard", "cappy", and "papery" (42, p. 913-933).

The overabundance of terms for describing the defect has led to confusion in the literature. Riel and Sommer (78, p. 1-2) attempted to make a distinction between the oxidized flavor defect of fluid milk and that of milk fat. They described the former as "oxidized flavor" and the latter as "oxidized fat flavor". Further confusion arises by the use of the term rancid to describe oxidized flavors

in other food lipids. In the dairy industry the term rancid refers to lipid hydrolysis and the flavor resulting from liberation of free fatty acids. Therefore the two terms are not synonymous and in this manuscript the term oxidized is used to describe the flavor defect which results from the autoxidation of milk fat.

Development of Autoxidation Theories

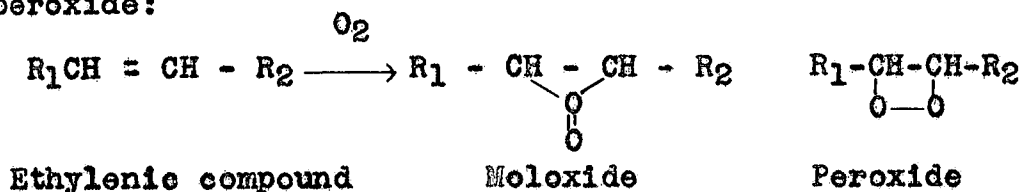
The reaction mechanisms resulting from the addition of oxygen to lipid material are not clearly understood. This may be attributed to the complexity of most natural lipid systems. The random distribution of fatty acids in the triglycerides coupled with lack of complete information on the composition of most lipids has forced chemists to study less complex systems when attempting to determine the reaction mechanism of autoxidation. The compounds most commonly used have been oleic and linoleic acids or their monoesters. Some of the original work on the generally accepted theories of autoxidation was conducted on cyclohexene (21, p. 75-96; 31, p. 228-236; and 32, p. 541-547). Generalizations made on the basis of simplified systems have been applied to natural lipids. This approach, while not entirely satisfactory, has been helpful in obtaining a better understanding of the autoxidation phenomenon.

The unsaturated fatty acids are the most susceptible sites for oxygen attack and their concentration in lipids largely influences the extent to which the system will readily oxidize. The type of unsaturation has an influence on the oxidation mechanism and these points will be considered below.

Lipid oxidation was recognized as early as 1827. Berzelius (8, p. 294) described experiments illustrating the inductive period, oxygen uptake, carbon dioxide formation, and polymerization of linseed oil. That oxygen and the double bonds of fatty acids were involved in the oxidation phenomenon was first reported by Schonbein (82, p. 328-340). It was believed that molecular oxygen was broken down into atomic or "active oxygen" which was responsible for autoxidation. Bach (5, p. 951-959) and Engler and coworkers (28, p. 3046-3055; and 29, p. 1669-1681) proposed, however, that oxidation of double bonds involved molecular oxygen rather than atomic oxygen and they called the oxygen "activated". The activated oxygen molecule was believed to react at the double bond to give a compound, $R_1-O-O-R_2$ which, like hydrogen peroxide, could oxidize another oxidizable substance.

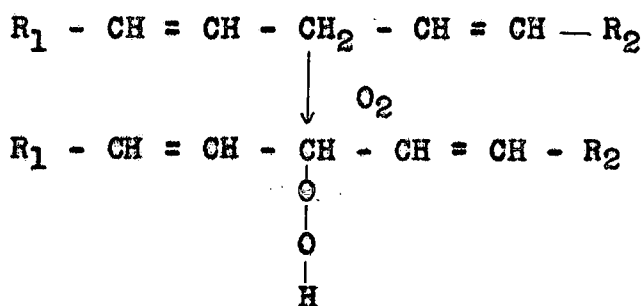
Staudinger (85, p. 1075-1079) modified Bach's theory when he reported that upon oxidation of a double bond, a moloxide was formed which then rearranged to a cyclic

peroxide:



Stephens (87, p. 568-571) in 1928 oxidized cyclohexane and obtained cyclohexane peroxide which he believed to be a cyclic peroxide across the double bond. However, Criegee (21, p. 75-96) showed that cyclohexane hydroperoxide still contained a double bond. This latter observation has been proven many times.

In 1937, Rieche (77, p. 520-524) proposed that oxidation of unsaturated substances might function by the formation of oxygen activated methylene groups giving products of the nature described by Criegee (21, p. 75-96):

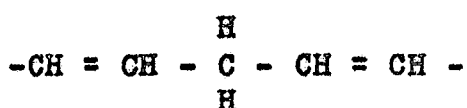


This statement by Rieche led Farmer and coworkers (30, p. 340-348; 31, p. 228-236; 32, p. 541-547; 33, p. 121-145; 34, p. 119-122; and 35, p. 10-13) to develop a theory which is generally accepted today for the explanation of

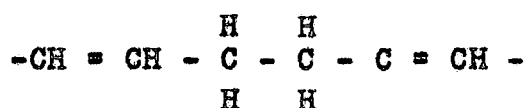
olefinic autoxidation.

For many years the reaction at the ethylenic bond of simple olefins was considered to be additive reactions with polar reagents. In such a case, where the molecule is unsymmetrical about a double bond, the direction of addition of a polar bond is determined by Markovnikov's rule. Farmer and coworkers pointed out that a large amount of experimental data did not fit into the polar scheme. Rather, the data suggested the occurrence of polar type reactions in which the participants were short lived neutral entities displaying free radical character. Farmer and coworkers further developed this reasoning into the hydroperoxide hypothesis of autoxidation and provided convincing experimental evidence to support it.

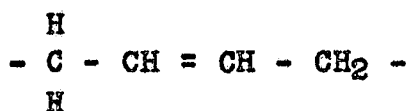
According to Farmer (32, p. 541-547), the formation of free radicals in an olefinic system is dependent largely by the type of unsaturation and by the presence or absence of substitution on the carbon atoms. Farmer arranged the unsaturated systems into four groups according to hydrogen lability.



Group I



Group II



Group III



Group IV

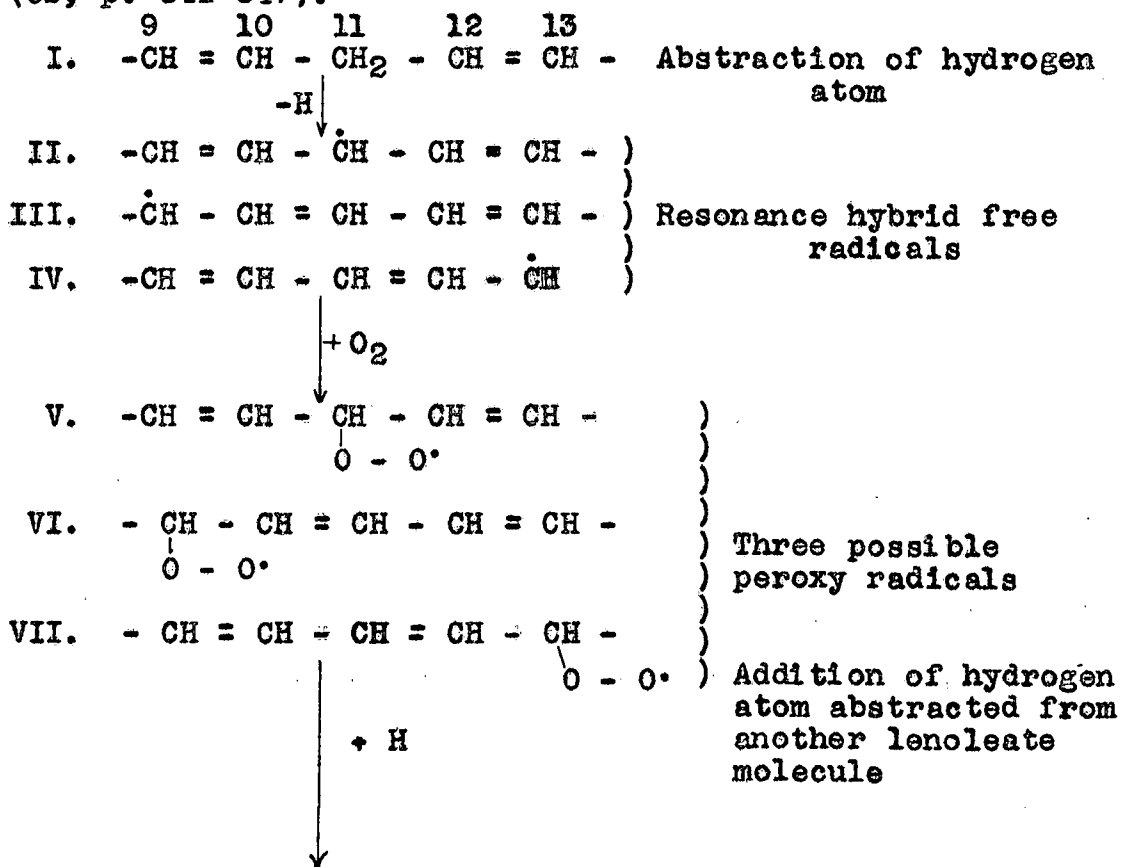
Group I has the highest degree of hydrogen lability because the alpha methylene group is flanked by double bonds. The influence of the double bonds are divided in Group II, and the hydrogen is less labile. Group III shows even less hydrogen lability whereas Group IV shows practically none due to double bond conjugation.

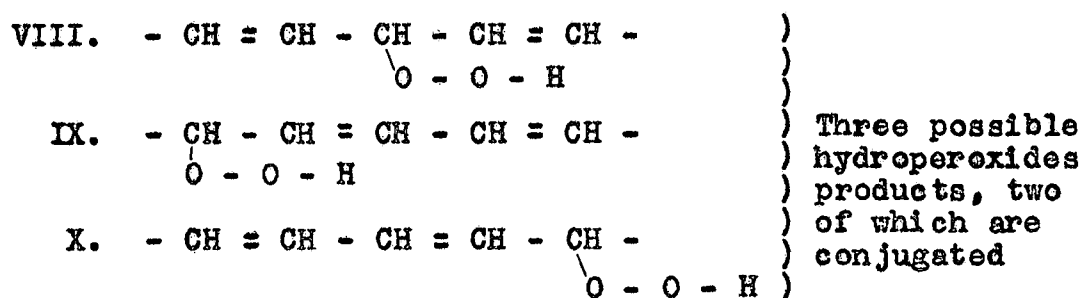
The pentadiene system (Group I) is the basis for the presently accepted theory, especially in the initial stages of the autoxidation process. When the system is excited by an external energy source, the energy may be expended through the escape of an electron which takes a proton with it to form a hydrogen atom. A free radical is first formed on the α -methylene group which sets up resonance isomers with either of the adjacent carbon to carbon unsaturated groups. The free radical absorbs an oxygen molecule to form a peroxide free radical which in turn accepts a hydrogen atom to form a hydroperoxide. The hydrogen usually come from another ester of a fatty acid thus forming another free radical, and this keeps the

chain reaction functioning.

Oxidation in Pentadiene Systems: Of the diethenoic acids in lipids, linoleic acid which contains the pentadiene system, is the most important and it has received the greatest attention.

The following oxidation mechanism for linoleate was originally proposed by Bolland and Kock (9, p. 445-447) and is based on the free radical theory of Farmer et al. (32, p. 541-547).





According to the above scheme, three hybrid free radicals are possible which give hydroperoxides in position 9, 11 and 13. Addition of oxygen to carbon 11 would yield a non-conjugated peroxide whereas the other hybrids would give conjugated systems.

Bolland and Koch (9, p. 445-447) by a comparison of the molecular extinction of oxidized linoleate with that of conjugated linoleic acid, calculated that approximately 70 per cent of the initial oxidation products were conjugated. Most other investigators, however, have obtained evidence which shows that most of the resonance hybrids are conjugated. Bergstrom (6, p. 1-8) chromatographed the hydrogenated products of linoleate oxidation and identified the 9 and 13-hydroxystearates. Cannon (18, p. 447-451) purified the hydroperoxides by countercurrent distribution and found 90 per cent to be conjugated. Privett (76, p. 23-27) obtained identical results through infrared spectrophotometric studies of highly purified hydroperoxides. The majority of the evidence suggests that the 9

and 13 carbon positions are the predominant sites of oxygen attack, but indirect evidence has been obtained which suggests at least some hydroperoxide formation at the number 11 carbon. The aldehydes 2,4-decadienal, 2-octenal and hexanal have been found in autoxidized cotton seed, corn and avocado oils (12, p. 238-242; and 90, p. 297-300). All of these oils contain relatively large amounts of linoleate and the aldehydes are believed to arise from the cleavage of the 9, 11, 13 hydroperoxides of the linoleate. No quantitative data were given so it is not possible to determine which of the three aldehydes predominated.

Aside from the evidence present above, only thermodynamic evidence supports the postulation of the formation of conjugated hydroperoxides in lieu of the unconjugated. The resonance energies associated with free radical conjugated and unconjugated systems are estimated to be 30.5 and 17.8 kcal respectively (10, p. 133-138). Thus the formation of the unconjugated type radical is much less probable than the conjugated.

There is disagreement in the literature on the mechanism of autoxidation of trienoic compounds. Linolenic acid, which is the most important of this group, has not been studied as extensively as the mono and

dienoic compounds. The data published in the literature are conflicting since some investigators have claimed that only small amounts of conjugated hydroperoxides are formed while other workers have reported the contrary. Most of the studies of autoxidized linolenate have consisted of fractionation of the oxidized materials followed by identification of the oxidation products. Fugger and coworkers (39, p.285-289) fractionated autoxidized methyl linolenate by countercurrent distribution techniques and came to the following conclusions:

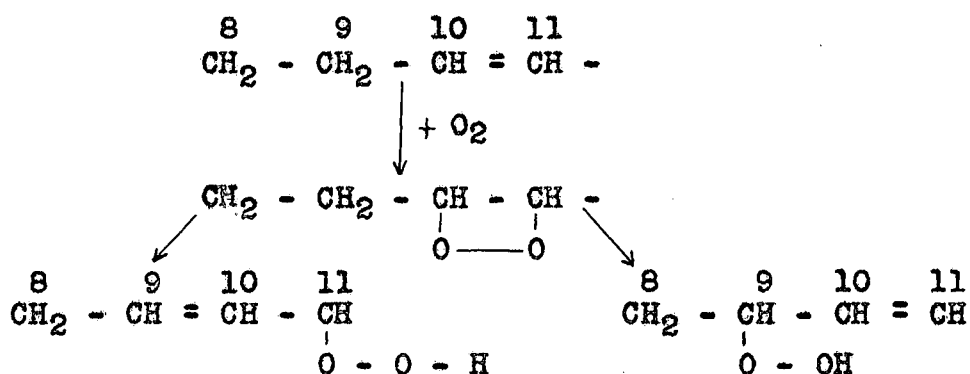
1. Monomeric hydroperoxides, if present, existed in very small quantities.
2. Dimers were formed during autoxidation or immediately thereafter.
3. Less than half of the oxidized linolenate was converted to a conjugated form and more than half of the double bonds were destroyed, probably through polymerization initiated by attack on the ethylenic bond.

These results suggest that the oxidation of linolenate differs significantly from that reported for the monene and diene systems. Contrary results were reported by Privett et al. (76, p. 23-27), who studied the oxidation products by means of infrared spectrophotometry. They concluded that:

1. Sixty per cent of the oxidative products consisted of cis-trans conjugated diene methyl octadecatrienoate monohydroperoxide.
2. No evidence of unconjugated hydroperoxides was found.
3. Only 15 per cent polymeric material was found.

The latter results would suggest that linolenate autoxidation is similar to that reported for the other unsaturated fatty acids. The differences in results may be due to the manner in which the systems were oxidized, the degree of oxidation and the methods used for studying the oxidation products.

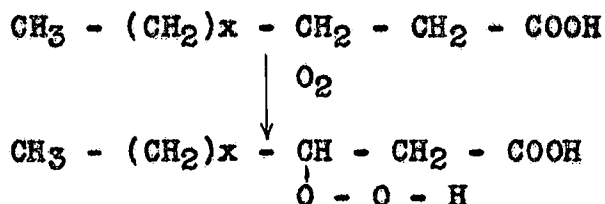
Oxidation of Monoethenoic Compounds: A study of the autoxidation of the monoethenoic fatty acids and esters has revealed some differences in the mechanisms relative to that of the poly-unsaturated acids. Farmer and Sutton (34, p. 119-122) oxidized methyl oleate and isolated a pure methyl oleate hydroperoxide in which there were two isomers, one on carbon eight and the other on carbon 11. Subsequently, Farmer et al. (35, p. 10-13) proposed a free radical mechanism for the monoethenoic acid oxidation which involved two, three carbon systems in resonance:



The above suggestion would mean that hydroperoxides can only occur on carbons 9 and 10. The nature of the bulk of the hydroperoxides found in oleate oxidation has not been elucidated. Hilditch has claimed that pure methyl oleate, in the absence of any polyethenoic compounds would not oxidize at normal temperature (below 50°C.). He explained that much of the methyl oleate used in experiments to determine reaction mechanisms has contained traces of poly-unsaturated compounds. This statement by Hilditch deserves appreciation in view of the fact that pure esters are still difficult to obtain absolutely free of impurities.

Knight et al. (58, p. 498-501) observed striking differences in the course of the oleate autoxidation reaction at different temperatures. Samples taken for analysis at different stages of oxidation therefore may lead to different conclusions relative to the main products. This could account for some of the confusion that has developed concerning the primary oxidation products of monoethenoic compounds.

Oxidation of Saturated Compounds: Even though it is known that the major site of lipid autoxidation lies in the unsaturated compounds, saturated fatty acids are known to undergo slow oxidation if stored at elevated temperatures. At temperatures above 100°C. the attack is predominantly at the beta carbon atom and the initial oxidation product is believed to be a hydroperoxide (50, p. 62). Formation of the initial product is believed to occur as follows:



Factors Affecting the Rate of Autoxidation of Lipids

The rate of lipid autoxidation is affected by the degree and types of unsaturation, the free fatty acid content, dilution of the fat, oxygen pressure, temperature, presence of pro-oxidants, antioxidants, and physical state of the substrate (50, p. 72-86).

Holman and Elmer (49, p. 127-129) reported that as the number of double bonds increased, the rate of oxidation increased. If the number of double bonds was great, a lower activating energy was needed to start the chain reaction. The additional increase in rate of oxidation

due to an added number of double bonds was in the order of two per additional double bond.

Disagreement appears in the literature on the effect of conjugation of double bonds on the rate of lipid oxidation. Myers et al. (68, p. 107-109) found that conjugated triene esters oxidized more rapidly than unconjugated triene esters. Allen et al. (1, p. 395-399) concluded that conjugated methyl linoleate oxidized slower than unconjugated methyl linoleate, whereas Holman and Elmer (49, p. 127-129) reported that the rates of oxidation of linoleic and conjugated linoleic to be the same.

Holman and Elmer (49, p. 127-129) found the maximum rates for the oxidation of unsaturated acids to be twice that of their esters. This was attributed to the participation of the carboxyl group in the decomposition of peroxides.

Dilution of the fat with some inert substances has the effect of reducing the rates of oxidation. The dilution decreases the chance of collision of free radicals and oxygen with the unoxidized fat, thus hindering the chain mechanism of lipid autoxidation.

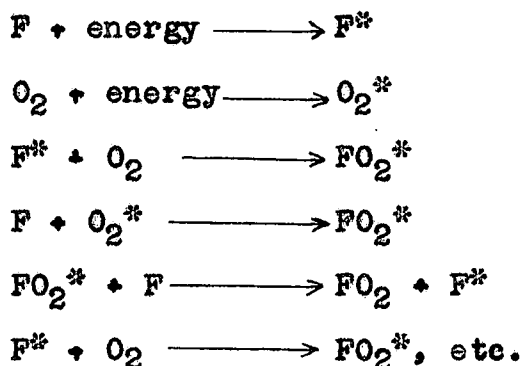
Paschke and Wheeler (70, p. 52-57) demonstrated the effect of temperature upon the rate of autoxidation. High temperature was shown to increase the rate of oxidation which is due to both the thermal activation of reacting

molecules and the thermal decomposition of the peroxides formed.

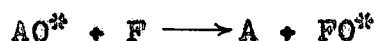
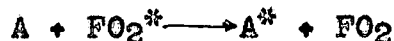
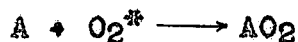
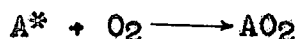
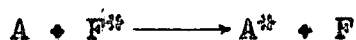
Pro-oxidants tend to increase the rate of oxidation. Metal catalysis, such as copper, iron, and cobalt have pro-oxidant action by catalyzing decomposition of peroxides to form free radicals (50, p. 79).

Antioxidants are substances which delay or prevent the oxidation of fats. Riemenschneider (80, p. 50-63) theorized that antioxidants functioned by interrupting the chain reaction of free radicals. He proposed the following scheme as a possible mode of action:

where F = fat; FO_2 = peroxide; * = activated molecule,



Antioxidants (A), if present, will interfere with the chain reaction by one or more of the following reactions:



Milk lipids contain several natural antioxidants (78, p. 13-16). These include tocopherol, carotene, and cephalin. The tocopherols have been reported to prevent an increase in the peroxide value of fats and oils (11, p. 1189-1196). Tocopherols were used by Godel (40, p. 28) to stabilize butter against oxidation. Carotene has been reported to be a natural antioxidant (59, p. 26-30), however Brown et al. (15, p. 925-935) found that the removal of carotene from milk did not increase its susceptibility towards oxidation. Olcott and Mattill (69, p. 98-102) reported that cephalin had oxidative inhibitory properties.

Formation of Carbonyl Compounds in Oxidizing Lipids

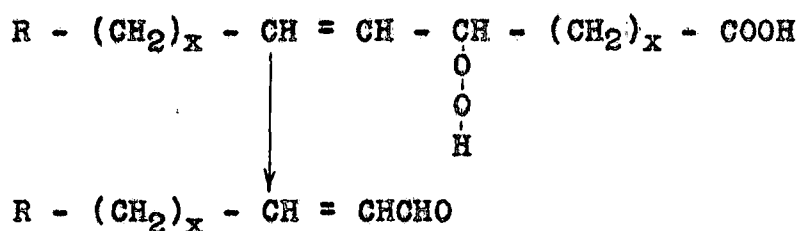
Most experimental evidence supports the view that hydroperoxides are the initial reaction products in autoxidizing lipids. The amount of peroxides present at any particular stage of oxidation is dependent upon the conditions which affect their stability. The

hydroperoxides are flavorless and it is their decomposition products that are of major concern in oxidized flavors. Due to the complexity of the secondary reactions of autoxidation, few of the products have been thoroughly studied. Because of their importance to oxidized flavor, the carbonyl compounds have received more attention than any other family of compounds. These compounds have been shown to be the major contributors to oxidized flavors (12, p. 238-242; 17, p. 975-999; 23, p. 585-597; 37, p. 91-102; 52, p. 372-377; 54, p. 713-718; 75, p. 323-360; and 91, p. 284-291).

The type of carbonyl compounds found in various autoxidized lipid system have been discussed in several comprehensive reviews (63, p. 1303-4309; 67, p. 125-132; and 78, p. 1-39). In the majority of cases, their identification has been tentative and as techniques and instrumentation have advanced, so has our knowledge of carbonyl products. It would appear, even from recent work, that the carbonyl picture is far from complete. Nevertheless, in studies of autoxidative mechanisms (23, p. 585-597; 37, p. 91-102; 38, p. 345-348; 48, p. 1-12; 56, p. 728-734; 79, p. 73; and 92, p. 487-498), carbonyl compounds of the following classes have been reported: n-alkan-2-ones, unsaturated ketones, n-alkanals,

alk-2-enals, alk-2,4-dienals.

Schemes for the origin of carbonyls, particularly the aldehydes, have been developed (52, p. 372-377; 63, p. 1303-1309; and 90, p. 297-300). The reaction schemes are based on the formation of the isomeric hydroperoxides followed by cleavage to yield an aldehyde:



It is notable that while these reaction schemes will explain the origin of many of the carbonyls, it is not possible to explain the presence of all of the carbonyl compounds reported to exist in autoxidized lipids. It would appear, therefore, that the secondary reaction products are undergoing further decomposition.

Not all of the classes of carbonyls listed above have been found in any particular lipid system by a single group of investigators. Saturated aldehydes were recognized as oxidative products by Scala in 1908 (81, p. 307-327) when he reported butanal, heptanal, and nonanal as components of the volatile material of oxidized milk fat. Keeney and Doan (54, p. 713-718; 55, p. 719-727, and 56, p. 728-738) isolated the volatile carbonyls by high heat-vacuum distillation of milk fat and reported that the

predominate odor compounds were ketones; that none of these ketones were methyl ketones and that a large portion was unsaturated. Tamsma (91, p. 284-291; and 92, p. 487-498) studied the volatile carbonyls from milk fat oxidized at 100°C. and concluded that the bulk of the oxidized flavor was comprised of unsaturated-unconjugated ketones. He also found conjugated dienals in the volatile material.

Contrary to the finding of Keeney and Doan (54, p. 713-718; 55, p. 719-727; and 56, p. 728-738) and Tamsma (90, p. 284-291; and 91, p. 487-498) are the recent results on autoxidized milk fat by Day and Lillard (23, p. 585-597). The latter workers reported that the bulk of the volatile carbonyl compounds were aldehydes. They conclusively identified the C₄ through C₁₁ alk-2-enals and C₁ through C₁₀ n-alkanals. Some 64 per cent the volatile carbonyls were n-alkanals and 34 per cent were the alk-2-enals. No evidence for alk-2,4-dienals was obtained and it was concluded that if these compounds were present, they occurred in trace quantities. The odd numbered C₅ through C₁₅ n-alkan-2-ones also were found in the milk fat but these compounds also were present in the control fat.

The findings of Day and Lillard have been supported to a large degree by the work of Forss et al. (36, p. 211-

219) who reported the same compounds in autoxidized milk fat to which propyl gallate, citric acid and nодиhydroguaiaretic acid had been added. Forss et al. (36, p. 211-219) also found octa-2,4-dienal and two unidentified fractions.

The carbonyls of autoxidized milk phospholipids have been studied by several workers. Forss et al. (37, p. 91-102; and 38, p. 395-398) identified propanal, ethanal, n-hexanal, 2-butenal, C_4 through C_{11} 2-enals, and C_6 through C_{11} 2,4-dienals from the steam distillates of skim milk. They proposed that these compounds originated from the oxidation of the highly unsaturated fatty acids in milk lipids. Riel and Sommer (79, p. 1-73) isolated the volatile components of autoxidized milk phospholipids and characterized them as carbonyl compounds. They concluded that five different carbonyls were present and the empirical formulae for three of them were $C_{15}H_{26}O_3$, $C_{10}H_{18}N_2O$, and $C_{11}H_{22}O$.

Duin (24, p. 81-89) after investigating the volatile material from oxidized milk phospholipids concluded that this material contains carbonyl compounds such as C_2 through C_{18} saturated aldehydes, C_5 through C_{11} alk-2-enals, C_8 and C_9 alk-2,4-dienals and alk-2-en-2-ones. The classes of carbonyls predominating in this case appear to differ somewhat from that of the milk fat. This has

been attributed largely to the higher concentration of polyunsaturated acids in the phospholipid fraction which are preferentially oxidized whereas in milk fat these acids do not exert the same degree of influence on the oxidation reaction.

Origin of Oxidized Flavors in Milk Lipids

Flavor defects resulting from lipid oxidation in dairy products are reported to differ depending upon the lipid composition and its physical state in the product. The phospholipids, due to their orientation on the fat globule and their high concentration of unsaturated fatty acids, are believed to be the initial site of oxidative attack and the origin of oxidized flavors in fluid milk products. The work of Brown, Dustman, and Thurston (14, p. 599-604) in which they oxidized various milk lipid fractions and added them back to milk for flavor evaluation was in agreement with this view. Further support was presented by Swanson and Sommer (88, p. 201-208) who found that the iodine number of the phospholipid fraction of oxidized milk was lowered while little or no change was observed in the triglyceride fraction. Tarassuk, Koop and Pette (93, p. 258-278) reported that the origin of trainy flavor in cream was in the fat globule membrane material and that it was due to the oxidation of the unsaturated

fatty acids of the phosphatides.

Investigations by Lea (62, p. 1-13) also have suggested that the phospholipids are the origin of oxidized flavors in milk. Lea points out that the phospholipid fraction of liquid milk oxidized preferentially and much more rapidly than the glyceride fraction. This conclusion is based on the fact that the content of polyunsaturated fatty acids in the milk phospholipids is considerably greater than in the glycerides.

A group of Australian workers do not completely agree with the above conclusions. Pont (73, p. 316-325) in his studies on phospholipid free milk fat obtained metallic and oily flavors which he stated were a part of the typical oxidized flavor encountered in whole milk. The oxidized flavor developed to the same extent in buttermilk and was unaffected by alteration of phospholipid content. Pont stated that if the oxidized flavor was due to oxidation of the globule membrane phospholipids only, one would not expect the flavor of equal extent to occur in skim milk, which he found to be the case.

It is generally believed that the flavor of autoxidized milk fat differs from that of whole milk (78, p. 1-2). In the case of milk fat the small amounts of phospholipids undoubtedly are distributed throughout the fat and have

less influence on the flavor compounds produced. The sensory character of the flavor may vary, depending upon environmental conditions and the composition of the fat.

The physical state of the milk lipids in the two systems discussed up to this point are different. In fluid milk a large portion of the phospholipids are located at the fat-serum interface (51, p. 269-274) whereas in milk fat the two fractions are interdispersed. Dried whole milk presents yet another system in which the lipids may form thin films over the non-lipid particles. The position of the phospholipids with respect to the triglycerides is not yet clear (43, p. 709). The oxidized flavor of dry whole milk, however, is described as tallowy and oily, which is suggestive of triglyceride oxidation (78, p. 1-2).

Direct chemical evidence to substantiate the views on the origin of oxidized flavor in dairy products is meager. Changes in the iodine number for instance have been shown not to correlate with the oxidized flavor (88, p. 201-208). It indicates the degree of unsaturation present in milk lipids, but does not decrease to a great extent during oxidation. This seems logical since the double bonds remain intact in the early stages of autoxidation (21, p. 75-96).

Quantitative and qualitative identification of flavor compounds should reveal difference in the various oxidized system if real differences exist. A few investigations have followed this approach in recent years (23, p. 585-597; 37, p. 91-102; and 38, p. 345-348), but the data is far from conclusive. These investigations, as described previously, have shown that the various oxidized systems produce carbonyls that are different; consequently, the flavor produced thereby may also be different.

Detection of the Autoxidized Flavor

Research workers in the food field have long sought objective chemical tests for detection of lipid oxidation which will correlate with psychometric methods. Many chemical tests have been developed but few, if any, are as reliable as organoleptic methods for detecting the defect. The search for objective tests continues, however, and for obvious reasons. This was pointed out by Trout and Sharp (94, p. 1-66) who indicated that organoleptic methods for evaluation of edible fats and oils are still the most suitable, but use of this method presents a number of problems. The selection, training and maintenance of an elaborate taste panel is costly and time consuming. Small laboratories may lack qualified personnel to serve as panel members. An additional serious factor

is that qualified personnel may be too close to the research program to render unbiased opinion.

The chemical tests that have been developed are dependent upon the measurement of the initial oxidation products (hydroperoxides), the oxidation-reduction potential of the system, or secondary products of the autoxidation reactions. A number of these tests were listed by Riel and Sommer (78, p. 17).

The tests that are dependent upon the initial oxidative products include the peroxide value, which is an expression of the iodine liberated by peroxide oxygen from an acid solution of potassium iodide and fat; ammonium thiocyanide and ferric ammonium sulfate reaction with fat peroxides (20, p. 133-139); the decrease in the iodine number of the fat due to the attack of oxygen at the double bonds in unsaturated fatty acids; a decrease in refractive index due to a decrease in unsaturation (86, p. 265-280); and the increase in the weight of the lipid, due to oxygen addition (16, p. 753-760).

The influence of autoxidation of lipids upon the oxidation-reduction system, as determined by measuring the increase in the potential of the system (89, p. 597-614) or by the reduction with methylene blue (13, p. 629-685), has been used as a method of detecting the defect.

The tests that are based on the formation of secondary products include the Kreis' test which was reported to be based upon a reaction between ephihydrin aldehyde and phloroglucinol (13, p. 629-685); the Schilsted's test in which a color is formed when aldehydes react with rosaniline-hydrochloric acid (88, p. 201-208); the 2-thiobarbituric acid reaction with malonaldehyde (72, p. 669-678); and the determination of the carbonyl content which will be discussed later. It has been found that most of these tests will reveal oxidative changes, but they have given poor correlations with sensory detection of the defect in milk lipids.

The 2-Thiobarbituric acid test (TBA) is one of the more sensitive tests that have been developed to measure oxidation in milk fat. Patton and Kurtz (72, p. 669-674) first investigated the reaction of this acid on oxidized milk fat and reported that malonaldehyde was the probable reactant. Later Dunkley and Jennings (26, p. 1064-1069) developed a TBA test for detecting the oxidized flavor in fluid milk. Dunkley (25, p. 342-346) found that the TBA test was highly correlated with organoleptic evaluations for determining the intensity of oxidized flavor while Aule and Storgard (3, p. 1076-1078) found no correlation in their work.

Yu and Sinnhuber (95, p. 104-108) adapted the TBA test for detection of fat oxidation in fishery products. Sinnhuber and Yu (83, p. 9-12) characterized the TBA value as milligrams of malonaldehyde per kilogram of fat. Later Sinnhuber et al. (84, p. 626-634) reported evidence that characterized the TBA reactant as malonaldehyde. This was done by comparing the TBA reaction product isolated from autoxidized lipid systems with the TBA-malonaldehyde reaction product.

The peroxide value has been reported to have a low correlation with oxidized flavor (47, p. 336-340; and 71, p. 693-701). The hydroperoxides do not exhibit oxidized flavors, rather it is their degradation which results in the flavor defects. The peroxides vary with temperature, type of fat, its acidity, age and treatment. These factors would tend to account for the low correlation of peroxides values with sensory detection of the flavor.

Since carbonyl compounds are products of autoxidation and are believed to be the flavor compounds in oxidized lipids, several investigators have attempted to develop suitable tests for their measurement (74, p. 215-218; 71, p. 541-545; and 45, p. 88-91). In 1920, Mathewson (66, p. 1277-1279) proposed a method for estimating small amounts of carbonyl compounds by converting the compounds into colored derivatives and measuring them spectrophoto-

metrically. Pool and Klose (74, p. 215-218) and Lappin and Clark (61, p. 541-545) utilized Mathewson's approach for estimating the total carbonyl content in oxidized foods. The procedures were based upon the formation of the 2,4-dinitrophenylhydrazones of the carbonyls directly in the lipids, reaction of the hydrazones with alcoholic potassium hydroxide, followed by spectrophotometric measurement of the resulting color at specific wave lengths. They found that the total carbonyl content did not correlate with peroxide values. While sufficient evidence was not given, they suggested that the total carbonyl content could be correlated to the oxidized flavor. Henick et al. (45, p. 89-91) further developed the method of Lappin and Clark into a test which they claimed correlated with the oxidized flavor in rancid foods.

Some fats contain compounds that interfere with the total carbonyls test. These compounds apparently do not contribute to the flavor and may be either non-volatile carbonyls or precursors which yield carbonyls during the course of the test. Consequently reliability of the total carbonyls test for measuring lipid oxidation is questionable. Chang and Kummerow (19, p. 341-344) overcame these difficulties by measuring only the volatile carbonyls, which are believed to be the flavor compounds. The volatiles were recovered on powdered dry ice by flushing the

fat with nitrogen and their concentrations measured by the alcoholic potassium hydroxide techniques as described by Lappin and Clark (61, p. 561-565). Berry and McKerrigan (7, p. 693-701) also recovered the volatile carbonyl compounds by flushing the sample with nitrogen and measuring them spectrophotometrically. They introduced the term "carbonyl index" as a unit of measure for the volatile carbonyl compounds and they reported a high correlation of the carbonyl index with oxidized flavor of fats and oils.

EXPERIMENTAL

Preparation and Autoxidation of Milk Fat Samples

In order for an objective chemical test to give reliable results on oxidized milk fat, it must be applicable to samples of varying composition and it should give comparable results over a relatively wide range of oxidative deterioration. The former criterion was met by collecting milk fat samples over a period of six months, from January through June, which gave samples covering the normal range of iodine numbers, 28 to 38. The latter criterion was fulfilled by allowing the samples to oxidize to different stages giving TBA numbers over the range of 0.51 to 30.90.

Sixteen milk fat samples, obtained from mixed herd milk, were collected over a six month period. The samples were prepared from sweet cream by the churning of large batches of butter in the Dairy Products Laboratory. The samples were taken from the churn prior to salting of the butter and phospholipid free milk fat was prepared by warming the butter to 55°C. and washing three times with distilled water. The water was removed after each washing by means of a small cream separator and after the final washing the fat was filtered for removal of the final

traces of water. The iodine number of each sample was determined by the Hanus method (4, p. 494), after which the butter oil was placed in quart milk bottles, sealed and stored in the dark at 23°C. After six months storage it was found that oxidation of the samples had not proceeded to the desired level and it became necessary to remove the samples from dark storage and expose them to light and atmospheric conditions to speed up the oxidation reaction. Samples were periodically removed from exposure to light and atmospheric conditions and placed in dark storage at -10°C. until analyses could be effected. It was possible to obtain samples with varying degrees of oxidation by this procedure. As a number of tests were involved, only one sample per week could be analyzed. Therefore, it was necessary to hold the oxidized samples in cold storage until time for analyses.

Control fat samples were prepared from fresh cream as described above and analyzed immediately after preparation.

Analyses of Fat Samples

The fat sample to be analyzed was removed from the -10°C. storage room and melted at 50°C. The peroxide value, TBA numbers, and total carbonyl values were determined immediately. The volatile carbonyls were then

isolated from the fat. This phase of the analysis required the better part of one day. The absolute flavor threshold value of the fat was determined on the following day and the analyses of the isolated volatile carbonyl compounds required the remainder of the week. Since autoxidized fat is subject to change during the course of its manipulation, the tests were conducted in the above sequence in order to limit the effect of such changes upon the results of the investigation.

The Peroxide Value

The peroxide value was determined by the A.O.C.S. method (2, p. C48-53). The peroxides and similar products were expressed as milli-equivalents of peroxide per kilogram of fat.

Determination of the Total Carbonyl Content of Milk Fat

The amount of total carbonyls in the various fat samples were determined by the procedure of Berry and McKerrigan (7, p. 693-701). The test involved the formation of 2,4-dinitrophenylhydrazones in the intact fat after which the solution was reacted with alcoholic KOH and the absorbancy at 430 $m\mu$ and 460 $m\mu$ was determined. The saturated and unsaturated carbonyls can be differentiated by this technique since the saturated components

in the mixture give a maximum absorption at 430 $m\mu$ and the unsaturated at 460 $m\mu$.

The absorbancy of the alkaline solutions were measured in a Beckman DU Spectrophotometer, using 1 cm matched quartz cells.

The formulae used for calculations of the total saturated and unsaturated carbonyl content were:

1. micromoles/50 ml. of unsaturated carbonyls:

$$V = (4.344)(A_{m460}) - (3.373)(A_{m430})$$

2. micromoles/50 ml. of saturated carbonyls:

$$S = (5.812)(A_{m430}) - (4.420)(A_{m460})$$

The values obtained were converted to millimoles per kilogram of fat by the formulae:

$$3. \quad U = \frac{ux5}{w}$$

$$4. \quad S = \frac{sx5}{w}$$

Where u and s are the values obtained from equations (1) and (2) and w is the weight of sample used for the determination.

2-Thiobarbituric Acid Number

The 2-thiobarbituric acid number is an expression of the milligrams of malonaldehyde per kilogram of fat. The procedure used in this investigation was essentially that described by Yu and Sinnhuber (95, p. 104-108). Since

pure fat samples were involved, it was unnecessary to use several of the reagents used by the above authors for the more complex food systems. Since several modifications were employed, the procedure as used in this investigation follows:

Reagents: The TBA reagent was prepared according to Kohn and Liversidge (60, p. 292-300). However, the charcoal treatment was found to be unnecessary and was omitted.

Method: The size of the milk fat sample ranged from two to five grams, depending upon the degree of oxidation of the fat. The larger size samples were required in the less intensely oxidized samples in order to obtain sufficient color development for reproducible measurements. The fat sample was weighed into a 250 ml. flat-bottom boiling flask with a ground glass top. Four ml. of distilled water, 15 ml. of 0.6N HCl and 6 ml. of TBA reagent was added to the flask. The flask was connected with a condenser and the mixture refluxed for 30 minutes, after which, 75 ml. of 0.6N HCl was added through the top of the condenser and refluxing was continued for ten minutes. The flask and contents were cooled to room temperature and the solution was transferred into a 250 ml. separatory funnel. The lower aqueous layer was drawn from the funnel and the absorbancy was measured with a Beckman DU

Spectrophotometer at 535 $m\mu$ using 1 cm. matched quartz cells. In the initial test the reaction mixture was placed in the separatory funnel and centrifuged at 1800 r.p.m. in order to obtain a clear aqueous phase for spectral analysis. Early in the work it was found that the centrifuging step could be eliminated merely by allowing the reaction mixture to stand in the separatory funnel for five minutes prior to drawing off the colored aqueous phase.

The results were expressed as TBA number, which is the milligrams of malonaldehyde per kilogram of fat sample.

Isolation of Volatile Monocarbonyl Compounds

The volatile carbonyl compounds were isolated from the fat by means of the distillation apparatus shown by Day and Lillard (23, p. 585-597) except that a 250 ml. flask was used in place of flask No. 3. A one-hundred gram sample was used in each distillation. The volatile carbonyls were removed by warming the fat to 40-50°C. at 2-4 mm H_g pressure and stripping the fat with steam generated at the same pressure. The distillation was stopped after 50 ml. of water had passed from the steam generator. The volatile carbonyl compounds were collected on traps cooled by powdered dry ice (-78.5°C.). After the distillation was completed, the frozen distillate was melted and

added to 50 ml. of 5N HCl saturated with 2,4-dinitrophenylhydrazine. The dry ice traps were washed with 50 ml. of ethanol, which was added to the distillate-reagent mixture to give a total volume of 150 ml. The mixture was allowed to stand twenty-four hours for the reaction to occur. The 2,4-dinitrophenylhydrazones of the volatile monocarbonyl compounds were then removed from the reaction mixture by extracting five times with twenty ml. portions of hexane. The hexane extracts were combined and the hexane was removed at reduced pressure to yield a dry residue. This residue, which had been previously shown to contain mostly monocarbonyl compounds (23, p. 585-597), was used for determining the quantity of total volatile unsaturated, volatile saturated, and individual volatile monocarbonyls compounds of the milk fat.

Quantitative Measurement of the Individual Volatile Monocarbonyl Compounds

Earlier work by Day and Lillard (23, p. 585-597) demonstrated that the 2,4-dinitrophenylhydrazones of the volatile monocarbonyl compounds from autoxidized milk fat were separable into 11 major bands by means of a hexane-nitromethane Celite partition column. In the course of identifying components of each of the major bands it was

revealed that each of the 11 chromatographic bands, except 10 and 11, represented a multi-component mixture. The compounds occurring in each chromatographic band were identified. Since the method used for measuring the individual compounds in this study is based upon the findings of Day and Lillard, Table 1 which describes the composition of the chromatography bands is included at this point for the sake of clarity. It will be noted in Table 1 that each of the chromatographic bands except bands one, ten and eleven contain a saturated and an unsaturated component. Furthermore, bands one, two, six and nine also contain saturated methyl ketones. In attempting to obtain quantitative data for each of the monocarbonyl compounds, it was impractical to separate each of the multicomponent bands listed by Day and Lillard (23, p. 585-597). Rather it was more feasible to apply the column chromatography technique to give the eleven major bands and then adapt the alcoholic KOH procedure of Berry and McKerrigan (7, p. 693-701) for measurements of the saturated and unsaturated components in each band. The procedure, as employed, follows:

Chromatographic Separation of the Monocarbonyl

Mixture: The column (method B) was prepared as described by Day et al. (22, p. 463-474). The 2,4-dinitrophenylhydrazones of the volatile monocarbonyls from 100 grams of

TABLE 1
COMPOSITION OF THE COLUMN CHROMATOGRAPHY BANDS
PRODUCED BY THE VOLATILE MONOCARBONYL
COMPOUNDS FROM AUTOXIDIZED MILK FAT

Band No.	Fraction	Compound
Forerun		2-Pentadecanone
1	1A	2-Tridecanone
	1B	2-Undecanone
2	2A	2-Nonanone
	2B	n-Decanal
	2C	Undec-2-enal
3	3A	n-Nonanal
	3B	Dec-2-enal
4	4A	2-Heptanone
	4B	n-Octanal
	4C	Non-2-enal
5	5A	n-Heptanal
	5B	Oct-2-enal
6	6A	2-Pentanone
	6B	n-Hexanal
	6C	Hept-2-enal
7	7A	n-Pentanal
	7B	Hex-2-enal
8	8A	n-Butanal
	8B	Pent-2-enal
9	9A	Acetone
	9B	Propanal
	9C	But-2-enal
10		Ethanal
11		Methanal

oxidized milk fat were dissolved in 15 ml. of carbonyl free hexane and 5 ml. was carefully added to the top of a 15 g. chromatography column. After the solution of derivatives moved into the column, hexane was added and the column was allowed to develop. Each band was collected as it was eluted from the column. The forerun and band one were collected together as were bands 10 and 11. The bands were collected in 250 ml. ground glass flasks and the hexane was removed under reduced pressure.

Measure of the Individual Compounds: The residue of each band was dissolved in 5 ml. of benzene. Usually one ml. (depending upon the concentration of the band and the level of oxidation in the sample) of the benzene solution was placed in a 50 ml. volumetric flask. Ten ml. of a 4 per cent alcoholic KOH solution was added and the contents of the flask diluted to the mark with carbonyl free absolute ethanol. After ten minutes, the absorbancy was measured at 430 and 460 $m\mu$ in a Beckman DU Spectrophotometer using 1 cm. matched quartz cells. After accounting for the dilutions involved in handling the contents of each band, the concentrations of saturated and unsaturated carbonyl compounds for each band were determined by the equation given previously (7, p. 693-701). The data were then converted to milligrams of carbonyl compound per

kilogram of fat by multiplying the millimoles of each compound by its molecular weight. All compounds were expressed as milligrams per kilogram of fat.

The above determinations did not distinguish between saturated aldehydes and ketones, and both of these classes of carbonyls were found in bands 1, 2, 6 and 9. Since the ketones were found only in trace amounts by Day and Lillard (23, p. 585-597), their presence in the autoxidized fat samples were ignored when expressing the saturated carbonyl content of the various chromatographic bands. The values for the saturated carbonyls, therefore, would include the traces of ketones in those bands where ketones were present.

Recovery Studies on the Distillation and 2,4-Dinitrophenylhydrazine Carbonyl Reaction

The efficiency of the distillation apparatus for removal of the volatile carbonyl compounds from milk fats was ascertained by adding weighted amounts of authentic but-2-enal, n-pentanal, n-heptanal, n-octanal, n-nonanal and n-undecanal to 100 grams of fresh milk fat. This mixture was distilled and received treatment identical to each of the autoxidized milk fat samples. For control purposes a 100 gram sample of fresh milk fat received the same treatment in order to account for carbonyls that were

originally present. The amount of each compound recovered was determined as previously described. The per cent recoveries found were but-2-enal, 23.4 per cent, n-pentanal, 30.73 per cent; n-heptanal, 36.16 per cent; n-octanal, 69.28 per cent; n-nonanal, 53.96 per cent; n-undecanal, 45.28 per cent.

The per cent reaction of each carbonyl compound with 2,4-dinitrophenylhydrazine was also accounted for by adding the above compounds to a reaction mixture identical to the one in which the oxidized fat distillates were treated. The per cent reaction were but-2-enal, 73.7 per cent; n-pentanal, 77.9 per cent, n-heptanal, 65.73 per cent; n-octanal, 83.0 per cent; n-nonanal, 92.3 per cent; n-undecanal, 95.01 per cent.

The true per cent recoveries including both the distillation and 2,4-dinitrophenylhydrazine reaction were butenal, 31.1 per cent; n-pentanal, 39.5 per cent; n-heptanal, 55.0 per cent; n-octanal, 83.46 per cent; n-nonanal, 58.6 per cent; n-undecanal, 47.65 per cent.

The data presented in Tables 7 and 8 were corrected on the basis of the recoveries studies. All of the carbonyls compounds found in the oxidized milk fat were not available in pure form for the recovery studies. The compounds used were selected to cover the entire range of

chain lengths and it was necessary to extrapolate for the compounds falling between those used.

Organoleptic Examination of Oxidized Milk Fat

The procedure described by Patton and Josephson (71, p. 316-318) was used to determine the absolute flavor threshold (FTV) of the oxidized fat samples.

Preparation of the Samples: A series of milk samples were prepared containing a range of concentrations of the oxidized milk fat. The fat sample to be evaluated was melted at 55°C. and twelve grams was emulsified into 288 ml. of fresh pasteurized skim milk by means of a small hand homogenizer. The skim milk was warmed to 55°C. to facilitate the homogenization step and the sample was passed four times through the homogenizer to assure adequate emulsification of the fat. This "oxidized" milk sample was then diluted with fresh homogenized milk to the proper dilution for the absolute flavor threshold determination. The desired range of dilutions depended upon the degree to which the fat sample had been oxidized. An ideal range of dilutions included concentration well below to well above the flavor threshold. Fresh homogenized milk was included in each evaluation as the control. The sample dilutions were expressed as grams of oxidized fat

per liter of milk. In all cases, the milk samples, constituting a series of dilutions of oxidized fat, always contained four per cent fat. For example, the dilution range for milk fat sample No. 4 in homogenized milk was 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 grams of oxidized fat per 1000 ml. of milk. Each of these dilutions contained 40 grams of fat per liter of milk, but the amount of oxidized fat ranged from 0 to 1.0 grams per liter of milk over the series.

Selection of Judges: The flavor judges were selected on their ability and consistency for detecting the oxidized flavor. Five judges were used to evaluate a particular fat, but it was necessary to use seven different judges throughout the investigation since every judge was not always available for the test.

Determination of the Absolute Flavor Threshold Value: The judge was seated beside a sink in a flavor judging laboratory in a position so that he could not see the samples. Five replications of each concentration was given to each judge at random. Five ml. of the sample, adjusted to room temperature, was drawn into a 10 ml. graduate pipette by means of an attached rubber bulb. The sample was then sprayed into the judge's mouth who, after evaluation of the sample, indicated his judgment as either

positive or negative concerning the presence of an oxidized flavor. One or two preliminary presentations of the control and highest concentration sample were made to each judge so that he was properly oriented to make judgement. Rinsing the mouth and rest periods between observations were left to the judges' discretion.

By using five judges, a total of 25 responses were obtained for a given concentration or a total of 150 observations for a series of dilutions. The per cents of positives responses versus concentration of oxidized fat in the milk were plotted on a graph and the 50 per cent level of positives responses was selected as the absolute flavor threshold value. A graph showing a typical curve is presented in Figure 1 for Sample No. 7A.

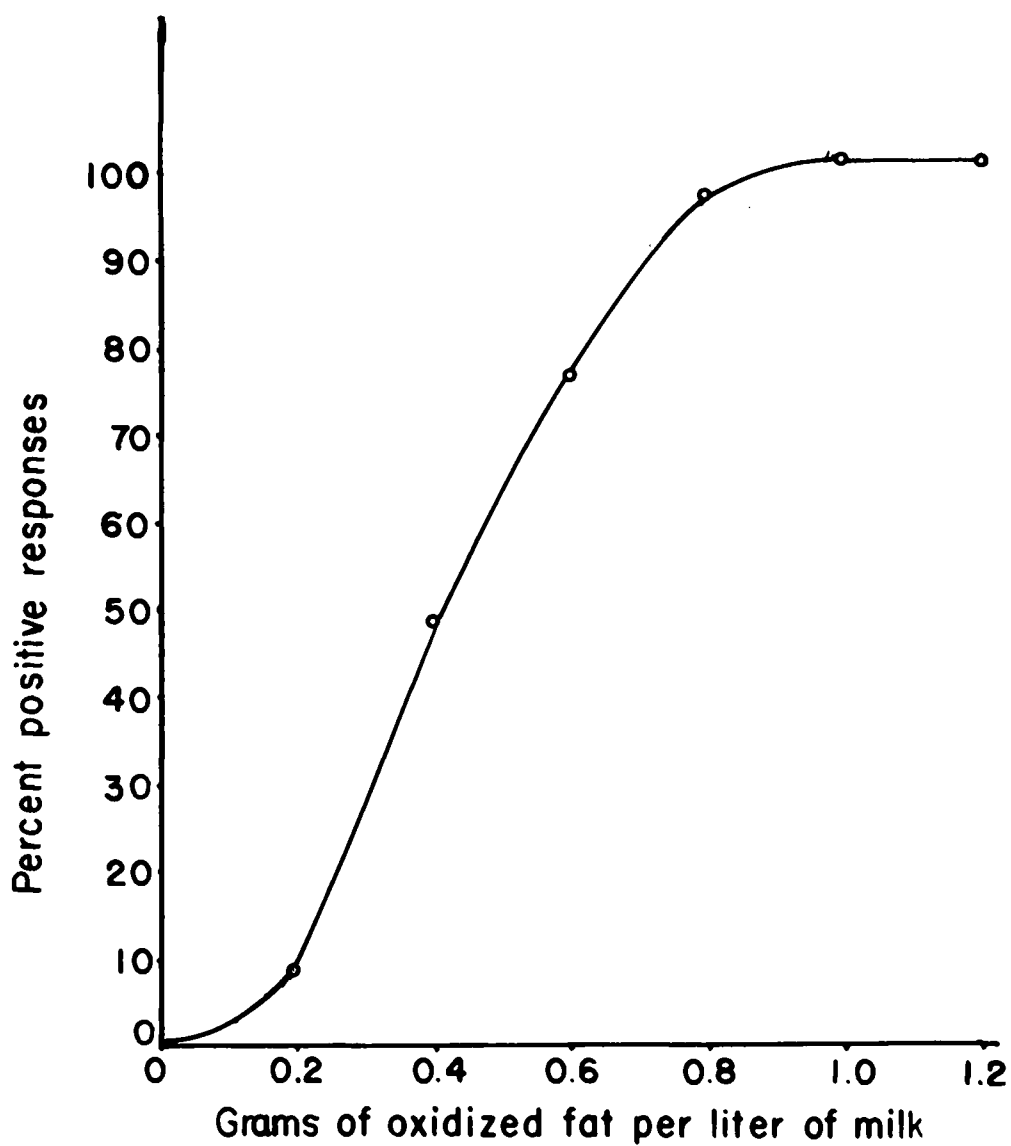


Fig. 1. Absolute flavor threshold curve for a sample of oxidized milk fat

RESULTS AND DISCUSSION

Quantitative Analysis

The data obtained for fresh milk fat and 16 oxidized samples, when analyzed by objective and sensory methods, are shown in Table 2. The samples are listed in the order of increasing oxidized flavor intensity, as measured by the absolute flavor threshold test. The data listed for the various chemical tests are the average of duplicate analysis.

Correlation of Chemical Tests to Each Other

Correlation coefficients were determined by the method described by Li 965, p. 268) and are listed in Table 3. All of the coefficients are significant at the one per cent level. This indicates that all of the chemical tests described herein are highly correlated with each other. The high correlation between peroxide value and the total carbonyl contents of oxidized fats has been reported by other workers (7, p. 693-701; and 45, p. 89-91). The high correlation of the peroxide values with the TBA numbers (0.962) and total carbonyls (0.961) indicates that the TBA and total carbonyl tests are largely an indirect measure of lipid hydroperoxides. The high correlation of the above chemical tests with each other

TABLE 2
OXIDATION INTENSITY OF MILK FATS AS MEASURED BY DIFFERENT TESTS

Sample Number	C ^e	1	2	3	4	5	6	7	8
Total Unsaturated ^a Carbonyls	0.61	0.99	0.91	2.94	2.83	4.53	3.55	6.24	3.73
Total Saturated ^a Carbonyls	12.10	8.76	7.47	14.05	15.90	10.00	14.77	12.03	16.08
Total Carbonyls ^a	12.71	9.75	8.38	16.99	18.73	14.53	18.32	18.27	19.81
Unsaturated ^a Volatile Carbonyls	0.0	0.0	0.0	0.0	0.03	0.02	0.03	0.04	0.05
Saturated ^a Volatile Carbonyls	0.05	0.11	0.24	0.28	0.26	0.20	0.26	0.24	0.35
Total ^a Volatile Carbonyls	0.05	0.11	0.24	0.28	0.29	0.22	0.29	0.28	0.40
TBA No. ^b	0.24	0.51	0.53	3.92	4.82	3.92	5.90	6.57	6.17
Peroxide Value ^c	0.0	0.0	0.0	11.98	12.54	16.69	20.91	18.66	13.35
Flavor Threshold ^d	40.00	5.70	3.10	0.80	0.42	0.41	0.39	0.35	0.34

TABLE 2, continued

Sample Number	9	10	11	12	13	14	15	16
Total Unsaturated Carbonyls ^a	4.87	4.26	5.92	13.25	5.04	3.68	7.91	6.79
Total Saturated Carbonyls ^a	13.95	10.26	9.43	28.25	15.01	16.45	17.30	23.15
Total Carbonyls ^a	18.82	14.52	15.35	41.50	20.05	20.13	25.21	29.49
Unsaturated Volatile Carbonyls ^a	0.02	0.04	0.05	0.12	0.04	0.06	0.07	0.13
Saturated Volatile Carbonyls ^a	0.42	0.17	0.42	0.48	0.41	0.43	0.55	0.82
Total Volatile Carbonyls ^a	0.44	0.21	0.47	0.60	0.45	0.49	0.62	0.95
TBA No. ^b	7.86	5.53	9.67	30.90	15.03	17.05	18.02	27.03
Peroxide Value ^c	17.30	13.33	17.80	64.60	26.45	28.65	29.72	45.72
Flavor Threshold ^d	0.30	0.25	0.25	0.17	0.16	0.15	0.14	0.12

^a Expressed as millimoles of carbonyl per kilogram of fat.

^b Expressed as milligrams of malonaldehyde per kilogram of fat.

^c Expressed as milliequivalents of peroxide per kilogram of fat.

^d Grams of oxidized fat per liter of 4% fat milk.

^e Control sample of fresh fat.

TABLE 3
CORRELATION COEFFICIENTS BETWEEN CHEMICAL TESTS
FOR MEASURING THE INTENSITY OF OXIDIZED FLAVOR

Chemical Test	Correlation Coefficient ^a
TBA No. and Peroxide value	.962*
TBA No. and Total Carbonyls	.897*
TBA No. and Total Volatile Carbonyls	.901*
Total Carbonyls and Peroxide Value	.961*
Total Carbonyls and Total Volatile Carbonyls	.714**
Peroxide Value and Volatile Unsaturated Carbonyls	.892*

^a Degree of freedom = 15.

TABLE 4
CORRELATION COEFFICIENTS BETWEEN RECIPROCAL OF
FLAVOR THRESHOLD AND THE CHEMICAL TESTS
FOR MEASURING THE INTENSITY OF OXIDIZED FLAVOR

Chemical Test	Correlation Coefficient ^a
TBA No.	.887*
Peroxide Value	.798*
Total Unsaturated Carbonyls	.666**
Total Saturated Carbonyls	.683**
Total Carbonyls	.714**
Volatile Unsaturated Carbonyls	.996*
Volatile Saturated Carbonyls	.896*
Total Volatile Carbonyls	.887*
Sum of Individual Volatile Unsaturated Carbonyls	.948*

^a Degree of freedom = 15.

* Significant at 0.1% level.

** Significant at 1.0% level.

may be explained partly by the fact that reaction conditions for the tests were conducive to hydroperoxide decomposition and each test measures a product of this reaction.

Correlation of Chemical Tests to the Flavor Threshold

The absolute flavor threshold values (FTV) for 16 oxidized milk fat samples are shown in Table 2. It will be noted that the more intense the oxidized flavor, the lower the FTV. Statistical analyses of the data in Table 2 revealed that the objective chemical tests gave a hyperbola relationship with FTV. Curve A in Figure 2 illustrates the relationship. Plots of the reciprocal of flavor threshold ($1/\text{FTV}$) against the chemical tests, however, approached linearity as shown in curve B, Figure 2.

The correlation coefficients between $1/\text{FTV}$ and the chemical tests are given in Table 4. It is notable that all tests employed were significant at the 1 per cent level. These findings were surprising in view of reports in the literature (3, p. 1076-1078; 7, p. 693-701; and 47, p. 336-340). This may be explained, in part, by the heretofore unreported relationship of the reciprocal of flavor intensity to the chemical tests and the precision of the flavor threshold test.

The unsaturated volatile carbonyls gave the highest correlation. The correlation coefficient of 0.992 could

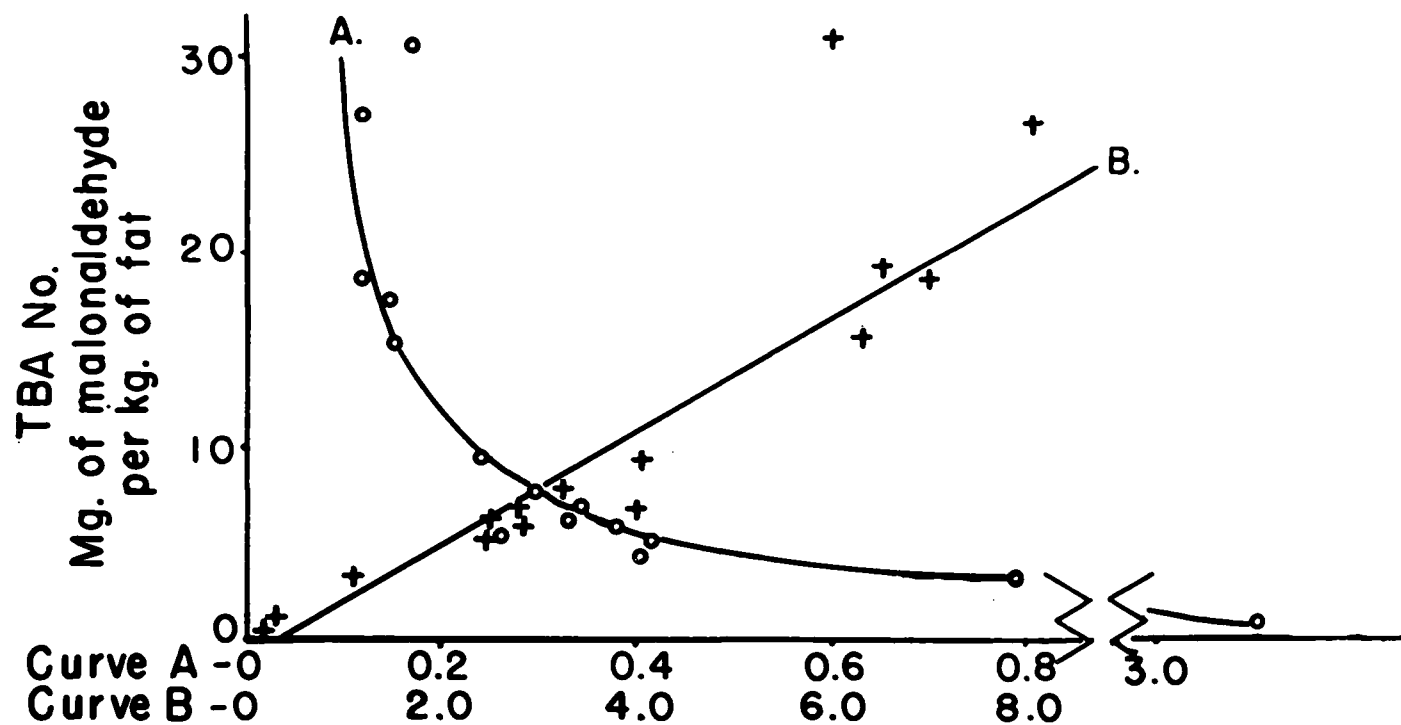


Fig. 2. Curve A. TBA No. vs. Absolute FTV (grams of oxidized fat per liter of milk)

Curve B. TBA No. vs. 1/FTV

have been influenced by the zero values obtained in the less oxidized samples, Table 2. However, the correlation of the total alk-2-enals for each fat, as taken from Table 7, with $1/FTV$ gave a coefficient of 0.948. Holm, Wode and Thome (48, p. 1-12) also found a high correlation between these compounds and the oily flavor of butter. The high correlation of the total volatile carbonyls with oxidized flavor is in agreement with the findings of Berry and McKerrigan (7, p. 693-701) for other fats and oils. These findings tend to indicate that a measure of the volatile unsaturated carbonyls would be the most precise way of relating sensory and chemical methods for oxidized flavor measurement. The unsaturated carbonyls have been reported as major constituents of oxidized flavor (37, p. 91-103; 38, p. 343-348; 48, p. 1-12; and 79, p. 1-73).

The total unsaturated and total carbonyls gave low correlations with $1/FTV$. This may be due to the presence of carbonyl material in the fat that interferes with the total carbonyls test. This carbonyl material apparently does not contribute to the flavor and may be non-volatile carbonyls or precursors which yield carbonyls during the course of the test. Therefore the use of the total carbonyls as a method for measuring lipid oxidation is questionable.

The correlation of the TBA number to $1/FTV$ was 0.884. This high correlation agrees with the work of Dunkley and Jennings (26, p. 1064-1069). On the other hand, Aule and Storgard (3, p. 1076-1078) did not observe a significant correlation of the TBA test with oxidized flavor.

The peroxide value had a correlation coefficient of 0.798 with $1/FTV$. Several investigators (7, p. 693-701; and 47, p. 336-340) have reported a low correlation between peroxide values and oxidized flavor intensity of fats and oils which is contrary to the data reported herein.

The slightly oxidized milk fat samples did not give positive peroxide values, even though an oxidized flavor had developed. Most dairy products exhibiting oxidized flavor defects would fall in this region which makes the peroxide test of questionable value in practical applications.

Individual Carbonyl Compounds

The quantities of individual carbonyl compounds that were found in the oxidized fat samples are given in Tables 5 and 6. In general, the concentration of the volatile aldehydes increased congruently with the degree of oxidation. Changes in the methanol-ethanol fraction were erratic and may be attributed to the inability to consistently trap this fraction during distillation. Solvent

TABLE 5
UNSATURATED VOLATILE MONOCARBONYL COMPOUNDS IN AUTOXIDIZED MILK FAT

Compounds (mg/kg fat)	Ca	Sample Number							
		1	2	3	4	5	6	7	8
C ₁₁ -enal	0.0	0.02	0.03	0.22	0.51	0.38	0.35	0.80	0.74
C ₁₀ -enal	0.0	0.02	0.07	0.06	0.61	0.48	0.37	0.85	0.90
C ₉ -enal	0.0	0.04	0.04	1.02	1.11	1.13	1.24	1.69	2.15
C ₈ -enal	0.0	0.06	0.05	0.18	0.14	0.44	0.44	0.87	0.46
C ₇ -enal	0.0	0.06	0.0	0.38	0.91	0.82	0.75	1.74	1.16
C ₆ -enal	0.0	0.0	0.0	0.16	0.27	0.02	0.19	0.49	0.02
C ₅ -enal	0.005	0.01	0.0	0.38	0.69	0.32	0.28	0.60	0.96
C ₄ -enal	0.0	0.0	0.0	0.17	0.31	0.17	0.07	0.12	0.21
TOTAL	0.005	0.21	0.19	2.57	4.55	3.76	3.72	7.16	6.60

TABLE 5. continued

Compound (mg/kg fat)	Sample Number							
	9	10	11	12	13	14	15	16
C ₁₁ -enal	0.50	1.51	0.69	1.41	0.96	0.93	1.32	2.08
C ₁₀ -enal	0.25	0.98	1.00	2.68	0.69	1.88	1.66	2.82
C ₉ -enal	3.24	2.35	4.53	7.26	5.61	7.16	7.88	6.24
C ₈ -enal	0.27	0.47	0.29	2.33	0.0	0.21	0.67	0.65
C ₇ -enal	1.52	1.32	1.85	2.81	1.99	2.59	2.92	1.47
C ₆ -enal	0.0	0.0	0.10	1.17	0.01	0.67	0.32	1.26
C ₅ -enal	1.19	0.58	1.20	3.21	1.44	1.87	2.40	3.80
C ₄ -enal	0.0	0.31	0.37	0.78	0.51	0.57	0.33	1.23
TOTAL	6.97	7.52	9.95	21.65	11.21	15.88	17.50	19.55

^a Control sample of fresh fat

TABLE 6
SATURATED VOLATILE MONOCARBONYL COMPOUNDS IN AUTOXIDIZED MILK FATS

Compounds (mg/kg fat)	C ^a	Sample Number							
		1	2	3	4	5	6	7	8
C ₁₃ -one	0.88	1.38	1.38	1.30	2.58	2.82	2.37	2.50	3.79
C ₁₀ -al	0.10	0.14	0.53	0.60	1.15	1.12	1.18	1.97	0.95
C ₉ -al	0.04	0.17	0.21	1.25	1.07	1.05	1.29	1.80	1.63
C ₈ -al	0.14	0.28	0.65	0.93	1.58	1.29	1.51	1.92	1.91
C ₇ -al	1.29	0.32	0.44	2.99	15.39	12.92	13.91	17.53	17.17
C ₆ -al	0.24	0.70	1.36	4.58	3.91	3.73	3.75	4.81	4.42
C ₅ -al	0.71	2.23	0.42	5.59	4.41	2.72	3.72	4.27	6.36
C ₄ -al	0.04	0.39	1.37	0.67	0.57	0.51	0.59	0.57	0.38
C ₃ -al	2.83	2.07	1.89	3.00	2.73	1.01	1.27	1.01	3.65
C ₂ -al and C ₁ -al	1.90	8.69	14.84	16.98	13.84	14.29	10.84	10.57	20.84
TOTAL	8.17	16.37	23.09	37.89	47.23	41.46	40.43	46.95	61.10

TABLE 6, continued

Compounds (mg/kg fat)	Sample Number							
	9	10	11	12	13	14	15	16
C ₁₃ -one	1.87	3.50	1.66	3.36	2.09	1.23	2.95	1.60
C ₁₀ -al	0.82	0.58	1.28	3.06	0.94	1.05	1.74	2.35
C ₉ -al	1.83	1.62	0.55	5.39	2.58	2.78	3.24	5.08
C ₈ -al	1.87	1.86	1.97	6.98	3.01	3.87	4.19	3.96
C ₇ -al	21.07	13.46	9.66	22.24	12.61	13.46	17.30	11.91
C ₆ -al	5.53	4.53	5.14	24.42	9.86	10.55	10.67	10.84
C ₅ -al	9.44	6.30	8.07	7.82	9.53	9.65	15.94	10.94
C ₄ -al	0.67	0.49	0.85	1.35	1.06	1.35	1.82	1.15
C ₃ -al	3.39	1.89	1.47	5.05	3.86	3.34	5.04	4.45
C ₂ -al and C ₁ -al	16.01	14.87	21.23	19.42	19.18	28.57	36.28	15.02
TOTAL	62.50	49.10	51.88	99.09	64.72	75.95	99.17	67.30

^a Control sample of fresh fat

contamination also may have contributed to the observed variability. It should be noted in Table 6 that in most oxidized samples n-heptanal occurred in the highest concentration and was followed by n-hexanal and n-pentanal respectively. The concentration of non-2-enal exceeded that of any other unsaturated aldehyde as shown in Table 5.

The percentage composition, mean percentage and standard deviation of the carbonyl mixture for the individual fat samples were calculated from the data of Tables 5 and 6 and are shown in Tables 7 and 8. It can be seen that the percentage composition varied considerably from one fat sample to the next. N-heptanal constituted from 1.13 to 32.39 per cent of the volatile monocarbonyl content of the samples studied. The different milk fat samples used in this investigation could account for some of the variation in percentage composition among the oxidized samples.

Forss et al. (36, p. 211-219) reported the percentage composition of the carbonyl mixture isolated from fishy flavored milk fat. The major compounds were heptanal, 20 per cent; hexanal, 25 per cent; non-2-enal, 5 per cent; hepta-2,4-dienal, 8 per cent; hepta-2-one, 10 per cent. The mean percentages of the carbonyls measured in this study, Tables 7 and 8, were heptanal, 18.9 per cent;

TABLE 7
PERCENTAGE COMPOSITION OF VOLATILE UNSATURATED
CARBONYL COMPOUNDS IN AUTOXIDIZED MILK FATS

Compounds	Sample Number									
	Ca	1	2	3	4	5	6	7	8	9
C ₁₁ -enal	0.0	0.15	0.07	0.54	0.99	0.85	0.80	1.48	1.09	0.63
C ₁₀ -enal	0.0	0.09	0.17	0.15	1.17	1.05	0.84	1.56	1.32	0.31
C ₉ -enal	0.0	0.25	0.10	2.52	2.15	2.49	2.82	3.13	3.18	4.08
C ₈ -enal	0.0	0.34	0.13	0.44	0.27	0.96	0.99	1.61	0.68	0.36
C ₇ -enal	0.0	0.37	0.0	0.93	1.77	1.82	1.76	3.21	1.72	1.92
C ₆ -enal	0.0	0.0	0.0	0.39	0.52	0.05	0.44	0.91	0.03	0.0
C ₅ -enal	0.06	0.02	0.0	0.93	1.34	0.72	0.63	1.11	1.41	1.49
C ₄ -enal	0.0	0.0	0.0	0.42	0.60	0.37	0.16	0.22	0.32	0.0
TOTAL	0.06	1.22	0.47	6.32	8.81	8.31	8.44	13.23	9.75	8.79

TABLE 7, continued

Compounds	Sample Number							MEAN	Standard Deviation
	10	11	12	13	14	15	16		
C ₁₁ -enal	2.66	1.02	1.17	1.26	1.01	1.13	2.40	1.08	0.81
C ₁₀ -enal	1.71	1.47	2.22	0.91	2.05	1.42	3.25	1.23	0.85
C ₉ -enal	4.16	6.70	6.01	7.40	7.87	6.75	7.19	4.18	2.52
C ₈ -enal	0.82	0.42	1.93	0.0	0.23	0.57	0.75	0.66	0.52
C ₇ -enal	2.33	2.73	2.33	2.62	2.82	2.50	1.70	1.91	0.88
C ₆ -enal	0.0	0.15	0.97	0.01	0.73	0.27	1.44	0.38	0.44
C ₅ -enal	1.03	1.65	2.66	1.90	2.04	2.06	4.38	1.46	1.06
C ₄ -enal	0.55	0.55	0.65	0.67	0.62	0.28	1.41	0.42	0.38
TOTAL	13.46	14.69	17.94	14.77	17.37	14.98	22.52	11.32	

^a Control sample of fresh fat

TABLE 8
PERCENTAGE COMPOSITION OF VOLATILE SATURATED
CARBONYL COMPOUNDS IN AUTOXIDIZED MILK FATS

Compounds	Sample Number									
	Ca	1	2	3	4	5	6	7	8	9
C ₁₃ -one	10.81	8.31	3.61	3.22	4.98	6.24	5.37	4.61	5.59	2.36
C ₁₀ -al	1.18	0.82	1.39	1.47	2.22	2.48	2.67	3.65	1.40	1.03
C ₉ -al	0.54	1.05	0.55	3.09	2.07	2.32	2.92	3.33	2.41	2.31
C ₈ -al	1.65	1.67	1.69	2.29	3.06	2.85	3.41	3.55	2.81	2.35
C ₇ -al	15.78	1.93	1.13	7.39	29.72	28.57	31.54	32.39	25.37	26.52
C ₆ -al	2.91	4.21	3.55	11.31	7.55	8.25	8.50	8.88	6.53	6.96
C ₅ -al	8.65	13.44	1.09	13.82	8.52	6.02	8.43	7.90	9.39	11.87
C ₄ -al	0.50	2.37	3.56	1.66	1.11	1.14	1.34	1.06	0.50	0.85
C ₃ -al	34.66	12.50	10.95	7.42	5.28	2.23	2.87	1.86	5.39	4.27
C ₂ -al & C ₁ -al	23.66	52.44	71.97	41.99	26.73	31.61	24.57	19.53	30.78	32.73
TOTAL	99.94	98.74	99.49	93.66	91.24	91.71	91.62	86.76	90.17	91.25

TABLE 8, continued

Compounds	Sample Number						Mean	Standard Deviation	
	10	11	12	13	14	15			16
C ₁₃ -one	6.17	2.45	2.78	2.75	1.39	2.53	1.84	4.01	1.94
C ₁₀ -al	1.02	1.89	2.54	1.24	1.14	1.48	2.71	1.82	0.79
C ₉ -al	2.85	0.81	4.46	3.40	3.02	2.77	5.85	2.70	1.31
C ₈ -al	3.28	2.89	5.78	3.96	4.22	3.60	4.55	3.28	3.03
C ₇ -al	23.71	14.23	18.42	16.61	14.66	14.82	13.71	18.80	10.00
C ₆ -al	7.98	7.58	20.23	12.99	11.50	9.15	12.47	9.23	3.95
C ₅ -al	11.10	11.90	6.48	12.55	10.52	13.66	12.60	9.96	3.45
C ₄ -al	0.86	1.25	1.11	1.40	1.47	1.56	1.33	1.41	0.71
C ₃ -al	3.33	2.19	4.18	5.08	3.64	4.32	5.12	5.04	2.99
C ₂ -al & C ₁ -al	26.21	40.13	16.08	25.26	31.13	31.09	17.30	32.47	14.07
TOTAL	86.51	85.32	82.06	85.64	82.69	84.98	77.48	88.72	

^a Control sample of fresh fat

hexanal, 9.2 per cent; pentanal, 10 per cent; non-2-enal, 4.2 per cent; the combined pent-2-enal and hepta-2,4-dienal fraction, 1.5 per cent. The differences in percentage composition of the carbonyls from fishy flavored fat (36, p. 211-219) and the fats included in this investigation may explain the differences in their sensory character.

Correlation of Individual Monocarboxyls to the Flavor Threshold

A reciprocal relationship was found to exist between the individual monocarbonyls and FTV. The correlations between the individual monocarbonyls and $1/FTV$ were determined and the coefficients are shown in Table 9. It should be noted that the unsaturated monocarbonyls tend to have a higher correlation with $1/FTV$ than the saturated monocarbonyls. This is in agreement with the observed high correlation of the total volatile unsaturated carbonyls with $1/FTV$. Non-2-enal gave the highest correlation, 0.942. Other alk-2-enals, which gave correlation coefficients, significant at the 1 per cent level were the C_5 , C_6 , C_7 , and C_{10} . Forss, Pont and Stark (37, p. 99-102) reported that the C_8 and C_9 alk-2-enals were the principal flavor components in the cardboard flavor of skim milk, whereas the correlation of oct-2-enal with $1/FTV$ was 0.372 in this study.

TABLE 9
CORRELATION COEFFICIENTS BETWEEN RECIPROCAL OF
FLAVOR THRESHOLD AND THE INDIVIDUAL VOLATILE
MONOCARBONYL COMPOUNDS IN OXIDIZED MILK FATS

Compound	Correlation Coefficient ^a	Compound	Correlation Coefficient ^a
C ₁₁ -enal	.439	C ₁₃ -one	.151
C ₁₀ -enal	.858*	C ₁₀ -al	.604***
C ₉ -enal	.942*	C ₉ -al	.844*
C ₈ -enal	.372	C ₈ -al	.824*
C ₇ -enal	.866*	C ₇ -al	.426
C ₆ -enal	.660**	C ₆ -al	.725**
C ₅ -enal	.894*	C ₅ -al	.857*
C ₄ -enal	.619***	C ₄ -al	.294
		C ₃ -al	.505***
		C ₂ and C ₁ -al	.455

TABLE 10
MULTILINEAR CORRELATION COEFFICIENTS OF VARIOUS
CARBONYL COMPOUNDS IN OXIDIZED MILK FATS WITH
THE RECIPROCAL OF THE FLAVOR THRESHOLD

Combination of carbonyls	Correlation Coefficients
C ₇ -al and C ₈ -enal ^b	.512***
C ₂ , C ₄ and C ₇ -al ^c	.639**
C ₂ , C ₆ and C ₇ -al ^c	.779*
C ₄ , C ₈ and C ₁₁ -enal ^c	.945*
C ₅ and C ₉ -enal ^b	.941*

^a Degree of freedom = 15

^b Degree of freedom = 14

^c Degree of freedom = 13

* Significant at 0.1% level

** Significant at 1.0% level

*** Significant at 5.0% level

Only four of the alkanals, found in oxidized milk fat gave correlation coefficients that were significant at the one per cent level. These were pentanal, hexanal, octanal and nonanal. Heptanal was present in the largest amount of any monocarbonyl, yet its correlation coefficient was 0.426. Heptanal was reported to be a major component of autoxidized milk fat as early as 1920 (75, p. 323-360) and at that time it was believed to be responsible for the oxidized flavor. While the results of this investigation showed a low correlation with $1/FTV$, heptanal may still be of importance to the sensory character of the oxidized flavor. Its high concentration in the oxidized fats coupled with reported flavor thresholds make it necessary to consider this compound in terms of oxidized flavor. This is illustrated by a recent report by Forss, Dunstone and Stark (36, p. 211-219) who found that n-hexanal, n-heptanal, and hex-2-enal were the significant compounds in the oily fraction isolated from fishy flavored milk fat.

Multiple Correlations Coefficients

Multiple correlation coefficients were calculated to determine if multiple combinations of compounds had an effect on correlations with $1/FTV$. The calculations were conducted only upon combinations of compounds that gave low individual correlations with $1/FTV$. These data are

shown in Table 10. The coefficients of but-2-enal, oct-2-enal and undeca-2-enal were 0.619, 0.372 and 0.439, respectively, all of which were insignificant at the one per cent level. The multiple correlation coefficient of these compounds to 1/FTV was 0.945. The fact that the multiple correlation coefficients exhibited an increase over the individual coefficients lends support to the postulation that the typical oxidized flavor of milk fat is due to the combined effect of many compounds rather than to an individual compound.

Recently El-Negoumy and Hammond (27, p. 840) reported that a hept-2-enal containing fraction, isolated from oxidized butter oil, produced the typical oxidized flavor, and that an isomer of hept-2-enal was the probable compound. The highly significant correlation coefficients of both individual and combinations of carbonyls to 1/FTV, as revealed in this investigation, does not support the findings of El-Negoumy and Hammond.

Concentration of Carbonyl Compounds at the Absolute Flavor Threshold of Oxidized Milk Fat

The concentration of each carbonyl compound at the absolute flavor threshold of the oxidized fat sample was calculated by the formula:

$$\frac{(\text{FTV})}{(1000)} \cdot (X) = Z$$

where X equals mg. of carbonyl per kg. of fat as listed in Tables 5 and 6 and Z equals the concentration of carbonyl per liter of milk. The concentration of each carbonyl, mean concentration and standard deviation are shown in Tables 11 and 12. All compounds occurred at parts per billion levels at the FTV of the fats. Flavor threshold values for all the monocarbonyls were not available. However, by comparing the data listed in Tables 11 and 12 with the flavor threshold reported for some of the carbonyls (64, p. 1289-1290), it can be concluded that most of the individual carbonyl compound occurred at marginal or sub-threshold levels in the oxidized milk fats at the FTV of each fat. The data further indicated that, at the absolute flavor threshold of the oxidized fats, the flavor may be due to the additive effects of compounds; each compound occurring at marginal or sub-threshold levels, but the combined concentrations equaling or exceeding the FTV of any one compound in the mixture. Compounds such as hept-2-enal, oct-2-enal and non-2-enal, which are very similar in sensory character, may act in such a way. The highly significant multiple correlations support this point.

TABLE 11
CONCENTRATION OF THE UNSATURATED CARBONYL COMPOUNDS
IN OXIDIZED MILK FATS AT THE ABSOLUTE FLAVOR THRESHOLD

Compounds ^a	Sample Number								
	1	2	3	4	5	6	7	8	9
C ₁₁ -enal	.114	.093	.176	.214	.156	.148	.280	.240	.150
C ₁₀ -enal	.114	.217	.048	.256	.197	.144	.298	.302	.075
C ₉ -enal	.228	.124	.816	.466	.463	.484	.592	.720	.972
C ₈ -enal	.342	.155	.144	.059	.180	.172	.305	.154	.081
C ₇ -enal	.342	0.0	.304	.382	.336	.293	.609	.389	.456
C ₆ -enal	0.0	0.0	.128	.113	.008	.074	.172	.007	0.0
C ₅ -enal	.057	0.0	.304	.290	.131	.109	.210	.322	.357
C ₄ -enal	0.0	0.0	.136	.130	.070	.027	.042	.070	0.0
TOTAL	1.197	.589	2.056	1.910	1.541	1.451	2.508	2.214	2.091

TABLE 11, continued

Compounds ^a	Sample Number						Mean	Standard Deviation
	10	11	12	13	14	15		
C ₁₁ -enal	.378	.169	.232	.149	.140	.185	.192	.134
C ₁₀ -enal	.245	.245	.442	.107	.282	.232	.221	.102
C ₉ -enal	.588	1.109	1.197	.870	1.074	1.103	.722	.322
C ₈ -enal	.118	.071	.384	.00	.032	.094	.148	.115
C ₇ -enal	.330	.453	.464	.308	.389	.409	.353	.130
C ₆ -enal	.00	.025	.193	.002	.101	.045	.057	.075
C ₅ -enal	.145	.274	.530	.232	.281	.336	.252	.141
C ₄ -enal	.078	.091	.129	.079	.086	.046	.071	.041
TOTAL	1.882	2.437	3.571	1.737	2.395	2.450	2.016	

^a Expressed as part per billion

TABLE 12
CONCENTRATION OF THE SATURATED CARBONYL COMPOUNDS
IN OXIDIZED MILK FATS AT THE ABSOLUTE FLAVOR THRESHOLD

Compounds ^a	Sample Number								
	1	2	3	4	5	6	7	8	9
C ₁₃ -one	7.78	4.28	1.04	1.08	1.16	0.92	0.88	1.27	0.56
C ₁₀ -al	0.80	1.64	0.48	0.48	0.46	0.46	0.69	0.32	0.25
C ₉ -al	0.97	0.65	1.00	0.45	0.43	0.50	0.63	0.55	0.55
C ₈ -al	1.60	2.02	0.74	0.66	0.53	0.59	0.67	0.64	0.56
C ₇ -al	1.82	1.36	2.39	6.46	5.30	5.42	6.14	5.75	6.32
C ₆ -al	3.99	4.22	3.66	1.64	1.53	1.46	1.68	1.48	1.66
C ₅ -al	12.71	1.30	4.47	1.85	1.12	1.45	1.49	2.13	2.83
C ₄ -al	2.22	4.25	0.53	0.24	0.21	0.23	0.20	0.13	0.20
C ₃ -al	11.80	5.86	2.40	1.15	0.41	0.50	0.35	1.22	1.02
C ₂ -al and C ₁ -al	49.53	46.00	13.58	5.81	5.93	4.23	3.70	6.98	4.80
TOTAL	93.27	71.58	30.29	19.82	17.08	15.76	16.43	20.47	18.75

TABLE 12, continued

Compounds ^a	Sample Number							Mean	Standard Deviation
	10	11	12	13	14	15	16		
C ₁₃ -one	0.88	0.41	0.55	0.32	0.18	0.41	0.19	1.38	1.91
C ₁₀ -al	0.15	0.31	0.50	0.15	0.16	0.24	0.28	0.47	0.11
C ₉ -al	0.41	0.13	0.89	0.40	0.42	0.45	0.61	0.57	0.21
C ₈ -al	0.47	0.48	1.15	0.47	0.58	0.59	0.48	0.78	0.42
C ₇ -al	2.37	2.37	3.67	1.95	2.02	2.42	1.43	3.58	1.92
C ₆ -al	1.13	1.26	4.03	1.53	1.58	1.49	1.30	2.11	1.11
C ₅ -al	1.58	1.98	1.29	1.48	1.43	2.23	1.31	2.55	2.83
C ₄ -al	0.12	0.21	0.22	0.16	0.20	0.25	0.14	0.59	1.10
C ₃ -al	0.47	0.36	0.83	0.60	0.50	0.71	0.53	1.79	0.76
C ₂ -al and C ₁ -al	3.72	5.20	3.20	2.97	4.29	5.08	1.80	10.43	14.80
TOTAL	12.30	12.71	16.33	10.03	11.38	13.87	8.07	24.25	23.33

^a Expressed as parts per billion

SUMMARY AND CONCLUSION

Sixteen milk fats were collected from large batches of unsalted, sweet cream butter. The samples were oxidized to different levels of oxidation under laboratory conditions. The TBA number, peroxide value, total saturated and unsaturated carbonyl content, total saturated and unsaturated volatile carbonyl content, and the absolute flavor threshold value (FTV) were determined on each sample. In addition the volatile monocarbonyl compounds were isolated, separated into ten fractions by column partition chromatography, and the concentration of each compound in each fraction was determined. Correlation coefficients were calculated to determine the relationships of the chemical tests and individual monocarbonyl compounds to FTV.

Findings of the investigation that are believed to be of significance to the problem of milk lipid oxidation were as follows:

1. Fresh milk fat contained traces of volatile carbonyl compounds. However, 99.94 per cent of the mixture was classified as alkanals or alkan-2-ones, with the rest being the unsaturated carbonyl, pent-2-enal.

2. The autoxidation of milk fat resulted in an increase in the volatile carbonyl material. The percentage composition of each individual compound varied with the sample and degree of oxidation, but in most instances the predominating compounds were the same for all samples.
3. Heptanal, with the exception of the methanal-ethanal fraction, was the major alkanal in the autoxidized milk fat, having a mean percentage of 18.80. Its correlation coefficient to $1/FTV$, 0.426, was insignificant at the 5 per cent level.
4. Non-2-enal was the major alk-2-enal present in autoxidized milk fat. It comprised 4.18 per cent of the mean composition of the volatile mixture and had a correlation coefficient to $1/FTV$ of 0.942.
5. The chemical tests and individual monocarbonyl compounds had a hyperbolic relationship with FTV .
6. All chemical tests employed in this investigation had a high correlation with $1/FTV$. This high correlation could have been due to the more precise sensory test, the unreported reciprocal

relationship between the chemical and sensory methods of detecting the intensity of the oxidized flavor, and the wide range of oxidation in the samples.

7. A method designed to measure the alk-2-enals of oxidized milk fat should give the best correlation with a sensory evaluation of the oxidized flavor intensity of milk fat.
8. That the individual alk-2-enals, with few exceptions, had high correlations with 1/FTV lends support to the previous results (23, p. 585-597) that unsaturated carbonyls are essential components of oxidized flavor in milk lipids.
9. Multiple correlation coefficients tend to suggest that the typical oxidized flavor is due to a combination of compounds rather than one individual compound.
10. The concentration of individual monocarbonyl compounds at the absolute threshold of oxidized flavor in the fats studied were not the same for all samples but they remained within a constant range. The carbonyl compounds at the FTV of the

fat were in marginal or sub-threshold levels.

This investigation produced further evidence on the relation of carbonyl compounds to oxidized flavor of milk fat. The relative importance of individual compounds awaits further research wherein the compounds can be evaluated in appropriate systems at concentration levels reported herein.

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