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Lipid autoxidation in fluid milk and a number of its products has been a concern of the dairy industry for a number of years. The need for low-temperature refrigeration of butter and butter oil, and inert-gas or vacuum packing of dry whole milks to prevent or retard lipid deterioration, in addition to the loss of fluid and condensed milks as a result of oxidative deterioration have been major problems of the industry.

The autoxidation of milk lipids is not unlike that of lipids in other edible products. However, the complex composition of dairy products, physical state of the product (liquid, solid, emulsion, etc.) presence of natural anti- or pro-oxidants, as well as processing, manufacturing, and storage conditions tend to influence both the rate of autoxidation and the composition and percentage of autoxidation products formed.

The literature dealing with the autoxidative mechanism involved in lipid deterioration has been concerned with investigations on pure unsaturated fatty acids and their esters. The reactions involved, however, are representative of those occurring in lipids and lipid-containing food products.

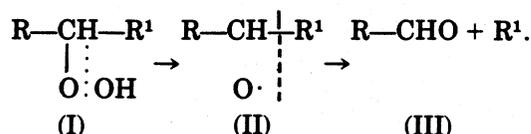
AUTOXIDATION MECHANISM

The initial step in the autoxidation of unsaturated fatty acids and their esters is the formation of free radicals. Although the initiation of such radicals is not completely understood, the resulting free-radical chain reaction has been elucidated in the investigations of Farmer and Sutton,⁸⁹ and others.^{23,32} In the case of monounsaturated and non-conjugated polyene fatty acids—the acids of significance in milk-fat—the reaction is initiated by the removal of a hydrogen atom from the methylene (α -methylene) group adjacent to the double bond (I). The resulting free radical, stabilized by resonance, adds oxygen to form peroxide-containing free radicals (II); these in turn react with another mole of unsaturated compound to produce 2 isomeric hydroperoxides in addition to free radicals (III) capable of continuing the chain reaction.

to-carbon polymerization, and the formation of epoxides and cyclic peroxides have been proposed or demonstrated in lipid oxidation—subjects which are not within the scope of this book.

PRODUCTS OF OXIDATION

The hydroperoxides formed in the autoxidation of unsaturated fatty acids are unstable and readily decompose. The main products of hydroperoxide decomposition are saturated and unsaturated aldehydes. The mechanism suggested for the formation of aldehydes involves cleavage of the isomeric hydroperoxide (I) to the alkoxy radical (II), which undergoes carbon-to-carbon fission to form the aldehyde (III).¹⁰⁵



Other products, such as unsaturated ketones,³³¹ saturated and unsaturated alcohols,^{153,332,333} saturated and unsaturated hydrocarbons,^{94,157,184} and semi-aldehydes,¹⁰⁵ have been observed in the decomposition of hydroperoxides of oxidized lipid systems.

A comprehensive review and study by Badings²⁰ includes a listing of the carbonyls which can result from the dismutation of the theoretical hydroperoxides formed in the autoxidation of the major unsaturated acids of butterfat and those which have been observed. In addition to those carbonyls theoretically possible, various others have been isolated and identified in the autoxidation of pure fatty acids or their esters. Their presence suggests that migration of double bonds,¹⁸ further oxidation of the unsaturated aldehydes initially formed,¹⁷ and/or isomerization of the theoretical geometric form²⁰ may occur during autoxidation.

In addition to the major fatty acids, milk also contains many minor polyunsaturated acids;²¹⁹ hence the autoxidation of dairy products can lead to a multitude of saturated and unsaturated aldehydes.

OXIDATION AND OFF-FLAVORS

The overwhelming consideration in regard to lipid deterioration is the resulting off-flavors. Aldehydes, both saturated and unsaturated, impart characteristic off-flavors at minute concentrations. Terms such

as painty, nutty, melon-like, grassy, tallowy, oily, cardboard, fishy, cucumber, etc. have been used to characterize the flavors imparted by individual saturated and unsaturated aldehydes, as well as by mixtures of these compounds. Moreover, the concentration necessary to impart off-flavors is so low that oxidative deterioration need not progress substantially before the off-flavors are detectable. For example, Patton *et al.*²⁶⁵ reported that 2, 4-decadienal, which imparts a deep-fried fat or oily flavor, is detectable in aqueous solution at levels approaching 0.5 part per billion.

In addition to aldehydes, other secondary products of lipid oxidation, such as unsaturated ketones and alcohols, impart characteristic flavors, and their presence in oxidized milk systems has been established.^{20,331,332}

Generally speaking, the flavor threshold values for aldehydes are governed to varying degrees by the number of carbon atoms; degree of unsaturation; location of unsaturation in the chain; form of geometric isomer; additive and/or antagonistic effects of mixtures of compounds; and the medium in which the flavor compounds are present.^{59,240} With respect to the latter point, the flavor potency of many aldehydes identified in oxidized lipids is up to 100 times greater in an aqueous medium than in a fat or oil. Hence, the extent of oxidative deterioration of fluid milk need not progress to the same point as that in butter oil before the onset of off-flavors in the fluid product.

The off-flavors which develop in dairy products as a result of oxidative deterioration are collectively referred to as the "oxidized flavor". However, the organoleptic properties of the off-flavor differ between products as well as in the same product, depending on the degree of deterioration. Descriptive terms, such as cappy and cardboard have been used to characterize the off-flavor in fluid milk, and the off-flavor in dry whole milk and butter oil has been referred to as oily or tallowy. Butter undergoes a continuous change in flavor defects during storage which usually develop in an order described as metallic, fatty, oily, trainy, and tallowy.²⁰

Although the conditions under which the above-mentioned products are normally stored undoubtedly influence the extent of deterioration and hence the character of off-flavor, the lipid constituents involved in the reaction also influence the resulting flavor. The site of oxidative deterioration in fluid milk and cream is the highly unsaturated phospholipid fraction associated with the fat globule membrane material.^{20,326,337} On the other hand, in products such as butter and dry whole milk, both the phospholipids and the triglycerides are subject to oxidative deterioration.²⁰ The off-flavor appearing in butter oil is understandably the result of triglyceride deterioration.

MEASUREMENT OF FAT OXIDATION

Various methods have been employed to measure the extent of autoxidation in lipids and lipid-containing food products. For obvious reasons, such methods should be capable of detecting the autoxidative process before the onset of off-flavor. Milk and its products, which develop characteristic off-flavors at low levels of oxidation, require procedures that are extremely sensitive to oxidation. Thus methods of measuring the decrease in unsaturation (Iodine No.) or increase in diene conjugation as a result of the reaction do not lend themselves to quality control procedures, although they have been used successfully in determining the extent of autoxidation in model systems.^{128,274}

Several methods have been introduced which express the degree of oxidative deterioration in terms of hydroperoxides per unit weight of fat. The Modified Stamm Method,¹³⁴ the most sensitive of the peroxide determinations, is based on the reaction of oxidized fat and 1,5-diphenylcarbohydrazide to yield a red color. The Lea Method^{6,225} depends on the liberation of iodine from potassium iodide, wherein the amount of iodine liberated by the hydroperoxides is used as the criterion of the extent of oxidative deterioration. The colorimetric ferric thiocyanate procedure adapted to dairy products by Loftus-Hills and Thiel,¹⁵ with modifications by various workers,^{271,335} involves conversion of the ferrous ion to the ferric state in the presence of ammonium thiocyanate, presumably by the hydroperoxides present, to yield the red pigment ferric thiocyanate. Hamm and Hammond¹³² have shown that the results of these three methods can be interrelated by use of the proper correction factors. However, those methods based on the direct or indirect determination of hydroperoxides which do not consider previous dismutations of these primary reaction products are not necessarily indicative of the extent of the reaction, nor do they tend to correlate well with the degree of off-flavors in the product.¹⁹⁸

Two variations of the Thiobarbituric Acid Method have been widely used to determine the degree of lipid oxidation in dairy products.^{78,187} The methods of approximately equal sensitivity are based on the condensation of two molecules of thiobarbituric acid with one of malonaldehyde,³⁰⁷ resulting in the formation of a red color complex with an absorption maximum at 532 to 540 $m\mu$. King¹⁸⁷ has shown (Table 5.4) that a correlation exists between the determined TBA values and the intensity of the oxidized flavor in fluid milks. Similar observations have been reported by others in fluid milks⁸⁵ and ultra-high temperature creams.⁶⁴ The TBA method of Dunley and Jennings⁷⁸ has been reported to be more applicable than the King method in determining the extent of autoxidation in butter, although no correlation with the

Table 5.4

RELATION BETWEEN ORGANOLEPTIC AND TBA VALUES
OF FLUID MILK¹⁸⁷

Flavor Score	Description	Range of Optical Density (432 m μ)
0	No oxidized flavor	0.010-0.023
1	Questionable to very slight	0.024-0.029
2	Slight but consistently detectable	0.030-0.040
3	Distinct or strong	0.041-0.055
4	Very strong	> 0.056

extent of the off-flavor is apparent.⁶⁴ Both methods have been used extensively in studies of the autoxidation of extracted milk components and model lipid systems.^{112,128,187} Lillard and Day reported²²⁸ a significant correlation between a modified TBA test and the reciprocal of the Average Flavor Threshold of oxidized butterfat. A similar correlation also existed between the Peroxide Value and the reciprocal of the Average Flavor Threshold of butterfat.

In addition to the previously mentioned chemical tests, methods based on the carbonyl content of oxidized fats have also been suggested^{143,228} as a measure of oxidative deterioration. The procedures determine the secondary products of autoxidation and have been reported to correlate significantly with the degree of off-flavor in butter oil²²⁸. The methods, however, are cumbersome and are not suited for routine analysis.

ANTIOXIDANTS

The use of synthetic antioxidants in the prevention or retardation of autoxidation in lipids and lipid-containing food products has been the subject of numerous investigations. Although the present U.S. standards do not permit antioxidants in dairy products, and hence the question of their effectiveness is one of only theoretical interest, they are of practical interest in countries where their use is permitted. Many compounds containing two or more phenolic hydroxy groups, such as esters of gallic acid, butylated hydroxyanisole, norhydroguaiaretic acid, hydroxyquinone and dihydroquercetin, have been employed as antioxidants in studies of dairy products. These compounds apparently exert their influence by interrupting the chain reaction in autoxidation by capture of the free radicals necessary for continuation of hydroperoxide formation.¹⁸

Considerations, other than legal, that must be taken into account regarding use of antioxidants in dairy products include off-flavors imparted by the antioxidant itself,^{113,294} ease of incorporation into the product,¹³⁵ and effectiveness of the antioxidant in different mediums. With regard to the latter point, studies of the use of antioxidants in dairy products reveal variations in their antioxidative properties in different products. Norhydroguaiaretic acid is effective in preventing the development of an oxidized flavor in fluid milk, but tends to increase the rate of autoxidation in milkfat.¹³⁵ The tocopherols, while of little value in dry whole milks³ and butter oil,²⁷² are highly effective in preventing spontaneous or copper-induced oxidation in fluid milk.^{79,189} Compounds reported to be among the most antioxidative in specific dairy products include: dodecyl gallate in spray-dried whole milks,^{2,341} ascorbyl palmitate in cold storage-cultured butter,²⁰¹ sodium gentisate in frozen whole milk,¹¹³ and quercetin and propyl gallate in butter oil.³⁷⁹

Synergists, such as the polybasic acids citric and phosphoric, have been used in conjunction with antioxidants. These compounds have no antioxidative value in themselves, but increase the effectiveness of antioxidants. Their synergistic influence on antioxidants may be due to the sequestering of metallic ions,^{18,161} inhibiting the antioxidant catalysis of peroxide decomposition,²⁸⁰ or regenerating the antioxidant in the system.³²³ It has been reported that these synergists, like the phenolic antioxidants, are capable of performing the dual role of retarding autoxidation at low levels and accelerating it at higher levels.²⁸⁰

In addition to antioxidants alone or in the presence of synergists, metal chelating compounds, such as the various salts of ethylenediaminetetraacetic acid,^{10,192} neocuproine³²⁵ among others,³⁰¹ have also proven their effectiveness as inhibitors of autoxidation.

OXIDATIVE DETERIORATION IN FLUID MILK

Fluid milks have been classified by Thurston³⁶² into three categories with regard to their ability to undergo oxidative deterioration: (a) spontaneous, for those milks that spontaneously develop off-flavor within 48 hr after milking; (b) susceptible, for those milks that develop off-flavor within 48 hr after contamination with cupric ion; and (c) resistant, for those milks that exhibit no flavor, even after contamination with copper and storage for 48 hr. A similar classification has been employed by Dunkley and Franke.⁷³

With the advent of noncorrodible dairy equipment, oxidative deterioration in fluid milks as a result of copper contamination has decreased

significantly, although it has not been completely eliminated.²⁹³ However, the incidence of spontaneous oxidation remains a major problem of the dairy industry. For example, Bruhn and Franke³⁴ have shown that 38% of samples produced in the Los Angeles milkshed are susceptible to spontaneous oxidation; and Potter and Hankinson reported²⁷⁶ that 23.1% of almost 3000 individual samples tasted were criticized for oxidized flavor after 24 to 48 hr storage. Significantly, certain animals consistently produce milk which develops oxidized flavor spontaneously, others occasionally, and still others not at all.²⁶⁰ Differences have even been observed in the milk from the different quarters of the same animal.¹²⁶

Greenbank¹²² attributed the resistance of certain milks to oxidation, even in the presence of added copper, to its poisoning action, i.e., the resistance of milk to a change in oxidation-reduction potential. That a correlation exists between the appearance of an oxidized flavor and conditions favoring a mild oxidation, as measured by the oxidation-reduction potential, was shown by Tracey *et al.*³⁶⁵ and by Greenbank.¹²² This apparent correlation, as well as other factors, tends to discredit theories as to the role of enzymes as catalytic agents in the development of oxidized flavor. Such a theory had been proposed initially by Kende,¹⁸¹ who claimed that milk contains "oleinase" which catalyzes the oxidation of oleic acid to produce the characteristic off-flavor. More recently, xanthine oxidase has been proposed^{12,13,15} as the catalytic agent in the development of spontaneously oxidized milk. The studies of Smith and Dunkley,³²² among others,²⁸² do not corroborate these studies, and they conclude that xanthine oxidase is itself not a limiting factor in the off-flavor.

Despite literature reports of anomalous behavior in several aspects, sufficient evidence has been accumulated in recent years to establish that the susceptibility or resistance of milk to oxidative deterioration is dependent on the percentage and/or distribution of naturally occurring pro- and antioxidants.

METALS

Metal-catalyzed lipid oxidative reactions were recognized in dairy products as early as 1905.¹¹⁶ Investigations through the years have shown that copper and iron are the important metal catalysts in the development of oxidized flavors. Of these two metals, copper exerts the greater catalytic effect, while ferrous ion is more influential than ferric ion.¹²²

Both copper and iron are normal components of milk. Disregarding

variations due to individuality, stage of lactation, and contamination, the former is present at average levels of 20 to 40 $\mu\text{g/liter}$ ^{157,203,227} and the latter at 100 to 250 $\mu\text{g/liter}$. Despite the greater abundance of iron in milk, copper has been shown by specific chelating agents to be the catalytic agent in the development of oxidized fluid milk.³²⁵

The natural copper content of milk originates in the cow's food, and is transmitted to the milk by way of the blood stream.¹³¹ The studies of Dunkley and co-workers^{75,287} suggest that an animal's feed can influence the natural copper content of its milk—a view which is not shared by others.²⁴⁹ Nevertheless, the total natural copper content of a milk is not the overall deciding factor in the spontaneous development of an oxidized flavor in fluid milk.

Poulsen and Jensen²⁷⁷ reported that "neither the absolute amount nor the range in content of naturally occurring copper during the lactation period has any significant influence on the tendency of milk to acquire oxidized flavor." Samuelsson³⁰⁰ investigated milks from cows of low and high yield production ranging in copper content from 0.023 to 0.204 ppm. He concluded that oxidation may occur irrespective of the copper content, but no oxidation faults have been observed in milks with a copper content less than 0.060 ppm. Similar results have been reported by others.¹⁹¹

Natural copper and iron exist in milk in the form of complexes with proteins and as such are not dialyzable at normal pH of milk.^{193,302} Copper and iron added to milk are, however, slightly dialyzable, the ease of dialysis of added copper increasing with a decrease in pH.³⁰² The latter observation suggests that the copper-protein bond of added copper is different from that of natural copper. King *et al.*¹⁹³ reported that 10 to 35% of the natural copper and 20 to 47% of the natural iron are associated with the fat globule membrane material. Only 2 to 3% of added copper and negligible percentages of added iron, however, become associated with the fat globule membrane. Similar trends in the distribution of natural and added copper in milk have been reported by others;²⁴⁸ the subject has recently been reviewed by Haase and Dunkley.¹³¹

Samuelsson observed³⁰⁰ that most of the natural copper associated with the cream phase can be removed by washing with water, and that the actual fat globule membrane proteins contain approximately 4% of the total natural copper content. Nevertheless, the value represents the highest concentration of copper per gram of protein in the milk system. Koops stated²⁰³ that "although the amount of natural copper in early lactation may be very high, the concentration of copper (average 11.0 $\mu\text{g}/100\text{ g fat globules}$) in the membrane does not deviate substantially from that of normal uncontaminated milk." King¹⁸⁶

observed that milks which developed oxidized flavor spontaneously had a higher total copper concentration in the fat globule membrane than did milks classified as susceptible or resistant.

Samuelsson concluded³⁰⁰ on the basis of his studies that the close proximity of a copper-protein complex to the phospholipids which are also associated with the fat globule membrane is an important consideration in the development of an oxidized flavor in fluid milks. Haas and Dunkley¹³¹ stated that although "some aspects of catalysis of oxidative reactions in milk by copper still appear anomalous . . . the mechanism of oxidized flavor development with copper as catalyst involves a specific grouping of lipoprotein-metal complexes in which the spatial orientation is a critical factor."

ROLE OF ASCORBIC ACID

That copper, naturally occurring or present as a contaminant, accelerates the development of oxidative deteriorations in fluid milk is evident. However, its presence is not the only consideration as to whether or not oxidative deterioration occurs. Olson and Brown²⁵⁶ showed that washed cream (free of ascorbic acid) from susceptible milk did not develop an oxidized flavor when contaminated with copper and stored for 3 days. Subsequently, the addition of ascorbic acid to washed cream, even in the absence of added copper, was observed²⁷⁰ to promote the development of an oxidized flavor. Krukovsky and Guthrie²¹⁰ and Krukovsky²⁰⁹ reported that 0.1 ppm of added copper did not promote oxidative flavors in milk or butter depleted of their vitamin C content by quick and complete oxidation of ascorbic acid to dehydroascorbic acid. Krukovsky and Guthrie^{208,210} further showed that the oxidative reaction in ascorbic acid-free milk could be initiated by the addition of ascorbic acid to such milk. Accordingly, these workers and others have concluded that ascorbic acid is an essential link in a chain of reactions resulting in the development of an oxidized flavor in fluid milk.

Various workers^{56,136,369} have observed a correlation between the oxidation of ascorbic acid to dehydroascorbic acid and the development of an oxidized flavor. Smith and Dunkley³²³ concluded, however, that ascorbic acid oxidation cannot be used as a criterion for lipid oxidation. Their studies showed that although ascorbic acid oxidation curves for homogenized and pasteurized milk were similar, the homogenized samples were significantly more resistant to oxidized flavor. Furthermore, whereas pasteurization caused an appreciable decrease in the rate of ascorbic acid oxidation compared to raw milk, the pasteurized samples were more susceptible to oxidation.

Haase and Dunkley^{129,130} reported, as a result of studies on model systems of potassium linoleate, that ascorbic acid functioned as a true catalyst, i.e., it accelerated the oxidation of linoleate but it itself was not oxidized. When copper was added to the system, however, the oxidation of ascorbic acid occurred simultaneously with the linoleate. In this respect, Smith and Dunkley³²⁴ reported that a significant correlation exists between the rate of ascorbic acid oxidation and the natural copper content of milk. Furthermore, King reported¹⁸⁸ a positive relation between lipid oxidation and ascorbic acid oxidation in model systems containing fat globule membrane material, the component of uncontaminated milk having the highest concentration of copper per gram of lipid. Although ascorbic acid alone in model systems of linoleate has been observed to be pro-oxidant, low concentrations of ascorbic acid in combination with copper exhibited greater catalytic activity than the additive activity of the two catalysts individually.¹³⁰ Possible explanations for the enhanced catalysis include reduction of copper by ascorbic acid to the more pro-oxidative cuprous form,^{24,130,325} increased concentration of a semidehydroascorbic acid radical,^{24,130} and the formation of a metal-ascorbic acid-oxygen complex.¹³⁰

The behavior of ascorbic acid in the oxidative reaction, however, is anomalous, as evidenced by the studies of several workers.^{27,28,45,211} Their results indicate that concentrations normal to milk (10 to 20 mg per l) promote oxidative deterioration, while higher concentrations (50 to 200 mg per l) inhibit the development of off-flavors.

Various researchers have proposed explanations for the inhibitory behavior of high concentrations of ascorbic acid in fluid milk. Chilson⁴⁵ reported that added ascorbic acid acts as a reducing agent which oxidizes more readily than milkfat. This either prevents or prolongs the time required for fat oxidation and the development of an oxidized flavor. Bell *et al.*²⁷ concluded that the addition of L-ascorbic acid to concentrated sweet cream lowers its oxidation-reduction potential and thus produces a medium less conducive to oxidation. In this respect, Campbell *et al.*³⁶ reported that the O-R potential of milk is entirely dependent on its vitamin C content, and Greenbank¹²³ has shown that the oxidation of ascorbic acid to dehydroascorbic acid is reflected in gradual increases in Eh. Krukovsky²⁰⁹ reported that the oxidative reaction is initiated more rapidly in milk when the ratio of ascorbic to dehydroascorbic acid is approximately 1 to 1 or lower. He states "that an unfavorable proportion of dehydroascorbic acid could not be accumulated if the rate of its oxidation to non-reducible substances surpassed that of ascorbic acid to dehydroascorbic acid. Consequently, the protective influence of ascorbic acid added in large but variable quantities to milk could be attributed to the exhaustion of occluded

oxygen prior to the establishment of a favorable equilibrium between these two forms of vitamin C". Smith and Dunkley³²⁵ disputed this theory and suggested that the results were influenced by higher than normal ascorbic acid contents when the ratio of ascorbic acid to dehydroascorbic acid was greater than 1 to 1 in the experimental milks. In this regard, King¹⁸⁶ was not able to duplicate Krukovsky's results in milks with normal ascorbic acid levels.

King theorized¹⁸⁸ that when the initial concentration of ascorbic acid increases beyond that necessary to saturate the copper in the system, the oxidation of ascorbic acid becomes so rapid and the products of the reaction accumulate so rapidly that they either block the reaction involving the lipids in the system or prevent the copper from acting as a catalyst.

Haase and Dunkley¹²⁹ reported that, although high concentrations of ascorbic acid in model systems of potassium linoleate were prooxidant, a decrease in the rate of oxidation was observed. They further noted¹³⁰ that certain concentrations of ascorbic acid and copper inhibited the formation of conjugated dienes, but not the oxidation of ascorbic acid, and caused a rapid loss of part of the conjugated dienes already present in the system. They theorized that certain combination concentrations of ascorbic acid and copper inhibit oxidation by the formation of free-radical inhibitors which terminate free-radical chain reactions, and that the inhibitors are complexes that include the free radicals.

ROLE OF α -TOCOPHEROL

The literature^{123,286} appears to be in general agreement that the use of green feeds tends to inhibit and that of dry feeds to promote the development of oxidized flavors in dairy products. Furthermore, the observation^{52,237} that milks produced during the winter months are more susceptible to oxidative deteriorations is the result, no doubt, of differences in feeding practices.

Investigations concerned with variations in the oxidative stability of milk as a result of feeding practices have centered on the transfer to milk of natural antioxidants. Although Kanno *et al.*¹⁷³ have reported the presence of γ -tocopherol, the only known natural antioxidant of consequence in milk is α -tocopherol.

Milk contains on the average approximately $25 \mu\text{g}$ of α -tocopherol per gram milkfat.^{34,86,173} Dicks⁶² has assembled a comprehensive bibliography of the literature on the α -tocopherol content of milk and its products, including data on the numerous variables which influence

Vitamin E content. Foremost among these variables is the feed of the animal as influenced by season of the year. Kanno *et al.*¹⁷³ reported that milk produced from May to October on pasture feeding averaged 33.8 μg α -tocopherol per gram fat, while that produced by dry-lot feeding from November to April contained an average of 21.6 μg α -tocopherol per gram fat. Similar results have been reported by others.^{190,217,312}

Krukovsky *et al.*²¹⁸ found a significant correlation between the tocopherol content of milkfat and the ability of milk to resist autoxidation. A high proportion of samples which contained less than 25 μg α -tocopherol per gram fat were unstable and developed oxidized flavors during storage. Erickson *et al.*⁸⁷ reported that the tocopherol concentration in the fat globule membrane lipids correlated more closely with oxidative stability of the milk than did the tocopherol content of the butter oil. Dunkley *et al.*⁷⁴ stated, however, that the concentration of α -tocopherol in milk is not satisfactory as a sole criterion for predicting oxidative stability, and that the concentration of copper must also be considered. In this regard, King *et al.*¹⁹⁴ found a direct relationship between the tocopherol level and the percentage of copper tolerated by milk. Spontaneous milk oxidation was reported by Bruhn and Franke³⁴ to be directly proportional to the copper content and inversely proportional to the α -tocopherol content of milk.

Erickson *et al.*⁸⁸ observed that, although containing only 8% of the total tocopherols in milk, the fat globule membrane contains the highest concentration of α -tocopherol per gram fat in milk (44.0 $\mu\text{g}/\text{g}$). Erickson and co-workers had previously concluded⁸⁷ that since "the lipids in the fat globule membrane are most susceptible to oxidation because of their unsaturation and their close association with the pro-oxidants copper and ascorbic acid, the α -tocopherol in the membrane is more important in inhibiting oxidation than that inside the fat globule". A similar conclusion has also been reached by King.¹⁸⁹

Recently, several studies have been concerned with increasing the α -tocopherol levels of milk to prevent the development of oxidized flavors when tocopherol-rich forages are not available for feed. Dunkley *et al.*,^{79,80} King *et al.*¹⁹⁴ and Merk and Crasemann²⁴¹ have reported increases in the α -tocopherol content of milk and increased resistance to spontaneous and copper-induced oxidation by supplementing the cow's ration with varying proportions of α -tocopherol acetate. Dunkley *et al.*⁸⁰ reported that supplementing the ration of an animal with 500 mg *d*- α -tocopherol acetate increased the total milk tocopherol content by 28.6 $\mu\text{g}/\text{g}$ lipid; and King *et al.*¹⁹⁰ reported that supplementing the feed to achieve a total intake of 1.0 g α -tocopherol/cow/day provides an effective control against oxidation in milk containing 0.1 ppm copper contamination. Several reports^{74,194} have shown that approximately

2% of the total α -tocopherol intake is transferred to milk and as such, supplementing the ration with α -tocopherol acetate is a relatively inefficient procedure. King¹⁸⁹ has reported, however, that the direct addition of *d*- α -tocopherol in an emulsified form at a concentration of 25 μ g/g milkfat would prevent the development of oxidized flavor in milk containing 0.1 ppm added copper—the same α -tocopherol concentration found to be effective when the ration was supplemented with α -tocopherol acetate. Control of oxidized flavor by direct addition of emulsified α -tocopherol to milk can be achieved with only 1% of the amount required by ration supplementation.

FACTORS AFFECTING OXIDATIVE DETERIORATION IN MILK AND ITS PRODUCTS

Storage Temperature

The role of storage temperature in the oxidative deterioration of dairy products is anomalous. Tracey³⁶⁴ recognized that fluid milk was more susceptible to oxidized flavor when stored at 4°C than at 20°C. Dunkley and Franke⁷³ also observed more intense oxidized flavors and higher TBA values in fluid milks stored at 0°C than at 4°C and 8°C. The flavor intensity and the TBA values decreased with increasing storage temperature. Bell²⁶ reported that, other conditions being equal, condensed milk stored at -17°C is more susceptible to the development of oxidized flavor than is condensed milk maintained at -7°C.

In contrast to the above, low storage temperatures tend to decrease the rate of light-induced oxidative deterioration⁷⁶ and to decrease or inhibit oxidative deterioration in other dairy products. Pyenson and Tracey²⁸¹ reported that storage temperatures of 2°C retarded the development of oxidative deterioration in dry whole milk, as determined by O₂ absorption and flavor scores, in comparison with samples stored at 38°C in an atmosphere of air. Downey⁶⁴ reported that oxidative deterioration in UHT cream occurred 2 to 3 times more rapidly at 18°C than at 10°C, while little or no oxidation occurred at 4°C. Holm *et al.*¹⁵⁶ showed that in the case of butter, approximately 4 times more storage time was necessary at -10°C to obtain the same 2-point decrease in flavor score given products held at 10°C. Sattler-Dornbacher³⁰⁴ reported an increase in the O-R potential of butter as the storage temperature increased with a corresponding increase in the rate of flavor deterioration. Hamm *et al.*¹³³ demonstrated the rates of oxidative deterioration in butter oils during storage at temperatures ranging from -10 to 50°C. Despite dramatic differences in the rate

of oxidation, increasing rates with increasing temperatures; they concluded that the same flavors were formed on storage, and that the reaction sequence for flavor formation was the same at all temperatures.

Oxygen Levels

The inhibition of oxidative deterioration in fluid milk held at higher storage temperatures has been attributed by various workers^{56,122,361} to a lowering of the oxygen content as a result of bacterial activity. In this respect, it has been noted that the increase in incidence of oxidized flavor in milk has paralleled the bacteriologically improved milk supply.¹⁶¹ Collins and Dunkley⁴⁷ have reported, however, that although large numbers of bacteria slightly retard development of oxidized flavor, the relatively small numbers of bacteria normally found in market milk are of no practical consequence in determining whether or not milk will develop off-flavor. Furthermore, Sharp *et al.*³¹⁶ stated that the number of bacteria necessary to reduce the oxygen content materially would be sufficient to cause other types of deterioration.

Removal of the dissolved oxygen in fluid milk or its replacement with nitrogen was shown by Dahle and Palmer⁵⁶ to inhibit the development of oxidized flavors. Sharp *et al.*³¹⁵ further showed that deaeration would inhibit the appearance of off-flavor even in the presence of 0.1 mg copper per l milk. Singleton *et al.*³¹⁷ confirmed previous observations that oxygen was required for the development of light-induced off-flavors. Schaffer *et al.*,³⁰⁶ applying deaeration to products other than fluid milk, concluded that, to prevent the production of tallowy flavor in butter oil, the available oxygen should be less than 0.8% of the volume of the fat. Similar storage conditions were also proposed by Lea *et al.*²²⁶ Although the deaeration of these products is of significance only from a scientific standpoint, the deaeration of dry milk products has practical applications.

Vacuum treatment or replacement of available oxygen with an inert gas has proved its reliability in preventing or retarding the onset of oxidation in dry whole milk for extended periods of storage. Greenbank *et al.*¹²⁵ showed that inert gas-packing to an oxygen level of 3 to 4% increased the storage life of whole milk powder 2 to 3 times that of air-packed samples, the length of storage being dependent on the initial quality of the product. Lea *et al.*²²⁶ showed that, whereas oxidative deterioration in milk powders packed at the 3 to 6% oxygen level was retarded significantly, inert gas containing 0.5 to 1.0% oxygen would prevent the development of recognizable tallowy flavors for an indefinite period. Tamsma *et al.*³⁴² showed statistically a highly

significant improvement in storage stability of whole milk powders packed in inert gases containing 0.1% oxygen over those packed at a 1% oxygen level. Schaffer *et al.*³⁰⁶ concluded that the time required for the production of a tallowy flavor is inversely proportional to the oxygen concentration.

Several deaeration techniques other than mechanical methods have been utilized to inhibit or retard the development of tallowy flavors in dry milks. Meyer and Jokay²⁴² reported that milk powders packed in the presence of an oxygen scavenger (glucose oxidase-catalase) and desiccant (calcium oxide) were comparable flavorwise to samples stored in the presence of an inert gas, the enzymes demonstrating the ability to reduce oxygen levels to 0.5% in one week. Jackson and Loo,¹⁵⁹ employing an oxygen-absorbing mixture (0.5 g Na₂SO₃ and 0.75 g CuSO₄ · 5H₂O) enclosed in porous paper pouches, demonstrated keeping qualities equal to those of dry milks stored in the presence of an inert gas. Abbot and Waite¹ reported favorable results in the keeping quality of dry whole milk by using a mixture of 90% nitrogen and 10% hydrogen in the presence of a palladium catalyst. The metal catalyzes the formation of water from the hydrogen and residual oxygen to produce an almost oxygen-free atmosphere in the pack. Tamsma *et al.*³³⁹ reported obtaining within 24 hr a pack containing less than 0.001% oxygen by use of an oxygen-scavenging system consisting of 95% nitrogen, 5% hydrogen and a platinum catalyst. Marked improvements in the keeping quality of milk powders packed in the scavenging system were reported.

Heat Treatment

Pasteurization of fluid milk leads to increased susceptibility to spontaneous,³¹ copper-induced,^{263,323} and light-induced oxidized flavor.⁹⁰ Heating to higher temperatures, however, reduces the susceptibility.^{30,323} A possible explanation for the increased incidence of oxidized flavor as a result of pasteurization temperatures is suggested by several studies. Sargent and Stine³⁰³ reported a substantial migration of added copper to the cream phase of milk at temperatures higher than 60°C. Van Duin and Bruns⁶⁹ also observed an increase in the copper content of creams prepared from pasteurized milk. Samuelsson³⁰¹ reported that washed cream made from milk heated to 80°C for 10 min contained twice as much copper as that prepared from unheated milk. The migration of the additional copper to the cream phase, which also contains the readily oxidized phospholipids, increased the potential of the system toward oxidative deterioration. Tarassuk *et al.*³⁵⁰ also observed that washed cream is very sensitive to the development of

trainy (fishy) flavor when heated to temperatures between 60 and 90°C. The effect of previous heat treatment on the copper content of butter was reported by Van Duin and Bruns.⁶⁹ They observed that pasteurization of cream at 78°C for 15 to 30 sec gave high copper concentrations in butter and low values in the buttermilk, the reverse being true when the cream was heated to above 82°C. They recommended that creams prepared from pasteurized milks should be heated to the higher temperatures to decrease the susceptibility of butter to oxidative deterioration during storage.

The inhibitory effect of high heat treatment on oxidative deterioration in fluid milk and its products has been reported by various workers.^{56,122,340} Gould and Sommer,¹¹⁹ in conjunction with studies on the development of a cooked flavor in heated milks, noted a decrease in the oxidation-reduction potential of the product. They attributed the cooked flavor to the formation of sulfhydryl compounds and correlated the liberation of these compounds to the heat retardation and prevention of oxidized flavor. The work of Josephson and Doan¹⁷¹ conducted simultaneously with these workers confirmed the relationship between sulfhydryl compounds, cooked flavor, decreased Eh, and inhibition of oxidized flavor. They further reported that most heated products do not become tallowy or oxidized until the sulfhydryls are first oxidized and the cooked flavor has disappeared. Wilson and Herreid³⁴² prolonged substantially the onset of oxidative deterioration of 30% sterilized cream by increasing to 13% the solids-not-fat content of the cream prior to sterilization, presumably by increasing the potential sulfhydryl content of the finished product. Gould and Keeney¹¹⁸ showed that oxidized flavor occurred in heated cream to which copper had been added when the active sulfhydryl compounds had decreased to a level approximating 3 mg per l of cystine HCl.

β -Lactoglobulin has been shown by Larson and Jenness²²²—and this finding was confirmed by Hutton and Patton¹⁵⁸—to be the major source of sulfhydryl groups in milk, while the fat globule membrane material contributes a minor portion of these reducing compounds.

Time-temperature relationships have been established by various workers as being optimum for preventing or retarding the development of oxidized flavors in dairy products: cream, 88°C for 5 min;¹¹⁸ condensed milk, 76.5°C for 8 min;²⁶ dry whole milk, preheat at 76.5°C for 20 min;⁴⁶ frozen whole milk, 76.5°C for 1 min.²⁹ Few, if any, instances of a tallowy flavor have been reported in evaporated milk; undoubtedly a major reason for its stability toward oxidation can be attributed to the sterilization temperatures employed in its manufacture.

Josephson reported¹⁷⁰ that butterfat prepared from butter heated to 149, 177, and 204.5°C was extremely stable to oxidation, while that

heated to 121°C oxidized readily when stored at 60°C. When butter oil itself was heated from 121 to 204.5°C it also oxidized rapidly. The addition of 1% skim milk powder to butter oil, however, prior to heating at 204.5°C for 10 min also resulted in a significant antioxidative effect, which Josephson concluded was the result of a protein-lactose reaction (carmelization). Wyatt and Day³⁷⁹ reported that the addition of 0.5% nonfat milk solids to butter oil followed by heating at 200°C and 15 mm Hg for 15 min caused the formation of antioxidants which protected the butter oil against oxidative deterioration for one year, thus surpassing the effectiveness of many synthetic antioxidants tested.

Exposure To Light

The catalytic effect of natural light in promoting off-flavor development in fluid milk has been recognized for some years. The extent of deterioration appears to be dependent on the wavelengths involved, intensity of the source, and the length of exposure.^{14,76,122,236} Off-flavors have also been reported to develop in butterfat which has been exposed to the action of natural light.³³⁴ In addition to natural light, incandescent or fluorescent lights employed in storage coolers may promote deteriorative reactions,³²¹ while the development of off-flavors is the limiting factor in the preservation of dairy products by high-energy radiation.^{58,152} Efforts to inhibit or retard the onset of off-flavors as a result of exposure to sunlight has led to the introduction of doorstep coolers and, in certain cases, of amber-colored milk bottles.

Two distinct flavors may develop in milk exposed to light:^{299,368} a burnt, activated or sunlight flavor which develops rapidly, and a typically oxidized flavor which develops on prolonged exposure.³³⁶ It is possible that the presence of contradictory statements in the literature regarding deterioration on exposure to light may be attributed to the failure of various investigators to recognize the existence of more than one off-flavor.

Studies^{264,368} have shown that riboflavin plays a significant role in the development of the activated flavor. Although removal of riboflavin from milk by passing through Florisil prevented the development of activated flavor, such treatments did not prevent the development of the oxidized flavor. The later observation does not agree with the reports of other workers,^{14,136,369} which indicate that riboflavin plays a significant role in the oxidized flavor. Ascorbic acid has also been implicated in the development of off-flavors in fluid milks exposed to light.^{14,77,299} The exact nature of its involvement, however, is not clear.

Limited studies have been conducted on the lipid components oxidized in milk exposed to sunlight. Finley *et al.*⁹⁰ observed a decrease in

the oleic and linoleic acid contents of an isolated low-density lipoprotein from milk, and implicated the lipoprotein as a major substrate for the photooxidation reaction. Although previous studies³⁷⁸ suggested that the monoene fatty acids are important oxidizing substrates in milk exposed to sunlight, Wishner noted³⁷⁷ that photooxidation of methyl linoleate in the presence of photosensitizers produces significant percentages of the less stable 11-hydroperoxide,¹⁸³ which on decomposition forms alk-2-enals, the significant carbonyls found in milk exposed to sunlight.

The sunlight flavor has been shown^{91,264} to originate in the proteins of milk. Hendrick¹⁴² concluded that the serum proteins are the main source of activated flavor in milk, with riboflavin as the photosensitizer. Similar results have been reported by Storgards and Ljungren.³³⁶ Singleton *et al.*³¹⁷ demonstrated a relationship between riboflavin destruction, tryptophan destruction, and the intensity of the sunlight flavor in milk, and implicated a tryptophan-containing protein rather than a single low molecular weight compound as one of the reactants. Finley *et al.* reported⁹⁰ that a low-density lipoprotein fraction associated with the fat globule membrane served as a carrier and a precursor for the light-induced off-flavor. Studies of the degradation of the lipoprotein on exposure to light showed that both the lipid and protein portions of the lipoprotein were degraded. In addition to tryptophan, they observed the destruction of methionine, tyrosine, cysteine, and lysine in the lipoprotein on exposure to light in the presence of riboflavin. The photooxidation of amino acids other than tryptophan has been observed in enzymes exposed to sunlight.³⁷⁷

Methional, formed by the degradation of the amino acid methionine, has been reported^{264,368} to be the principal contributor to the activated flavor. Samuelsson²⁹⁸ reported, in studies of di- and tripeptides containing methionine, that irradiation did not result in any hydrolysis of the peptides, and the presence of methional in the reaction products could not be demonstrated. He concluded that methional can only occur in irradiated milks from the free methionine in the milk serum. Thiols, sulfides and disulfides observed as products of the irradiated peptides may be of greater significance in the activated flavor.

Acidity

The development of a fishy flavor in butter is well known, and its association with salted butter made from acid cream was first demonstrated by Rogers in 1909.²⁹¹ Cream acidities ranging from 0.20 to 0.30% appear to represent those levels where flavor development is marginal.^{156,292} Although the development of fishy flavors in unsalted

butters is rarely encountered,²⁹² it is not restricted to those products containing salt. Pont *et al.*²⁷³ induced the development of a fishy flavor in commercial butterfat by the addition of nordihydroguaiaretic acid and citric or lactic acid. In addition, Tarassuk *et al.*³⁵⁰ reported the development of fishy flavors in washed cream adjusted to pH 4.6.

Koops²⁰² conducted a comprehensive study of the development of trainy (fishy) flavor which occurs in butter prepared from cultured cream (pH 4.6) during cold storage. He observed²⁰³ that, although the acidification of milk or cream to pH 4.6 did not result in a transfer of natural copper from the plasma proteins to the fat globule membrane, 30 to 40% of added copper migrated to the membrane proteins at pH 4.6. He concluded²⁰² that the development of a trainy flavor in cultured butter is the result of the migration of the plasma-bound added (contaminated) copper to the fat globule membrane and the enhanced interaction between the cephalin fraction of the membrane phospholipids, which is highly susceptible to oxygen,²⁰⁰ and the copper-containing membrane protein.

Although not studied extensively, reports on other dairy products suggest that titratable acidity as well as hydrogen-ion concentration tend to influence the development of oxidative deteriorations. Anderson⁷ found a relationship between the titratable acidity and the development of an oxidized flavor in milk. Furthermore, his results showed that, while milks developed an oxidized flavor at a titratable acidity of 0.19%, the deteriorative mechanism was inhibited when the milks were neutralized to acidities of 0.145% or lower. Greenbank¹²² found that an increase in pH of 0.1 was sufficient to inhibit the development of oxidized flavors in fluid milks for 24 hr. Anderson⁷ reported similar results. In addition to fluid milk, Dahle and Folkes⁵⁴ attributed the development of oxidized flavors in strawberry ice cream to the presence of copper and the acid content of the fruit.

Homogenization

Homogenization was found in 1933 by Tracey *et al.*³⁶⁵ to inhibit the development of an oxidized flavor in fluid milk. Subsequently, similar observations were reported on cream,³⁵⁰ ice cream,⁵⁵ dry whole milk,¹⁵⁵ and frozen condensed milk.²⁶ The inhibitory effect, however, is not absolute. Roadhouse and Henderson²⁸⁸ found that the absolute pressure required varies with different milks contaminated with the same concentration of cupric ion. The results of Larsen *et al.*²²⁰ and Smith and Dunkley³²³ indicate that the inhibitory effect of homogenization is dependent on the degree of metallic contamination.

Various workers have proposed explanations for the inhibitory effect

of homogenization on oxidative deterioration. Tracey *et al.*³⁶⁵ considered it to be apparent rather than actual, resulting from changes in the physical consistency of the milk, which may alter the taste. These workers based their proposal on the observation that homogenization has no apparent effect on the Eh of milk. Similar observations have been noted by others.²²¹ Still others have proposed that the inhibition is real, and is due to migration of the phospholipids into either the serum phase³⁶³ or interior of the fat globule,²⁰⁷ to general redistribution of the phospholipids in the milk proper,¹²⁴ or to denaturation of proteins resulting in an increase in the number of available -SH groups.¹⁰⁰ King¹⁸⁶ proposed that homogenization effects an irreversible change in the structural configuration of the copper-protein complex in such a way that ascorbic acid is no longer able to initiate the formation of lipid free radicals. Smith and Dunkley³²³ theorized that homogenization causes a change in the copper-protein binding by the formation of a chelate that is less active in ascorbic acid oxidation and inactive in lipid peroxidation. Tarassuk and Koops³⁴⁹ stated that "the decrease in concentration of phospholipids and the copper-protein complex per unit of newly formed fat globule surface appears to be the most important factor, if not the only one, that retards the development of oxidized flavor in homogenized milk."

Dunkley *et al.*⁷⁷ demonstrated, by the use of TBA values and a highly trained taste panel, that although homogenization inhibits light-induced lipid oxidation, the process increases the susceptibility of milk to development of the activated flavor. An increase in the intensity of off-flavors in homogenized milks exposed to sunlight has been reported by several workers.^{53,177} Finley concluded⁹⁰ as a result of his studies that any treatment (e.g., homogenization) which affects the fat globule membrane increases the susceptibility of milk to light-induced off-flavors. It is evident from the literature that homogenization affords a degree of protection against oxidative deterioration in fluid milks provided excessive metallic contamination and undue exposure to light are avoided.

CARBONYL CONTENT OF OXIDIZED DAIRY PRODUCTS

Considerable effort has been expended in recent years on the odorous compounds formed in autoxidized dairy products. Although some of the early identification studies lack present-day sophisticated methodology, may be incomplete, and do not differentiate between isomeric forms of the various compounds, their contribution to the

knowledge of the products of autoxidation in dairy products is invaluable.

Table 5.5 summarizes the carbonyls that have been identified in several selected dairy products. Despite the general similarity in the qualitative carbonyl content of oxidized dairy products, flavor differences are apparent. Attempts to correlate the off-flavors with specific compounds or groups of compounds, however, are made difficult for several reasons. These include: (a) the multitude of compounds produced; (b) difficulties arising in the quantitative analyses of oxidized dairy products; (c) differences in threshold values of individual compounds; (d) similarity of flavors imparted by individual compounds near threshold; (e) a possible additive and/or antagonistic effect, flavor-wise and with regard to threshold values of mixtures of compounds; (f) the possible existence of a compound or group of compounds heretofore not identified; and (g) the difficulties involved in adding pure compounds to dairy products as a means of evaluating their flavor characteristics.

Several individual compounds formed by the autoxidation of milk lipids, however, have been implicated in specific off-flavors. Stark and Forss³³¹ have identified 1-octen-3-one as the compound responsible for the metallic flavor which develops in dairy products. This compound has also been shown to be an integral part of other oxidized flavor defects.^{20,95,96}

4-*cis*-Heptenal, responsible for the creamy flavor of butter,²⁵ results

Table 5.5

CARBONYLS IDENTIFIED IN AUTOXIDIZED DAIRY PRODUCTS

Product	Alkanal	Alk-2-enal	Alk-2,4-dienal
Skimmilk, copper-induced ^{98,99a}	C ₂ , C ₆	C ₄ to C ₁₁	C ₆ to C ₁₁
Whole milk, spontaneous oxidation ²⁶⁰	C ₅ to C ₁₀	C ₆ to C ₁₁	C ₆ to C ₁₂
Whole milk, light-induced ³⁷⁸	—	C ₄ , C ₆ to C ₁₁	—
Dry whole milk air-packed ²⁶¹	C ₁ to C ₃ C ₅ to C ₁₀	C ₅ to C ₁₁	traces
Butter oil, exposed to air ^{88a,88a}	C ₁ to C ₁₀	C ₄ to C ₁₁	C ₇ , C ₁₀
Butter, cold storage ^b defects ²⁰	C ₅ to C ₁₂	C ₅ to C ₁₁	C ₇ ^c , C ₉ , C ₁₀ ^c , C ₁₁

^aReferences

^bMiscellaneous carbonyls: 4-heptenal^c; 2,6-nonadienal^c; 2,5-octadienal^c; 2,4,6-nonatrienal^c; 2,4,7-decatrienal^c; 1-penten-3-one; 1-octen-3-one; 3,5-octadien-3-one; 3,5-undecadien-3-one.

^cIncludes *cis/trans* geometric isomers.

from autoxidation of minor isolinoleic acids in butterfat.¹⁶⁹ At higher concentrations this compound has also been implicated in the trainy flavor which develops in cold storage butter.¹⁹⁶ *trans*-Nonenal has been identified as the compound responsible for the "drier" flavor²⁶² which frequently appears in freshly prepared foam spray-dried milks—an off-flavor which is peculiar to this particular product. Although the evidence suggests that it is formed in foam spray-dried milk by trace ozonolysis of minor milk lipids, it has also been identified in stored sterile milks.²⁵⁹ The latter observation suggests it may also appear in dairy products as a result of autoxidation reactions.¹⁸²

The findings of other studies suggest that the preponderance of certain carbonyls or groups of carbonyls is involved in the off-flavors of various dairy products. Forss *et al.*^{98,99} reported that the C₆ to C₁₁ 2-enals and the C₆ to C₁₁ 2,4-dienals—and more specifically 2-octenal, 2-nonenal, 2,4-heptadienal, and 2,4-nonadienal—constitute a basic and characteristic factor in copper-induced cardboard flavor in skim milk. The same workers concluded that "while these compounds in milk closely simulate the cardboard flavor, the resemblance is not complete" and that "the defect contains further subsidiary flavor elements".

Bassette and Keeney²² ascribed the cereal-type flavor in dry skim-milk to a homologous series of saturated aldehydes resulting from lipid oxidation in conjunction with products of the browning reaction. The results of Parks and Patton²⁶¹ suggest that saturated and unsaturated aldehydes at levels near threshold may impart an off-flavor suggestive of staleness in dry whole milk. Wishner and Keeney³⁷⁸ concluded from studies on milk exposed to sunlight that C₆ to C₁₁ alk-2-enals are important contributors to the oxidized flavor in this product. Parks *et al.*²⁶⁰ concluded, as a result of quantitative carbonyl analysis and flavor studies, that alk-2,4-dienals, especially 2,4-decadienal, constitute a major portion of the off-flavor associated with spontaneously oxidized fluid milk. Forss *et al.*^{95,96} reported that the fishy flavor in butterfat and washed cream is in reality a mixture of an oily fraction in addition to 1-octene-3-one, the compound responsible for the metallic flavor. *n*-Heptanal, *n*-hexanal, and 2-hexenal were found to be constituents of the oily fraction in washed cream, and these three carbonyls plus heptanone-2 were constituents of the oily fraction isolated from fishy butterfat. Badings²⁰ identified 40 volatile compounds in cold storage cultured butter which had a trainy (fishy) off-flavor. Included among the 14 compounds which were present in above-threshold levels were: 4-*cis*-heptenal; 2-*trans*, 4-*cis*-decadienal; 2-*trans*, 6-*cis*-nonadienal; 2,4,7-decatrienal; 3-*trans*, 5-*cis*-octadien-2-one; 1-octene-3-one; and 1-octen-3-ol.

Comparative studies by Forss and co-workers^{95,97} on the fishy, tal-

lowy, and painty flavors of butterfat tend to emphasize the importance of the relative and total carbonyl contents in dairy products with different off-flavors. These researchers showed that three factors distinguished painty and tallowy butterfat from fishy flavored butterfat. First, there was a relative increase in the *n*-heptanal, *n*-octanal, *n*-nonanal, heptanone-2, 2-heptenal, and 2-nonenal in the tallowy butterfat, and a relative increase in the *n*-pentanal, and the C₅ to C₁₀ alk-2-enals in the painty butterfat. Secondly, 1-octen-3-one was present in such low concentrations in both the tallowy and painty butterfats as to have no effect on the flavor. Thirdly, the total weight of the volatile carbonyl compounds was about ten times greater in the tallowy and 100 times greater in the painty butterfat than in the fishy-flavored butterfat.

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