INDICATORS OF LIPID DETERIORATION IN MARKET CREAM

by

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J. Warren Stull — Date

Professor of Nutrition and Food Science

Dedicated to the memory of my father,

Jesus Barron Encinas

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ABSTRACT

Hydrolytic and oxidative rancidities were studied in five commercial brands of market cream (whipping cream and half-and-half). Hydrolytic rancidity was measured by the acid degree value (ADV). Oxidative rancidity was determined by measuring the content of aldehydes by the thiobarbituric acid test (TBA) and peroxide levels (PV). Correlation was made between chemical determinations and the sensory evaluation of samples.

Sensory evaluation was performed by one experienced individual who analyzed the samples for rancid and oxidized flavors using the American Dairy Science Association's scoring guide. Statistical treatment of the data includes analysis of variance of the parameters involved as well as regression analysis between objective and subjective results.

ADV was determined by direct titration of the free fatty acids in the cream fat previously isolated by detergent deemulsification techniques. The cream products analyzed did not show any perceptible rancid flavor. Moreover, rancidity seemed not to be a serious problem in the samples studied.

The TBA test was employed to evaluate the extent of oxidation as a function of the amount of aldehydes present. Oxidized flavor was not perceived in cream samples with TBA values lower than 0.200. PV's were analyzed by colorimetric procedures. Statistical analysis showed that PV had a somewhat better correlation with flavor scores than the TBA test.

CHAPTER 1

INTRODUCTION

Hydrolytic and oxidative rancidity in market cream has not been extensively studied. Most of the investigations concerning rancidity in dairy products have been carried out in fluid milk, butter, dry whole milk, butteroil and other commodities with little or no attention given to cream products.

Since cream may be considered a fluid milk byproduct, most of the reactions which occur in fluid milk may also occur in cream. Therefore, in studying the phenomenon of rancidity, the majority of the characteristics of fluid milk may also be found in cream, the major difference being that cream contains a higher percentage of fat.

In the present work, oxidized and rancid flavor characteristics of cream products were studied for possible correlation between chemical determinations and sensory evaluation.

Two types of cream products were studied (whipping cream and half-and-half). The samples were collected from local food markets at suitable intervals. Subjective flavor characteristics were evaluated by one experienced person using the American Dairy Science Association official flavor scoring system. Each sample was analyzed for rancidity determined as a function of the total free fatty acids in the sample (ADV). Oxidative rancidity was related to: a) aldehyde compounds determined by the thiobarbituric acid test (TBA), and peroxides (PV).

The sampling procedure used made it possible to draw conclusions regarding the lipid deterioration in these products being offered for sale in a market of 2.3 million potential consumers.

In order to establish reliability of the data, statistical treatments were performed which included regression analysis and analysis of variance.

CHAPTER 2

LITERATURE REVIEW

The review of literature in this report considers two different types of rancidity; hydrolytic and oxidative. Each one was detailed separately.

Hydrolytic Rancidity

The term rancidity is used in dairy industry to denot the flavor due to the presence of fatty acids. The lower volatile fatty acids are more noticeable in their pronunced effect on flavor. Free fatty acids are products of hydrolytic action of lipases acting on the milk fat.

The development of the rancid flavor in milk and its products is undesirable because it affects the acceptability of these products. Therefore, a better understanding of the factors involved in the development of rancidity is of great importance for the dairy industry.

Lipases is the name of a wide class of hydrolytic enzymes or esterases which act on various esters. Lipases are consider a system which involves multiple enzymes (39).

Individual cows maintained under similar conditions seem to vary in their susceptibility of their milk toward rancidity (50). Numerous investigators have associated an increase incidence of rancidity with advanced lactation. However, some have failed to show a direct

correlation (10, 11). Cow's feed has also been shown to be an important practical factor in influencing the susceptibility of milk to rancidity (81). Regardless of the factors involved, the fact remains that in some cases up to 22% of the cows in a herd produced milk which becomes rancid quickly (36). Milk which inherently possesses the quality of high susceptibility toward rancidity has been termed "spontaneous" (82).

Some years ago, it was believed that all milk contains a minimum of two true lipplytic enzymes. One of these has been termed the plasma lipase and other the membrane lipase. Evidence has been shown that when freshly drawn milk is cooled, irreversible adsorption of the membrane lipase on the material enveloping the fat globules takes place. The other plasma lipase remains in the skimmilk and is intimately associated with the caseinate fraction (83). Later, it was found that only the α-casein fraction possessed lipolytic action.

Based in the data accumulated upon the existence of plasma and membrane lipases, Tarassuk and Frankel (83) attempted to explain the phenomenon of spontaneous rancidity on the basis of concentration of lipase (membrane lipase) in milk. The so-called plasma lipase is assumed not to be adsorbed onto the fat globule and is supposedly not implicated to any great extent in spontaneous rancidity. In order to facilitate lipolysis by this enzyme, certain activation treatments such as homogenization, shaking or thermal manipulation are required (83).

Much of the early work should be interpreted with caution because it is difficult to ascertain whether these investigators were dealing with the same, different, one or more lipases. Also, the conditions of temperature, centrifugal force, pH, size and number of fat

globules, age, and history of the milk, undoubtedly affect the concentration and distribution of lipase activity in various fractions (72). All of these conditions are manifested in the many contradictory reports in literature (28, 42, 60, 64).

Activation and Inactivation of Lipases

Activations treatments which have been found to accelerate lipolysis in normal milk are homogenization, thermal manipulation, various forms of agitation such as shaking and churning, and addition of chemicals.

As early as 1939, it was shown that homogenization of raw milk containing liquid fat resulted in rapid lipolysis. It was established at that time that the intensity of the resulting rancid flavor was related to the homogenization pressure employed (14). In the same manner, churning of cold cream containing liquid fat enhances lipolysis.

It has been generally accepted that homogenization and most forms of mechanical agitation are alike in that they accomplish activation by increasing the surface of the substrate available to enzymes (38). This explanation is not entirely satisfactory for churning, however, which unlike homogenization and shaking, reduces rather than increases the surface area of the substrate.

According to Tarassuk and Frankel (83), foam promotes lipolysis by providing: a) great increase in surface area, b) selective concentration of enzymes at the air-liquid interface, c) "activation" of the substrate by surface denaturation of the membrane materials around the fat globule, and d) intimate contact of the enzyme(s) and the activated substrate. In the case of shaking and churning, foam formation can

readily take place as well as the disruption of the natural membrane materials (83).

Krukovsky and Herrington (53) were the first to demonstrate that lipolysis in normal milk can be hastened by warming cold milk to near 30°C; then cooling below the solidification point of the fat. This type of activation is of practical importance, because it can happen accidentally.

The principle involved in inducing lipolysis by temperature fluctuation is probably quite different from that involved in activation by homogenization and shaking (35). This is manifested by the fact that lipolysis in milk activated by temperature changes proceeds faster in the cold. On the other hand, lipolysis caused by homogenization and shaking proceeds better at temperatures normally employed in enzymatic reactions, 37°C. However, spontaneous lipolysis in milk is unaffected by temperature incubation, once the milk has been cooled to an appropriate temperature (84). In this respect, it has been noted that rapid cooling of raw milk gives more higher free fatty acids than does slow cooling (44).

Several hypothesis have been proposed to explain the effect of temperature fluctuation in rancidity. Tarassuk and Richardson (87) agreed that this phenomenon is related to the permeability of the fat globule membrane to the enzyme.

Another important form of activation of the lipolytic enzymes is by chemicals. Dunkley and Smith (20) stated that calcium chloride in small amounts will accelerate lipolysis. Mercuric chloride apparently activates the milk lipase system. More recently, it was

found that there is a bivalent cation requirement for full milk lipase activity (72). Packard and Jezeski (62) reported that surface active agents such as sodium heptadecylsulphate slightly stimulates lipase action in normal milk both at 5°C and 37°C. Pictocin, a hormone, was reported to increase lipolysis (47). Another hormone, diethylstilbestrol was found to increase lipolysis toward tributyrin but not toward milkfat (72).

The effect of heat in the inhibition and complete inactivation of the milk lipase system has been extensively investigated. Frankel and Tarassuk (27) reported that heat inactivation of milk lipases follows a first order kinetics. Discrepancies concerning the temperature-time relationship necessary for partial or complete inactivation of lipases are probably due to several factors, such as the reproducibility of assay procedure, length of the incubation period following heating, the presence and concentration of fat and solids non-fat in the milk at the time of heating, and the type and condition of the substrate (72).

Light is another source of lipase inactivation. Kay (46) found that light by itself can inactivate up to 3/4 lipase activity. He also noticed that oxygen plays an important role in this type of inactivation. Frankel and Tarassuk (27) found that inactivation of milk lipases by daylight was independent of milk temperature, and that enzymes were markedly protected from light inactivation by the presence of fat. Another important finding in this respect, is that the destructive effect of light on the milk lipase system can be prevented by several compound such as hydrogen sulfide and cystein, but

once the lipase has been inactivated by light, reactivation is not possible by using these compounds (27).

A large variety of chemical compounds have been studied in their effect on lipase activity. Heavy metals usually affect enzymes adversely, and this is true also for milk lipases. Frankel and Tarassuk (27) found that inhibition of lipase systems by copper was temperature dependent and that fat exerts a protective effect against this inhibition by copper. Krukovsky and Sharp (54) found that inhibition of lipase systems by metals was oxygen dependent; moreover, that oxygen itself is an active inhibitor, and that its effect is greatly enhanced by small amounts of copper. Herald and Brunner (34) found that the fat membrane material contains copper in considerable amounts, suggesting that copper added to non-homogenized milk tends to be absorbed onto the natural membrane. Since this membrane is the natural environment for lipases, inactivation may occur through whatever mechanism before the enzyme is able to penetrate the membrane material.

A number of salts inhibits lipolysis with the most effective being sodium chloride (91). Lipolysis was found to be insignificant in cream containing 4% sodium chloride (28).

It has been concluded, from various experiment, that sulphydryl groups are essential sites of activity in milk lipases. This is supported by Frankel and Tarassuk (27) who showed that glutathione, hydroquinone, and potassium thiocyanate markedly increases the storage stability of milk. Of this reducing agents, glutathione was the most effective.

Chandan and Shahani (9) have reported that aureomycin, penicillin, streptomycin, and terramycin reduce lipase activity in milk up to 49%. It was also shown that trypsin reduced lipolytic activity in skimmilk, and that this inactivation was not affected by the presence of milk fat (9).

Formaldehyde has been the most studied chemical inhibitor of milk lipolysis. Many investigators have information concerning the effect of formaldehyde in the milk lipase system or in prevention of lipolysis (2, 67). An extensive study of the effect of formalin on milk lipolysis inhibition has been published by Schwartz et al. (73). They found that formaldehyde acts as a competitive inhibitor and also, under proper conditions, selectively inhibits lipases of raw skimmilk. They also stated that the inhibitory effect of formaldehyde depends on factors such as pH, time of addition of inhibitor, length of the incubation period, concentration and availability of the substrate, and concentration of the inhibitor.

Properties of the Milk Lipase System

A study of lipids specificity requires that the enzyme and substrate be virtually pure. Contamination with other esterases will give rise to misleading results. Pure substrates of known configuration are required.

Purified lipase shows an apparent specificity for triglycerides containing short chain fatty acids rather than those containing long chain fatty acids including milk fat (72). Fatty acids in the 1-and-3 positions are hydrolyzed at a greatly accelerated rate compared to fatty acids in the 2-position (72).

Lipases are sensitive to extreme pH, and even in the vecinity of the pH optimum, were stability reaches its great point, marked inhibition may occur (26). It has been demonstrated that milk lipase subjected to incubation to pH 5.0 is almost completely destroyed (72). Furthermore, milk lipase activity ceases on the acid side of the pH curve. This is of practical importance but there is still controversy (65, 74, 88). Schwartz and Harper (74) found that the pH curve of lipase system varies with the concentration of fat use as a substrate. In general, when the substrate concentration is not limiting, the pH optimum most frequently observed for the milk lipase system lies between pH 9.5 and 9.0.

A rise in temperature tends to increase the enzyme catalyzed reaction but also tends to increase the rate of thermal inactivation of the enzyme itself. The apparent temperature optimum for the milk lipase system is reported to be 37°C in both milk lipase and tributyrin (25). This temperature has been recorded at pH 8.7 and 6.6 for milk fat, and 8.0 and 6.6 for tributyrin. Nevertheless, other authors do not agree with this, and they suggested 15°C as the apparent temperature optimum for the milk lipase system (72).

Some investigators have studied the stability of the milk lipase system under different circumstances. Schwartz and Harper (74) found no loss in activity at pH from 6.2 to 8.5 after the milk has been stored at pH 6.6 for 6 hours in the dark at 4°C prior to assay. Frankel and Tarassuk (27) permitted raw milk to stand for 1 hour at 37°C at pH levels ranging from 5.2-9.8, and found greatest activity in pH range of 6.6-7.6 when the milk was subsequently incubated with

substrate at pH 8.9. Therefore, it appears that normal milk is in an optimum pH range for stability of the lipases. The same pH range of optimum stability was found in whole milk.

Effect of Lipolysis

The most serious effect of lipolysis is the appearence of the so-called rancid flavor. The various fatty acids and their soaps which are thought to be implicated in the flavor have been studied in an effort to assess the role of a indivitual fatty acids in the rancid flavor picture. Later, it was found that only the even-numbered fatty acids from C_4-C_{12} account for the contribution of the fatty acids to the flavor, but no single fatty acids exerts predominant influence (70). Another study implicates that sodium and calcium salts of capric and lauric acids as a major contributor to the rancid flavor (3). presumably, butyric acid is the compound most intimately associated with the rancid flavor but it has not been proved to be uniquely involved.

A variety of other effects, besides the rancid flavor, come as a result of lipolysis. One of the most noticeable, is the lowering of surface tension as lipolysis proceeds (16). As little as 0.1% milk fat in the rancid state proved to be an effective foam depressant while condensing milk and whey (8). The inhibitory effect of rancid milk on the growth of S. lactis has been reported (72). Other investigators have attributed the inhibitory effect of rancid milk to changes in surface tension (88). However, some believed that this is due to the toxic effect of individual fatty acids (12, 13).

Rancid milk decreases the quality of cream, butter, and buttermilk made from it. Moreover, it has been proposed to limit the acid

degree value of milk from which butter is to be made. Nevertheless, whole milk powder made from lipase-modified milk has generally met acceptance among chocolate manufacturers. It imparts a rich and distinctive flavor to milk chocolate (93).

Methods to Determine Lipase Activity

A number of methods are available for following lipase activity. Although numerous modifications and variations have been introduced, the basic methods may be listed as (a) titration of the liberated fatty acids, (b) changes in surface tension, (c) colorimetric determination of the fatty acids, (d) use of gas-liquid chromatography, and (e) use of radioactive substrates.

Titration of fatty acids formed by action of the milk lipase system has been the procedure most widely used. Titration has been conducted directly in the reaction medium in presence of added organic solvents (20, 65). Titration has been also used after separation of the lipid phase by extraction, distillation, churning, and adsoption followed by elusion of fatty acids (33). The most widely used laboratory method appears to be the silica-gel extraction or the method of Thomas (33, 86).

Oxidative Rancidity

Lipid oxidation of dairy products has been a major problem for many years. Certain special treatments such as low-temperature refrigeration of butter and butteroil, inert-gas or vacuum packing of dry whole milks have been used to prevent or retard lipid

deterioration. The loss of fluid and condensed milks as a result of oxidative deterioration have been matters of great concern.

Autoxidation of milk lipids is different from that of lipids in other edible products. The rate of autoxidation and the composition and percentage of autoxidation products formed are influenced by factors as the complex composition of dairy products, physical state of the products, presence of natural antioxidants or prooxidants as well as processing, manufacture and storage (63).

Oxidation and Its Concern

The main concern of lipid oxidation is the resulting off-flavor. Aldehydes, both saturated and unsaturated, impart characteristic off-flavors at minute concentrations. Terms such as painty, nutty, melon like, grassy, tallowy, cardboard, fishy, etc. have been used to characterize the flavor imparted by saturated and unsaturated aldehydes as well as by mixtures of these compounds. Moreover, the concentration of compounds necessary to impart off-flavor is so low that oxidative deterioration needs to progress only slightly before the off-flavors are detected (63).

In addition to aldehydes, other secondary products of lipid oxidation such as unsaturated ketones and alcohols impart characteristic flavors and their presence in oxidized milks have been established (78, 79).

The off-flavor which develops in dairy products as a result of lipid oxidation is reported as the "oxidized flavor"; however, the sensory properties differ between products as well as in the same product depending on the degree of deterioration.

The conditions under which milk and the various dairy products are stored undoubtedly influence the extent of deterioration and therefore the character of off-flavor. The lipid constituents involved in the reaction also influence the resulting flavor (63). The site of oxidation deterioration in fluid milk and cream is the highly unsaturated phospholipid fraction associated with the fat globule membrane material (76). On the other hand, products such as butter and dry whole milk, both the phospholipids and triglycerides are subject to oxidative deterioration. The off-flavor appearing in butteroil is understandably the result of triglyceride deterioration (63).

Important Factors in Considering Oxidative Deterioration

Temperature affects oxidative deterioration in milk and its products; eventhough, the specific role played by temperature has not been established yet. In early reports, the conclusion is that flavor scores and TBA values decreased with increasing storage temperatures (17). In contrast to the preceding information, low temperatures tend to decrease the rate of light induced oxidative deterioration. Downey (15) reported that oxidative deterioration in UHT creams occurred 2 or 3 times more rapidly at 18°C than at 10°C while little or no oxidation occurred at 4°C. Results from other investigations have stated that the rate of flavor deteriration and O-R potential of butter vary directly with storage temperature (61).

The inhibition of oxidative deterioration in fluid milks at higher storage temperatures has been attributed to the lowering of

oxygen content as a result of bacterial activity. However, recent studies have demonstrated that although a large number of bacteria slightly retard the development of oxidized flavor, the relatively small number of bacteria normally found im milk are of no practical consequence in determining whether or not milk will develop off-flavor (10).

Later, it was found that removal of disolved oxygen or its replacement with other gases, such as nitrogen, inhibited the development of oxidized flavors (71). Today, vacuum treatment or replacement of oxigen with an inert gas has proved its reliability in preventing or retarding the onset of oxidation in dry whole milk for long periods of storage (81). Several mixtures of inert gases have been used to replace the oxygen available. Abbot and Waite (1) reported the use of a mixture of 90% nitrogen and 10% hydrogen in presence of palladium catalyst. The metal catalyzes the formation of water from hydrogen and residual oxygen to produce almost oxygenfree atmosphere. Tamsma et al. (81) reported achieving an atmosphere of less than 0.001% oxygen using an oxygen-scavenging system containing 95% nitrogen, 5% hydrogen, and platinum catalyst.

Heat treatment of dairy products is another important factor which influence their susceptibility to oxidative deterioration. Pasteurization tends to increase the availability of fluid milk to spontaneous, copper-induced, and light-induce oxidized flavors (6, 77). However, heating to higher temperatures reduces susceptibility (5, 77). A possible explanation for the incidence of oxidized flavor as a result

of pasteurization temperatures is suggested by several studies. Samuelsson (68) reported that washed creams made from milk heated at 80°C for 10 minutes contained twice as much copper than those prepared from unheated milk. The explanation to this, probably, is that additional copper migrates to the cream phase, which also contains the readily oxidized phospholipids, increasing the potential of the system toward oxidative deterioration. Sargent and Stine (69) reported a substantial migration of added copper to the cream phase of milk at temperature higher than 60°C.

The inhibitory effect of higher temperatures on oxidative deterioration in fluid milk and its products has been reported by various workers. In all of the investigations related to the topic, it was found that oxidized flavor retardation was related to the sulphydryl groups and the resultant cooked flavor produced by the high temperature treatments. They further reported that most heated products do not become oxidized until the sulphydryls are first oxidized and the cooked flavor has disappeared (82). In another report, it was found that the oxidative deterioration of 30% creams could be prolonged substantially by increasing the solids-non-fat content of the cream to 13% prior to sterilization (61).

Exposure to light is another agent known to induce the development of oxidized flavor in milk and its products (90). Removal of riboflabin content has proved to prevent the development of other off-flavors caused by exposure to light, but such treatment did not prevent the development of oxidized flavor (90). However, controversy exists in the role playe by riboflabin in the light-induced

oxidized flavor in milk (4). Limited studies have been conducted on the lipid components of milk exposed to sunlight. A recent study by Wishner (92) noted that photooxidation of methyl linoleate in presence of photosensitizers produced significant percentage of less stable 11-hydroperoxide, which in decomposition forms alk-2-enals, the carbonyls found in milk exposed to sunlight.

As early as 1933, homogenization was found to inhibit the development of an oxidized flavor in fluid milk. Later, the inhibitory effect was found in other dairy products such as cream, dry whole milk, and frozen condensed milk. However, the inhibitory effect of homogenization on the oxidized flavor is not absolute, and it is dependent on the degree of metalic contamination (56, 77).

Various investigators have tried to explain the inhibitory effect of homogenization on the oxidized flavor. Some have proposed that the inhibition is nor real (57). Others have proposed that the inhibition is real, and due to: (a) the migration of the phospholipids either to the serum phase or to the interior of the fat globule, (b) the general redistribution of the phospholipids in the milk proper, or (c) the denaturation of the protein resulting in as increase in the number of available -SH groups (24, 51). Tarassuk and Koops (85) have proposed that there is a decrease in the phospholipid concentration and the copper-protein complex per unit of newly formed fat globule surface. This appears to be the most important factor that retards the development of oxidized flavor in homogenized milk. Dunkley et al. (18) demonstrated that, although homogenization inhibits light-induced lipid oxidation, the process increase the susceptibility of

milk to the development of activated flavor. As a conclusion, homogenization affords a degree of protection against oxidative deterioration.

Although it has not been studied extensively, certain reports suggest that hydrogen-ion concentration tends to influence the development of oxidative deterioration. Nelson and Trout (61) found a link between titratable acidity and the development of an oxidized flavor in milk. They also found that the deteriorative mechanism was inhibited when milks were neutralized to acidities lower than 0.14% (61). Greenbank found that an increase of 0.1 in pH was sufficient to inhibit the development of oxidized flavors in fluid milk for 24 hours (29).

Influence of Metals

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

Both copper and iron are normal components of milk. Taking into consideration variations due to individuality, stage of lactation, and contamination, copper is present in an average levels of 20-40 μg per liter (41) and iron at 100-250 μg per liter. Even though iron is found in great amount compared with copper, the latter is the catalytic agent of greatest concern in the development of oxidized flavor in fluid milk. This has been shown by the use of chelating agents.

King (48) observed that milks which develops oxidized flavor spontaneously had a higher total copper concentration in the fat globule membrane than did milks classified as susceptible or resistant. Other investigators claim that the close proximity of the copper-protein complex to the phospholipids which are associated with the fat globule membrane is an important consideration in the development of an oxidized flavor (60). Nevertheless, some aspects of the catalytic effect of copper in the oxidative deterioration in milks still appears anomalous.

Influence of Other Milk constituents

The presence of copper is not the only consideration as to whether or not oxidative deterioration occurs. As early as 1942, it was shown that washed creams free of ascorbic acid did not develop oxidized flavor when contaminated with copper and stored for 3 days (66). Subsequently, the addition of ascorbic acid to washed creams, even in the absence of added copper was observed to promote the development of oxidized flavor. Moreover, with the studies of Krukovsky (52), it was observed that oxidative reaction in ascorbic acid-free milk could be initiated by the addition of ascorbic acid to that milk. Other investigators have reported that ascorbic acid functions as a true catalyst. They found that it accelerates the oxidation of linoleate, but itself was not oxidized. When copper was added to the system, however, the oxidation of ascorbic acid occurs simultaneously with the linoleate (30, 31).

Another finding reveals that low concentration of ascorbic acid in combination with copper exhibits greater catalytic effect than the additive activity of the two catalysts individually (31). An explanation for this could be the reduction of copper by ascorbic acid to more prooxidative cuprous form, increased concentration of semihydroascorbic acid radical, and the formation of metal-ascorbic acid-oxygen complex (31).

It is known that the use of green feeds tends to inhibits and that of dry feeds to promote the development of oxidized flavor in dairy products. Furthermore, the observation that milk produced during the winter months is more susceptible to oxidative deteriration is the result of differences in feeding practices. Therefore, investigations concern with variations in the oxidative stability of milk as a result of feeding practices have centered on the transfer to milk of natural antioxidants. The only natural antioxidant in milk is α-tocopherol. Milk contains an average of 25 μg of α-tocopherol per gram of milk fat (7).

Kanno et al. (45) reported that milk produced from May-October on pasture feeding average of 33.8 μg of α-tocopherol per gram of fat, while that produced by dry-lot feeding from November-April contained an average of 21.6 μg of α-tocopherol per gram of fat. Similar results have been reported by others (49, 65). Krukovsky et al. (55) found a similar correlation between the tocopherol content of milk fat and the ability of milk to resist autoxidation.

Spontaneous milk oxidation was reported to be directly proportional to the copper content and inversely proportional to the α-tocopherol content of milk (7). As a conclusion, both the copper

content and the α-tocopherol content of milk, should be taken in consideration in predicting the oxidative stability of dairy products.

Compounds Found in Oxidized Dairy Products

Odorous compounds formed in autooxidized dairy products have been a matter of great investigation in recent years. Several carbonyls have been identified in various dairy products. These carbonyls differ greatly in flavor, regardless of their similarity in formulation. Individual compounds formed by the autooxidation of milk lipids have been implicated in specific off-flavors. For example, 1-octen-3-one has been identified as the compound responsible for the metallic flavor (78). Forss et al. (23) reported that the C_6-C_{11} 2-enals and C_6-C_{11} 2,4-dienals constituted a basic and characteristic flavor in copper-induced carboard flavor in skimmilk. Parks (63) in their quantitative analysis of carbonyls concluded that alk-2,4-dienals, specially 2,4-decadienal constitute a major portion of the off-flavor associated with the spontaneously oxidized fluid milk. In the case of creams, Forss et al. (22) found that fishy flavor in butter fat and washed cream is a mixture of 1-octen-3-one, the compound responsible for the metalic flavor, and n-heptanal, n-hexanal, plus heptanone-2. Another important studies about the carbonyl content of dairy products and their characteristic off-flavor have been found in the literature (21, 23).

Measurement of Oxidative Deterioration

Various methods have been employed to measure the extent of autooxidation in lipids and lipid containing products. Milk and its products develop off-flavors at low levels of oxidation; therefore, extremely sensitive procedures to detect oxidation are required.

Several methods have been introduced which express the degree of oxidative deterioration in terms of hydroperoxides per unit of fat weight. The modified Stamm method (32), the most sensitive of the peroxides determinations, is based in the reaction of oxidized fat and 1,5-diphenylcarbohydrazide to yield a red color. The Lea method (58), depends on the liberation of iodine from potassium iodide, where the amount of iodine liberated by the hydroperoxides is used as a criterion of the extent of oxidative deterioration. The colorimetric ferric thiocyanate procedure adapted to dairy products by Hills and Thiel (37) with modification of various workers, involves conversion of the ferric ion to ferric state in the presence of ferric thiocyanate, presumably by the presence of hydroperoxides present, to yield the red pigment ferric thiocyanate. All of these methods based in the direct or indirect measurement of hydroperoxides which do not concider previous dismutation of these primary reaction products, are not necessarily indicative of the extent of the reaction, nor do they tend to correlate with the degree of off-flavors in the product (60). Two variations of the thiobarbituric acid test have been widely used to determine the degree of lipid oxidation in dairy products (19, 48). The two TBA methods are based in the condensation of two molecules of thiobarbituric acid with one of malonaldehyde, resulting in the formation of a red

color complex with an absorption maximum at 532-540 mu. King (48) has shown that a correlation exist between the determined TBA value and the intensity of the oxidized flavor in fluid milks. Similar observations have been reported by others in ultra high-temperature pasteurized creams (15).

Besides the previous mentioned chemical tests, other methods based in the carbonyl content of oxidized fats have been suggested as a measure of oxidative deterioration. These procedures determine the secundary products of oxidation and have been reported to correlate significantly with the degree of off-flavor in butteroil (59). These methods, however, are not suited for routine analysis.

CHAPTER 3

MATERIALS AND METHODS

Source of Materials

Five commercial brands each of whipping cream and half-and-half were collected. A total of 8 lots from each brand were examined during this study which included the period August 30-October 21, 1977.

These samples were obtained from various food markets with the same being utilized each time for specific brands and products. Samples were coded for pull date and collection date. All samples were kept under refrigeration at 4°C until analyses were completed.

A uniform schedule of observations and analyses was arranged in the following sequence:

1. Sensory evaluation
2. Acid degree value (ADV)
3. Peroxide value (PV)
4. Thiobarbituric acid test (TBA)

Sample collection dates together with pull dates were recorded for the possibility of relating chemical analyses to the relative freshness of the samples at the time of collection.

The ADV and PV tests were carried out in rapid sucession, using the advantage that both determinations begin with the separation of the fat. The TBA test was performed 24 hours after the sensory analyses.

Sensory Evaluation

Flavor evaluation of the samples was made within 24 hours after collection. Ten different product were evaluated each time the sensory determination was carried out. Each product was evaluated for rancid and oxidized flavor. Before each sample was evaluated, it was held at room temperature for at least 15 minutes. All samples were scored using the American Dairy Science Association's scoring guide.

Because the sensory observations were made at the begining, the samples were kept in their own containers under refrigeration until this evaluation was performed. Once this evaluation was made, the samples were transfered to carefully cleaned glass-stoppered containers and kept under refrigeration until the chemical determinations were carried out.

The sensory observations were made by one experienced individual who analyzed the samples for rancid and oxidized flavors. That person had no idea of the identity of the product being analyzed in order to control any subconscious bias or preference.

Figures 1, 2 present the format of the scoring cards used in this investigation. Table 1 describes the scoring guide according to the American Dairy Science Association.

Acid Degree Value (ADV)

Fluid milk and products manufactured from milk may, at times, possess a flavor described as rancid. The term is used in dairy industry to denote the accumulation of free fatty acids (FFA) released hydrolytically from milk fat under the catalytic influence of lipases.

OXIDIZED FLAVOR				
Sample	Relative Intensity and Score			
	None 10	Slight 6	Definite 4	Pronounced 1
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Week No. _____

Date _____

Judge _____

Figure 1. Format of the Scoring Card Used to Evaluate Oxidized Flavor.

RANCID FLAVOR				
Sample	Flavor Intensity and Score			
	None 10	Slight 4	Definite 1	Pronounced 0
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Week No. _____

Date _____

Judge _____

Figure 2. Format of the Scoring Card Used to Evaluate Rancid Flavor.

Table 1. Scoring Card According to the American Dairy Science Association.

Flavor	None	Slight	Definite	Pronounced
Oxidized	10	5	3	1
Rancid	10	4	1	0

Although pasteurization inactivates all lipases in milk, the phenomenon of reactivation of these agents after treatment (flash pasteurization) has been suggested by some workers (72). Nevertheless, Downey (15) stated that there was no evidence of this phenomenon of reactivation in creams.

The methods used for quantitative determination of the extent of lipolysis can be grouped into two classes: (a) those which require total fat extraction and subsequent direct titration, and (b) those which use colorimetric procedures using comparative standards.

The method outlined by Thomas et al. (89) was the choice for measuring total FFA as a possible indication of the degree of rancidity in the samples analyzed. The method involves the use of a nonionic surface active agent (BDI reagent) for the fat isolation and titration with alcoholic KOH of suitable normality.

Experimental Method

Equipment:

1. A speed controlled centrifuge that will hold standard 18 gm, 50% Babcock test bottles.
2. Water bath for maintaining test bottles at boiling temperature, equipped with rack to hold the bottles.
3. Water bath to hold test bottles at 55-60°C for tempering the fat column. Some boiling-water baths are equipped with an additional compartment where this tempering of the fat column can be achieved.
4. Standard 9 ml T.D. pipettes.
5. 1.0 ml disposable serological pipettes to extract fat from the test bottles.
6. 50 ml Erlenmeyer flasks.
7. 5 ml microburette or 25 ml burette.

Reagents:

1. BDI reagent -- Thirty gm of Triton X-100 (a nonionic surface active agent manufactured by Rohn and Hass, Philadelphia, Pennsylvania) and 70 gm of sodium tetraphosphate are made up to 1 liter with distilled water.
2. Alcoholic KOH -- Absolute ethanol is used in the preparation of standard KOH. This solution should be standardize frequently against standard potassium acid phthalate or other suitable standard. In this investigation the KOH was either 0.01 or 0.02 N.

3. Indicator solution--1 gm of phenolphthalein dissolved in 100 ml of absolute ethanol.
4. Absolute ethanol was prepared by refluxing 1 liter of commercial ethanol over 12 gm of zinc dust and 5 gm of KOH for 4 hours followed by distillation.
5. Petroleum ether (B.P. range 30-60°C).
6. Aqueous Methyl Ethamol-- This was prepared by mixing equal volumes of chemical pure ethanol and distilled water.
7. Fat Solvent--Two parts of petroleum ether and one part of absolute ethanol (v/v).

Procedure

Recovery of Fat:

1. Transfer 9 ml of well mixed sample to a 18 gm, 50% Babcock cream test bottle, using a 9 ml, T.D. pipette.
2. Add BDI reagent as needed in portions of 5 ml (10 ml for the whipping cream products and 5 ml for the half-and-half) trying to wash traces of cream in the walls of the bottle.
3. Shake to mix.
4. Transfer the bottles to a boiling-water bath.
5. After 5 minutes, remix and return the bottles to the bath for an extra 10 minutes.
6. Remove from the bath without shaking.
7. Add 50% (v/v) methyl alcohol to the top of the graduate neck; allow alcohol to run down side of the neck.

8. Transfer the bottles to a unheated centrifuge and maintain speed for 2 minutes.
9. Transfer the bottles to a water bath (55-60°C) inmersing to the level of the fat column. Allow the bottles to remain until the temperature is in equilibrium (approximately 15 minutes).
10. Remove the bottles from the bath and withdraw a fat sample.

Titration:

1. Transfer 1 ml of fat from the test bottles to a 50 ml Erlenmeyer flask with 1 ml serological pipette.
2. Dissolve the fat in 10 ml of petroleum ether and 5 ml of absolute ethanol.
3. Add 10 drops of indicator.
4. Titrate to the first definite color change with standard KOH. Sometimes turbidity in the mixture will be observed during titration. In this case, the addition of 2 or 3 ml of additional solvents usually clear the mixture.
5. Express the results in terms of ml of 1 N base to titrate 100 gm of fat.

Calculations of ADV:

1. Calculate the gm of milk fat in the sample titrated by multiplying the ml of fat by its density (0.88 gm/ml).
2. $$\text{ADV} = \frac{(\text{ml KOH used in titration} - \text{blank})}{\text{weight of fat}} \times N \times 100$$

3. Blank is obtained as the titration value of the fat solvent in absence of fat using 10 drops of indicator. Blank determinations should be run in each new batch of solvent and then retitrated at frequent intervals thereafter.

Thiobarbituric Acid Test (TBA)

The method used in this work was outlined by King (48) which is essentially a simplified version of the Dunkley method (19). King found that lactose may undergo degradation in the TBA reaction due to the high temperatures at which the test is carried out in fluid milk. This may interfer in the TBA reaction and values obtained by this test. A satisfactory application of the method to milk includes trichloroacetic acid to remove the fat nad protein, and ethanolic-TBA to increase the rate of color formation at 60°C, temperature at which lactose degradation is minimized.

The modifications to the method of King introduced in this work are those proposed by Downey (15) which include: (a) the use of stoppered test tubes to hold 20 ml of cream, (b) incubation period following addition of reagents of 15 minutes at 60°C, and (c) the amount of filtrate used to react with 0.5 ml of TBA solution was 2.0 ml.

Experimental Method

Equipment:

1. Stoppered test tubes (1"x5½") to hold 20 ml of sample.
2. Temperature-controlled water bath (60°C).
3. Filter paper, Whatman No. 42.
4. Spectronic 20 or suitable spectrophotometer.

Reagents:

1. Trichloroacetic acid solution (1 gm/ml).
2. Ethanol A.R. grade 95%.
3. TBA solution (0.1M) prepared by dissolving 1.4 gm of TBA in 100 ml of 95% ethanol. The reagent tends to undergo deterioration and should not be stored longer than 3 days.

Procedure

1. Place 20 ml of cream in a stoppered test tube.
2. Incubate the tube in a temperature-controlled bath ($60^{\circ}C$) for 15 minutes.
3. Add 1 ml of trichloroacetic acid solution. Invert the tube once and return it to the temperature-controlled bath for 15 minutes.
4. Add 2 ml of ethanol 95%, shake vigorously for 5 seconds and return to the water bath for 15 minutes.
5. Take the tube out of the bath and filter the contents through a Whatman No. 42 filter paper.
6. Take a 2 ml aliquot of the filtrate and add 0.5 ml TBA solution.
7. Incubate the mixture for 60 minutes at $60^{\circ}C$.
8. Take the tube out of the bath, cool to room temperature and read the optical density at 532 nm.
9. Express the results as:

$$E^{1\ cm}_{532} \ / \ 20 \text{ ml of cream.}$$

10. Preparation of the blanks is made by applying the same procedure described above but using 20 ml of water instead of cream in the first step.

Peroxide Value (PV)

This chemical constant is a specific characteristic of the oxygen bound as a peroxide such as can be found, for example, in the primary products of autooxidation. For this reason, the peroxide value is one of the most important chemical constants for appraising the degree of deterioration of fats. The peroxide value gives the milliequivalents of oxygen contained in 1 kilogram of fat by using suitable procedures and strictly observed conditions.

Several attempts have been made to find a suitable procedure for peroxide determination in dairy products. The oxidation of ferrous ion to ferric ion and its estimation by the thiocyanate reaction has been the basis for a number of tests to determine peroxides in fats and oils.

In the early literature, Hills and Thiel (37) modified the method employed by Chapman and McFarlane in which the thiocyanate was first used to determine peroxides as a measure of fat oxidation. Hills and Thiel modified the solvents used and the wavelength range for this colorimetric determination. Stine et al. (80) also used the method proposed by hills and Thiel with its modifications. However, they centered their attention to find a better method for the isolation of the fat from the dairy samples.

The method employed in this work was a recent modification by Holloway (40) on the work of Stine. This author used chloroform-methanol as a solvent for the milk fat insted of benzene-methanol proposed by Stine.

Experimental Method

Isolation of the Milk Fat. The same technique utilized for the recovery of fat by the method of Thomas et al. (89) in the determination of ADV is suitable for the isolation of fat to be used in the analysis of peroxides.

Apparatus. The equipment neeeded is the same as used for the ADV determination (page 29). The use of a suitable spectrophotometer such as a Spectronic 20 is the only different part of equipment used. All the equipment was cleaned to be free of iron and oxidized fat by detergent washing and soaking for 2 hours in 1:3 HNO_3 followed by distilled water rinsings.

Reagents. All chemicals should be analytical reagent grade.

1. Fat Solvent: Chloroform-methanol 70:30 (v/v).
2. Ferrous Chloride Solution (0.014M): Barium chloride (0.4 g) dissolved in 50 ml glass distilled water is added slowly, with stirring to ferrous sulphate (0.5 g) dissolved in 50 ml of distilled water. To this mixture is added 2.0 ml of 10N HCL. The barium sulphate precipitate is allowed to settle by gravity or by centrifuging and the clear solution is then decanted into a bottle protected from light. The solution remains stable for about a week.

3. Ammonium Thiocyanate Solution: 30 g of anhydrous salt is dissolved in distilled water and the volume made up to 100 ml.
4. Standard Ferric Ion Solution: Iron wire (0.250 g) is dissolved in 25 ml of 10N hydrochloric acid and oxidized with 1-2 ml of hydrogen peroxide. The excess peroxide is removed by boiling and the solution is diluted to 250 ml. This solution will contain 1 mg/ml.

Preparation of the Standard Curve

1. Dilute 1 ml of the standard ferric iron solution to 100 ml with chloroform-methanol 70:30 (v/v) obtaining a working standard solution of 10 g/ml.
2. With the preceding solution and appropriate amounts of the chloroform-methanol solvent, prepare four tubes containing 5, 10, 15, and 20 µg of ferric iron in 9.9 ml of solution.
3. Add one drop of ammonium thiocyanate solution and one drop of water containing 2 ml of 10N HCl per 100 ml.
4. Shake and hold the tubes for 5 minutes in subdued light and read at 505 nm in a suitable colorimeter against a blank of chloroform-methanol solvent. Plot optical density against concentration of ferric iron. A straight line is obtained indicating close adherence to Beer's law for the concentration used.

Procedure

1. A volume of chloroform-methanol equal to 9.9 ml less the volume of fat to be taken is pipetted into a screw cap test

tube. The volume of fat depends on the expected peroxide value. For PV below 0.5, 0.2 to 0.5 ml of fat is convenient. PV's between 0.5 to 5.0 require 0.1 ml or less.

2. One drop each of ammonium thiocyanate and ferrous chloride solutions are added in that order. The test tube is then shaken to dissolve the fat and reagents.
3. The color is developed under subdued light conditions and after 5 minutes the color is determined at 505 nm as indicated in the preparation of the standard curve.
4. A fat blank is necessary to compensate for some of the red components in the color of the fat. This blank contains fat and thiocyanate but no ferrous salt.
5. The reagent blank is also prepared with each batch of tests and subjected to the same treatment. This reagent blank contains no fat.

Calculations

The peroxide value is expressed in terms of milliequivalents of oxygen per kilogram of fat. In making this calculation, the percent transmittance for the blanks must be converted to micrograms of iron per 10 ml of solvent by means of the standard curve (Figure 3). The net volume of the unknown in terms of micrograms of iron/10 ml of solvent is then calculated by deducting the sum of the fat and reagent blanks.

$$PV = \frac{\text{Net ug of iron/ 10 ml}}{\text{weight of fat in grams X } 55.85}$$

(as m.eq O_2/ Kg fat).

The K value is the ratio between concentration of ferric iron and O.D.

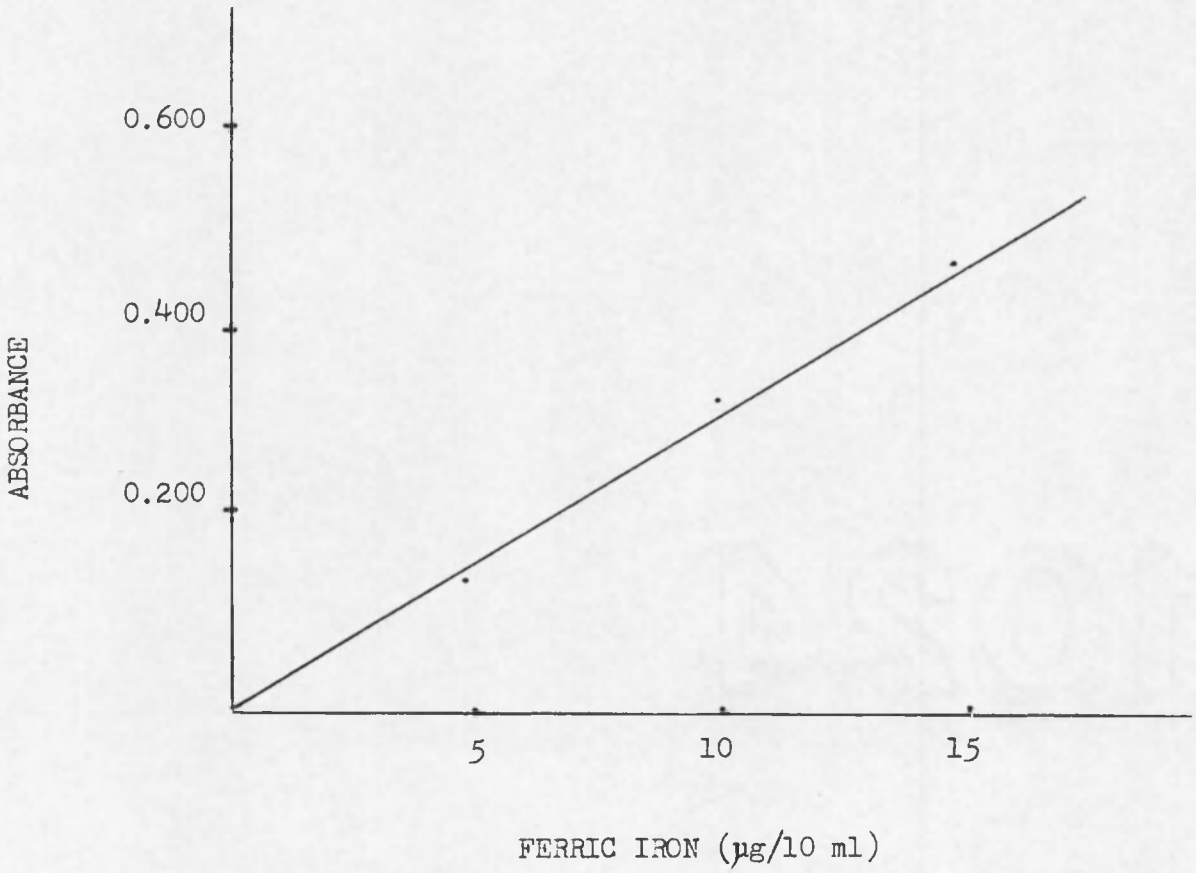

Figure 3. Peroxide Test Standard Curve.

CHAPTER 4

RESULTS AND DISCUSSION

Sensory (Subjective) Evaluation

Rancid and oxidized flavor scores for whipping cream products by individual brand are listed in Table 2. Oxidized and rancid flavor scores for the half-and-half products also by individual brand are listed in Table 3.

In the case of half-and-half products, there was no rancid flavor score recorded as low as "slight" (6.0) for the five different brands during the eight weeks of the study. The average rancid flavor scores were 9.8, 9.3, 9.3, 9.6, and 9.0 for brands A, B, C, D, and E, respectively. Brand E received the lowest average rancid score (9.0). However, neither this one nor any of the other four gave indications of rancidity (hydrolytic) as measured by flavor scores.

Whipping cream products of the five brands were as free of rancidity as were the corresponding half-and-half products. No product was given a rancid flavor score lower than 6.0 or "slight" intensity. The average rancid flavor scores for the whipping cream were 9.1, 9.5, 9.0, 9.5, and 9.3 for brands A, B, C, D, and E, respectively. Brands A, B, and C received at least one 6.0 score. Brands B and D had slightly higher average rancid flavor score.

Table 2. Flavor Scores for Various Whipping Cream Products

Week No.	Brand A		Brand B		Brand C		Brand D		Brand E	
	Ranc.	Oxid.	Ranc.	Oxid.	Ranc.	Oxid.	Ranc.	Oxid.	Ranc.	Oxid.
1	10.0	10.0	10.0	9.0	10.0	6.0	10.0	10.0	10.0	9.0
2	10.0	8.0	10.0	9.0	10.0	9.0	10.0	10.0	8.0	8.0
3	10.0	8.0	6.0	10.0	8.0	9.0	10.0	7.0	10.0	9.0
4	6.0	9.0	10.0	9.0	9.0	8.0	10.0	10.0	9.0	4.0
5	9.0	9.0	10.0	8.0	10.0	10.0	9.0	6.0	8.0	8.0
6	9.0	2.0	10.0	6.0	6.0	4.0	8.0	6.0	10.0	8.0
7	9.0	7.0	10.0	10.0	9.0	8.0	9.0	9.0	10.0	10.0
8	10.0	8.0	10.0	10.0	10.0	8.0	10.0	10.0	10.0	10.0
$\bar{x}$	9.1	7.6	9.5	8.8	9.0	7.7	9.5	8.5	9.3	8.3

Table 3. Flavor Scores for Various Half-and-Half Products.

Week No.	Brand A		Brand B		Brand C		Brand D		Brand E	
	Ranc.	Oxid.	Ranc.	Oxid.	Ranc.	Oxid.	Ranc.	Oxid.	Ranc.	Oxid.
1	10.0	10.0	10.0	10.0	10.0	7.5	10.0	8.0	10.0	8.5
2	10.0	10.0	9.0	9.0	10.0	10.0	10.0	10.0	9.0	4.0
3	10.0	8.0	8.0	9.0	9.0	7.0	8.0	8.0	8.0	7.0
4	9.0	10.0	10.0	8.0	8.0	10.0	10.0	8.0	9.0	8.0
5	10.0	8.0	10.0	10.0	8.0	10.0	10.0	8.0	10.0	9.0
6	10.0	8.0	7.0	8.0	9.0	6.0	9.0	8.0	7.0	10.0
7	10.0	9.0	10.0	8.0	10.0	9.0	10.0	10.0	9.0	8.0
8	10.0	10.0	10.0	10.0	10.0	8.0	10.0	10.0	10.0	7.0
$\bar{x}$	9.8	9.1	9.3	9.0	9.3	8.4	9.6	8.7	9.0	7.7

In the case of oxidized flavor scores, the whipping cream products averaged 7.6, 8.8, 7.7, 8.5, and 8.3 for brands A, B, C, D, and E, respectively. All the five brands of whipping cream products recorded at least one 6.0 or lower score which falls in the category of "slightly" oxidized. In some specific cases, such in brand A, week 6; brand C, week 6; brand E, week 4 the flavor scores were in the category of pronounced oxidation according with the scoring guide given by the American Dairy Science Association (page 28).

Half-and-half products were generally given higher oxidized flavor scores than the corresponding whipping cream products. Only in two cases, brand C, week 6 and brand E, week 2 were the oxidized flavor scores graded as "slight" in the first case and "pronounced" in the second. In all other cases, the oxidized flavor scores were 8.0 or higher. The average oxidized flavor scores for the Half-and-half products were 9.1, 9.0, 8.4, 8.7, and 7.7 for brands A, B, C, D, and E, respectively.

In generally, oxidative rancidity was found to be more pronounced in the whipping cream than in the half-and-half products. Nevertheless, none of the brands in any of the two products were graded below the category of "slight" as shown by the average oxidized flavor scores.

Hydrolytic rancidity flavor was not apparently found in any of the five brands of the two different products during the eight weeks of the study. As shown by the rancid flavor scores averages, none of the five brands of the two different products received lower score than 9.0.

Statistical Treatment of the Data

Statistical treatment of the data was based on multiple regression analysis applied to the five combined brands in each of the two categories: namely, whipping cream and half-and-half. In this way, the ADV, TBA, and PV data for the five combined brands were correlated with oxidized and rancid flavor scores, respectively. Possible correlation between chemical determinations and chemical scores was obtained for both whipping cream and half-and-half products. One-way analysis of variance was carried out to determine the significance in the results for each chemical determination. By doing this, the degree of consistency in the values obtained for each chemical determination could be observed between brands.

Relative Freshness of Samples

In order to establish the relative freshness of samples, collection and pull dates of the samples were recorded. Samples were collected at weekly intervals. Since two products were chosen (whipping cream and half-and-half) for each brand, a total of 16 different items were involved for each brand during the eight week study.

Tables 4, 5, 6, 7, and 8 show the "Difference in days" between the date on which the sample was collected from the food market and the pull date on the product's level. A product with less "Difference in days" characterized one with shorter expected remaining shelf life. Consequently, the possibility that this product undergoing any further deterioration will be minimized. In this comparison, both products for brands B and C, and brand E (half-and-half) had the lowest average in "Difference in days" indicating a somewhat slower rate of turn over

Table 4. Collection and Pull Dates for Experimental Samples (Brand A).

Code	Collection Date	Pull Date	Difference (days)
	Whipping Cream		
AW1	09-01-'76	09-19-'76	18
AW2	09-09-'76	09-19-'76	10
AW3	09-16-'76	09-23-'76	7
AW4	09-21-'76	09-27-'76	6
AW5	09-28-'76	10-06-'76	8
AW6	10-05-'76	10-29-'76	24
AW7	10-12-'76	11-01-'76	19
AW8	10-19-'76	11-19-'76	30
	Half-and-Half		
AH1	09-01-'76	09-19-'76	18
AH2	09-09-'76	09-19-'76	10
AH3	09-16-'76	09-22-'76	6
AH4	09-21-'76	09-28-'76	7
AH5	09-28-'76	09-29-'76	1
AH6	10-05-'76	10-16-'76	11
AH7	10-12-'76	10-23-'76	11
AH8	10-19-'76	10-30-'76	11

Table 5. Collection and Pull Dates for Experimental Samples (Brand B).

Code	Collection Date	Pull Date	Difference (days)
	Whipping Cream		
BW1	08-30-'76	09-05-'76	5
BW2	09-08-'76	09-12-'76	4
BW3	09-16-'76	09-20-'76	4
BW4	09-21-'76	09-23-'76	2
BW5	09-28-'76	10-05-'76	7
BW6	10-05-'76	10-09-'76	4
BW7	10-12-'76	10-21-'76	9
BW8	10-19-'76	11-02-'76	13
	Half-and-Half		
BH1	08-30-'76	09-05-'76	5
BH2	09-08-'76	09-14-'76	6
BH3	09-16-'76	09-21-'76	5
BH4	09-21-'76	09-25-'76	4
BH5	09-28-'76	10-06-'76	8
BH6	10-05-'76	10-10-'76	5
BH7	10-12-'76	10-17-'76	5
BH8	10-19-'76	10-26-'76	7

Table 6. Collection and Pull Dates for Experimental Samples (Brand C).

Code	Collection Date	Pull Date	Difference (days)
	Whipping Cream		
CW1	08-30-'76	09-07-'76	7
CW2	09-08-'76	09-15-'76	7
CW3	09-16-'76	09-24-'76	8
CW4	09-21-'76	09-29-'76	8
CW5	09-28-'76	10-06-'76	8
CW6	10-05-'76	10-12-'76	7
CW7	10-12-'76	10-21-'76	9
CW8	10-19-'76	10-31-'76	12
	Half-and-Half		
CH1	08-30-'76	09-06-'76	6
CH2	09-08-'76	09-15-'76	7
CH3	09-16-'76	09-23-'76	7
CH4	09-21-'76	09-28-'76	7
CH5	09-28-'76	10-06-'76	8
CH6	10-05-'76	10-08-'76	3
CH7	10-12-'76	10-20-'76	8
CH8	10-19-'76	10-27-'76	8

Table 7. Collection and Pull Dates for Experimental Samples (Brand D).

Code	Collection Date	Pull Date	Difference (days)
	Whipping Cream		
DW1	08-30-'76	09-23-'76	23
DW2	09-08-'76	09-21-'76	13
DW3	09-16-'76	10-09-'76	23
DW4	09-21-'76	10-20-'76	29
DW5	09-28-'76	10-08-'76	10
DW6	10-05-'76	10-23-'76	18
DW7	10-12-'76	11-02-'76	21
DW8	10-19-'76	11-18-'76	30
	Half-and-Half		
DH1	08-30-'76	09-03-'76	3
DH2	09-08-'76	09-15-'76	7
DH3	09-16-'76	09-24-'76	8
DH4	09-21-'76	10-01-'76	10
DH5	09-28-'76	10-20-'76	22
DH6	10-05-'76	10-15-'76	10
DH7	10-12-'76	10-22-'76	10
DH8	10-19-'76	10-29-'76	10

Table 8. Collection and Pull Dates for Experimental Samples (Brand E).

Code	Collection Date	Pull Date	Difference (days)
	Whipping Cream		
EW1	08-30-'76	09-06-'76	6
EW2	09-08-'76	09-16-'76	8
EW3	09-16-'76	09-21-'76	5
EW4	09-21-'76	09-25-'76	4
EW5	09-28-'76	10-04-'76	6
EW6	10-05-'76	10-27-'76	22
EW7	10-12-'76	10-27-'76	15
EW8	10-19-'76	11-03-'76	15
	Half-and-Half		
EH1	08-30-'76	09-05-'76	5
EH2	09-08-'76	09-13-'76	5
EH3	09-16-'76	09-22-'76	6
EH4	09-21-'76	09-26-'76	5
EH5	09-28-'76	10-03-'76	5
EH6	10-05-'76	10-11-'76	6
EH7	10-12-'76	10-21-'76	9
EH8	10-19-'76	10-26-'76	7

in the food market. Later in this report, comparison will be made between shelf life of the different products and the oxidized flavor scores obtained. Shelf life will also be compared with chemical indicators of rancid and oxidized defects. Another comparison that can be made from relative freshness data is the possibility of determining how oxidative and hydrolytic rancidities vary during the storage life of the products. Since the collection interval was on a weekly basis, sometimes the elapse time between consecutive collections did not allow the processor to change the product in a given market. This resulted in the same product being collected on two consecutive weeks in the case of brand A, weeks 1-2 (whipping cream and half-and-half) and brand E, weeks 6-7 (whipping cream).

Acid Degree Value (ADV)

The titratable acidity of all fresh creams varies with the fat percentage of the cream. There is an inverse relationship between the percentage of fat and the percentage of titratable acidity. Unfortunately, this relationship is not always fully appreciated. Since the titratable acidity of freshly separated cream is always lower from that of milk from which it was separated, those not familiar with this relatioship often suspect that the acidity of cream has been standardized (61).

Thomas et al. (89), in their study made to correlate ADV and the extent of rancidity in pasteurized milk, found that rancidity was detected unequivocally when ADV reached values of 1.3 to 1.6.

Dunkley (16) studied the relationship between fat acidity and rancid flavor in creams. He found that rancidity could be detected in a few samples with acid degrees as low as 1.5, but it was not detected consistently in all samples until the acid degree exceeded 2.1. Furthermore, in the same investigation, poor correlation between acid degree and sensory intensity of rancidity was reported. Dunkley finally stated that acid degree determinations, applied to fat obtained from cream samples, are helpful in classifying cream as "not rancid" or "rancid", but they are of little value as measure of the intensity of rancidity when compared with sensory determinations as a standard.

On the same subject, Nair and Bentham (in 61, p. 320) suggested that upper limits of acidity permissible in creams to be correspond to a serum acidity of approximately 0.18%. This means then that a 20% cream may have a titratable acidity not exceeding 0.144%.

No reports have been found in the literature regarding the correlation of sensory evaluation of creams with ADV as determined by the Thomas method. Nevertheless, it has been stated that titratable acidity of creams is always lower than that of milk. The American Dairy Science Association acceptable limits of ADV is 1.1 in milk.

In this investigation, ADV for brands A, B, C, D, and E in the half-and-half products were 0.929, 0.753, 0.764, 0.812, and 0.890, respectively. In the case of whipping cream, the ADV for brands A, B, C, D, and E averaged 0.920, 0.781, 0.738, 0.794, and 0.874, respectively. Considering the ADV limit of acceptability in milk (1.1), the cream products analyzed (whipping cream and half-and-half) would

not be expected to show any perceptible rancid flavor. These results are in good agreement with the flavor scores assigned by the sensory evaluation.

Tables 9 and 10 present the average sensory scores for rancidity and the corresponding ADV's for the two products. None of the average ADV's exceeded the acceptable limit of 1.1 recommended for milk.

In specific cases such as in brand A, week 1 and 2 (whipping cream and half-and-half) and brand E, weeks 6 and 7 (whipping cream) the products collected had the same pull dates for the two consecutive weeks. In the first case, the ADV increased from 0.729 to 0.972 (whipping cream) and from 0.729 to 0.851 (half-and-half). In the second case, the ADV increased from 0.960 to 1.034 (whipping cream). In both instances, the increase was not perceived by sensory evaluation.

The correlation coefficient for the ADV results and rancid flavor scores for the combined five brands of whipping cream products was 0.164 which represents a relatively low correlation. In the half-and-half products, the correlation coefficient (r) was 0.244 which is somewhat better than that for the whipping cream products but still low correlation.

In view of the rather low correlation coefficients obtained between ADV and rancid flavor scores, a one-way analysis of variance was performed to find out a possible significance in the ADV obtained for each brand. The analysis of variance showed that the half-and-half products were similar in their ADV's at a 0.01% level of significance. The whipping cream products were similar in their ADV's at a 0.05% level of significance.

Table 9. ADV and Rancid Flavor Scores for Half-and-Half Products.

Week No.	Brand A		Brand B		Brand C		Brand D		Brand E	
	ADV	Flavor Score	ADV	Flavor Score	ADV	Flavor Score	ADV	Flavor Score	ADV	Flavor Score
1	0.729	10.0	0.729	10.0	0.851	10.0	0.729	10.0	0.851	10.0
2	0.851	10.0	0.851	9.0	0.607	10.0	0.607	10.0	0.729	9.0
3	0.972	10.0	0.729	8.0	0.729	9.0	0.851	8.0	0.851	8.0
4	0.851	9.0	0.486	10.0	0.607	8.0	0.607	10.0	0.729	9.0
5	0.856	10.0	0.797	10.0	0.736	8.0	0.675	10.0	0.859	10.0
6	1.107	10.0	0.518	7.0	0.738	9.0	0.960	9.0	0.886	7.0
7	1.034	10.0	0.886	10.0	0.960	10.0	1.034	10.0	1.181	9.0
8	1.034	10.0	1.034	10.0	0.886	10.0	1.034	10.0	1.034	10.0
$\bar{x}$	0.929	9.8	0.753	9.3	0.764	9.3	0.812	9.6	0.890	9.0

Table 10. ADV and Rancid Flavor Scores for Whipping Cream Products.

Week No.	Brand A		Brand B		Brand C		Brand D		Brand E	
	ADV	Flavor Score	ADV	Flavor Score	ADV	Flavor Score	ADV	Flavor Score	ADV	Flavor Score
1	0.729	10.0	0.607	10.0	0.729	10.0	0.607	10.0	0.851	10.0
2	0.972	10.0	0.729	10.0	0.729	10.0	0.729	10.0	0.851	8.0
3	0.729	10.0	0.668	6.0	0.668	8.0	0.729	10.0	0.729	10.0
4	0.851	6.0	0.729	10.0	0.607	9.0	0.607	10.0	0.729	9.0
5	0.981	9.0	0.859	10.0	0.736	10.0	0.797	9.0	0.736	8.0
6	0.738	9.0	0.886	10.0	0.738	6.0	0.738	8.0	0.960	10.0
7	1.181	9.0	0.886	10.0	0.812	9.0	1.034	9.0	1.034	10.0
8	1.181	10.0	0.886	10.0	0.886	10.0	1.107	10.0	1.107	10.0
$\bar{x}$	0.920	9.1	0.781	9.5	0.738	9.0	0.794	9.5	0.874	9.3

Thiobarbituric Acid Test (TBA)

The application of the reaction to evaluate oxidation in dairy products was proposed by Dunkley and Jennings (19) who demonstrated that TBA correlated closely with numerical flavor scores of milk samples having oxidized flavor of varied intensity. The TBA test is based upon the reaction involving the formation of a red color when oxidized milk is acidified and heated with 2-thiobarbituric acid.

In order to have some meaningful interpretation of the validity of the test, previous correlated objective/subjective values should be taken as a basis. Table 11 indicates the relation derived from the work of Downey (15) which was employed in this work.

Table 11. Frequency Distribution of Creams According to Flavor Scores and Chemical Assessment.

Flavor Scores	Thiobarbituric Acid (TBA) Ranges ≤ 0.08	0.08-0.16	≥0.16	No. of Samples
Acceptable	181 (90%)	49 (44.5%)	18 (6.9%)	248
Doubtful	13 (6.5%)	45 (40.9%)	48 (18.2%)	106
Unacceptable	7 (3.5%)	16 (14.6%)	198 (75.0%)	221
Total	201 (100%)	110 (100%)	264 (100%)	575

In this report, Downey (15) stated that 80% of the creams with TBA values equal to 0.08 were considered by the test panel as to have an acceptable flavor, while only 5% had attained unacceptable levels of off-flavor. The possibility of cream having acceptable flavor is increased at lower TBA values. As a result, this investigation pointed out that creams with TBA values up to and including 0.08 were considered

to have good flavor quality, while those with TBA values greater than and including 0.16 were deemed to have attained objectional levels of off-flavor.

In the present study, TBA values and the corresponding flavor scores for the various brands of the whipping cream products are listed in tables 12, 13, 14, 15, and 16. Tables 17, 18, 19, 20, and 21 summarize the results for the various brands of the half-and-half products.

The data reveal that the TBA values are well correlated, in some cases, to the oxidized flavor scores according to the criterion suggested by Downey (15).

In a few individual cases of whipping cream products (brand D, week 6; brand B, week 6; brand C, week 6; and brand E, week 4) good correlation between TBA values and flavor scores was shown. Other than in these specific examples, there was relatively poor correlation between TBA values and flavor scores in whipping cream products. This is shown by the correlation coefficient (r) of -0.106 obtained between TBA values and the oxidized flavor scores for the five combined brands. The TBA values for the five combined brands were not significantly similar between brands as shown by the one-way analysis of variance. The average TBA values for brands A, B, C, D, and E were 0.089, 0.122, 0.114, 0.095, and 0.096, respectively. All of these fell in the category of "slightly" oxidized according to the criterion suggested by Downey. However, the average flavor scores for the five brands would indicate a level below that for any significantly oxidized flavor.

Similarly, in the case of half-and-half, only in a few specific cases such as brand E, week 2 did TBA values correlated well with

Table 12. Oxidative Rancidity Related Determinations in Whipping Cream (Brand A).

Week No.	TBA (O.D.)	PV (Meq. O_2/Kg Fat)	Flavor Score
1	0.066	0.508	10.0
2	0.066	0.305	8.0
3	0.076	0.284	8.0
4	0.009	0.508	9.0
5	0.153	0.408	9.0
6	0.174	0.264	2.0
7	0.119	0.406	7.0
8	0.155	0.172	8.0
$\bar{x}$	0.089	0.356	7.6

Table 13. Oxidative Rancidity Related Determinations in Whipping Cream (Brand B).

Week No.	TBA (O.D.)	PV (Meq. O_2/Kg Fat)	Flavor Score
1	0.092	0.081	9.0
2	0.114	0.183	9.0
3	0.155	0.152	10.0
4	0.041	0.274	9.0
5	0.053	0.285	8.0
6	0.092	0.203	6.0
7	0.114	0.223	10.0
8	0.319	0.295	10.0
$\bar{x}$	0.122	0.212	8.8

Table 14. Oxidative Rancidity Related Determinations in Whipping Cream (Brand C).

Week No.	TBA (O.D.)	PV (Meq. O_2/Kg Fat)	Flavor Score
1	0.114	0.305	6.0
2	0.081	0.183	9.0
3	0.063	0.396	9.0
4	0.108	0.305	8.0
5	0.022	0.285	10.0
6	0.137	0.284	4.0
7	0.184	0.244	8.0
8	0.208	0.295	8.0
$\bar{x}$	0.114	0.249	7.7

Table 15. Oxidative Rancidity Related Determinations in Whipping Cream (Brand D).

Week No.	TBA (O.D.)	PV (Meq. O_2/Kg Fat)	Flavor Score
1	0.078	0.162	10.0
2	0.004	0.203	10.0
3	0.105	0.284	7.0
4	0.061	0.427	10.0
5	0.068	0.183	6.0
6	0.081	0.223	6.0
7	0.174	0.284	9.0
8	0.194	0.213	10.0
$\bar{x}$	0.095	0.247	8.5

Table 16. Oxidative Rancidity Related Determinations in Whipping Cream (Brand E).

Week No.	TBA (O.D.)	PV (Meq. O_2/Kg Fat)	Flavor Score
1	0.125	0.305	9.0
2	0.041	0.223	8.0
3	0.066	0.515	9.0
4	0.168	0.233	4.0
5	0.004	0.122	8.0
6	0.051	0.162	8.0
7	0.240	0.335	10.0
8	0.073	0.233	10.0
$\bar{x}$	0.096	0.241	8.3

Table 17. Oxidative Rancidity Related Determinations in Half-and-Half (Brand A).

Week No.	TBA (O.D.)	PV (Meq. O_2/Kg Fat)	Flavor Score
1	0.022	0.305	10.0
2	0.009	0.366	10.0
3	0.043	0.203	8.0
4	0.025	0.427	10.0
5	0.032	0.183	8.0
6	0.032	0.142	8.0
7	0.552	0.284	9.0
8	0.114	0.295	10.0
$\bar{x}$	0.096	0.275	9.1

Table 18. Oxidative Rancidity Related Determinations in Half-and-Half (Brand B).

Week No.	TBA (O.D.)	PV (Meq. O_2/Kg Fat)	Flavor Score
1	0.022	0.284	10.0
2	0.032	0.376	9.0
3	0.034	0.223	9.0
4	0.004	0.447	8.0
5	0.034	0.143	10.0
6	0.032	0.183	8.0
7	0.128	0.264	8.0
8	0.038	0.213	10.0
$\bar{x}$	0.036	0.266	9.0

Table 19. Oxidative Rancidity Related Determinations in Half-and-Half (Brand C).

Week No.	TBA (O.D.)	PV (Meq. O_2/Kg Fat)	Flavor Score
1	0.063	0.366	7.6
2	0.071	0.345	10.0
3	0.086	0.223	7.0
4	0.046	0.396	10.0
5	0.092	0.143	10.0
6	0.086	0.183	6.0
7	0.086	0.183	9.0
8	0.131	0.142	8.0
$\bar{x}$	0.082	0.247	8.4

Table 20. Oxidative Rancidity Related Determinations in Half-and-Half (Brand D).

Week No.	TBA (O.D.)	PV (Meq. O_2/Kg Fat)	Flavor Score
1	0.094	0.185	8.0
2	0.018	0.325	10.0
3	0.068	0.315	8.0
4	0.013	0.549	8.0
5	0.036	0.265	8.0
6	0.011	0.305	8.0
7	0.058	0.488	10.0
8	0.058	0.295	10.0
$\bar{x}$	0.044	0.341	8.7

Table 21. Oxidative Rancidity Related Determinations in Half-and-Half (Brand E).

Week No.	TBA (O.D.)	PV (Meq. O_2/Kg Fat)	Flavor Score
1	0.056	0.305	8.5
2	0.155	0.813	4.0
3	0.076	0.203	7.0
4	0.094	0.549	8.0
5	0.097	0.143	9.0
6	0.002	0.142	10.0
7	0.357	0.223	8.0
8	0.097	0.244	7.0
$\bar{x}$	0.105	0.327	7.7

the flavor score. Other than that, TBA values and flavor scores in the half-and-half products did not agree consistently. The correlation coefficient between the TBA values and flavor scores for the five combined brands was -0.166. The TBA values were shown not to be significantly different between the five brands by the one-way analysis of variance. The average TBA values for brands A, B, C, D, and E were 0.096, 0.036, 0.082, 0.044, and 0.105, respectively. Brands A, C, and E exceeded the acceptable limits of TBA values as proposed by Downey (15) corresponding to products with "noticeable" oxidized flavor. However, the sensory evaluation indicated no perceptible in any of the five brands.

The majority of both whipping cream and half-and-half products gave TBA values which did not correlate well flavor scores according with the criterion suggested by Downey (15) and as employed in this investigation. This suggests that oxidized flavor is not perceived in cream samples with TBA values lower than 0.200. A better correlation between TBA and oxidized flavor scores is reported when TBA approches higher values. The last observation is in agreement with the findings of King (48).

In three specific examples, brand A, weeks 1 and 2 (whipping cream and half-and-half) and brand E, weeks 6 and 7 (whipping cream) it was possible to compare changes in TBA during one week. Only in the latter case did TBA values change; namely, from 0.051 to 0.240. However, the change was not perceived by the sensory evaluation.

Peroxide Value (PV)

The oxidation of ferrous to ferric ion and its estimation by thiocyanate has been the basis for a number of tests used to determine peroxides in fats and oils. Lea (58) found that the method gives excellent reproducibility and that the peroxide value obtained by this method is proportional to iodometric value.

Most of the publications previously mentioned in the ferric thiocyanate test used to determine peroxides were conducted to establish the standard conditions for the test. Nevertheless, Hills and Thiel (37) reported values of PV of 0.14-0.17 for cream. Stine et al. (80) gave values of PV of the order of 0.16-0.18 for cream. In the investigation conducted by Downey (15) to find a correlation between flavor scores and PV in creams, the following figures were found (Table 22).

Table 22. Frequency Distribution of Creams According to Flavor Score and Chemical Assessment.

Flavor Scores	Peroxide Ranges ≤ 2.0	≥ 2.0	No. of Samples
Acceptable	118 (43.5%)	3 (4.9%)	121
Doubtful	66 (24.4%)	2 (3.3%)	68
Unacceptable	87 (32.1%)	56 (91.8%)	143
Total	271 (100%)	61 (100%)	332

In this investigation, low peroxide values were not necessarily an indication of an acceptable flavor. However, high peroxide values were indicative of poor flavor and 75% of creams with peroxide values equal to 2.0 had unacceptable flavor.

In the results of this study, first in the whipping cream, the highest PV detected was 0.508 and the lowest 0.081. These values are listed in tables 12, 13, 14, 15, and 16. PV averaged 0.356, 0.212, 0.249, 0.247, and 0.241 for brands A, B, C, D, and E, respectively. None of these were represented by flavor scores which were indicative of significant oxidative rancidity. Brand A was given the lowest average flavor score (7.6). Even this was above the category of "slightly" oxidized as indicated by the scoring guide given by the American Dairy Science Association. In general, PV correlated poorly with sensory evaluation in the five individual brands or in the five brands combined as a group. The correlation coefficient (r) between PV and oxidized flavor scores for the five combined brands was 0.168.

In the half-and-half products, the highest PV detected was 0.813 and the lowest 0.142. These values are listed in tables 17, 18, 19, 20, and 21. PV averages for brands A, B, C, D, and E were 0.275, 0.266, 0.247, 0.341, and 0.327, respectively. Brand E received the lowest average flavor score (7.7) which corresponds to one of the highest average PV (0.327). In this product (half-and-half), PV levels correlated better with sensory evaluation than in the whipping cream products. Only in one case (brand E, week 2) did the flavor score indicate an intensity of "slightly" oxidized. In this sample a PV of 0.813 was the highest observed. Therefore, it seems that a better correlation exists between PV and flavor scores when PV is higher. Brand E received the lowest average flavor score (7.7) and brand A the highest (9.1). The correlation coefficient of the PV and the oxidized flavor scores was -0.244 for the five combined brands.

This correaltion coefficient (r) was somewhat better than that obtained for the whipping cream products.

It is concluded that low levels of oxidative rancidity could not be detected in creams using PV as the only indicator. The PV values obtained for the various brands in the two products ranged from 0.08 to 0.80. According to Downey, this is indicative of no oxidative deterioration. Downey (19) stated that oxidized flavors could be detected when PV reached 2.0, and that PV lower than 2.0 were not necessarily indicative of acceptable flavors. Since the PV in this study were all well below 2.0, there was no consistent trend between scores and corresponding PV.

PV results were found to be similar at a 0.05% level of significance for the whipping cream products. On the other hand, PV were found significantly different for the half-and-half products. This was elucidated by the one-way analysis of variance for the PV of the five combined brands in the two different products (whipping cream and half-and-half). Statistical analysis also showed that PV has a somewhat better correlation with flavor scores than TBA. Combining TBA and PV values, still gave a relatively poor degree of correlation with sensory evaluations, being a little better for whipping cream ($r = 0.168$) than for half-and-half products ($r = 0.021$).

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