THE ASSOCIATION OF AN UDDER BORNE MICROCOCCUS WITH THE OXIDIZED FLAVOR OF MILK

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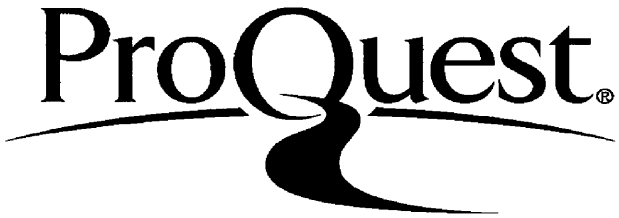

C O N T E N T S

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INTRODUCTION

Studies on the occurrence and prevention or control of oxidized flavor in milk have been reported for twenty years. The same flavor, though called by several other names, has been the basis of research in the milk by-products fields for a much longer time. This is especially true of the products butter and powdered milk. The exact origin of this flavor has never been determined, though it is now well known that a number of control measures will prevent it.

Oxidized flavor continues to be a major problem in the dairy industry. This is shown by the growing number of markets that report having it and the more intense effort expended in its control. One intriguing observation has been made by many investigators. That is that the occurrence and intensity of the flavor increases, as quality measured in other ways, improves. Consumer education in nutrition and sanitation have led the entire dairy industry toward supplying a better product; therefore more emphasis is constantly being placed on the production, processing and distribution of a more nourishing and a public health-wise safe product. The flavor is definitely associated with the rich portion of the milk and is further characteristic of milk that has been handled so as to make it bacteriologically good.

It is unfortunate that a food industry constantly progressing toward the goal of perfection in product quality, should be simultaneously plagued by a serious fault. Flavor is the consumer's final basis of judgment.

HISTORICAL REVIEW

Metals and Light as Catalysts

Feeds, Vitamins and Oxidized Flavor

Heat and Deaeration

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Lipolytic and Oxidizing Organisms

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METALS AND LIGHT AS CATALYSTS

Metals, primarily copper and iron, have been given much attention as aids to oxidized flavor development. Guthrie and Brueckner (31) contributed the significant fact that the mechanism of oxidation catalysis by oleinase is entirely different from that of copper, since the former does not require high oxidation-reduction potentials and the latter does. Of the many references to the catalytic effects of metals the more pertinent works of Kende (37) (38) (40) appear to be a logical summary. He showed that copper, either that contained in the udder or outside, was responsible for fat oxidation according to the relation of copper to the oleinase and/or the reducing substances present. Reductase present in the udder or produced by bacteria were factors reciprocating with oleinase and the metal. The progress or durability of the "oily" or "emery" flavor, its intensity, the time of develop-

ment and the eventual disappearance were dependent upon and were measured by this reciprocating action. Feeds may supply substances sufficient to protect the milk from becoming oxidized, or living cultures of reducing bacteria or their metabolic products may be used.

Frazier (24) held that neither enzymes nor bacteria were necessary for milk fat oxidation. He kept raw and pasteurized milk samples at about freezing for eight hours in diffused light. Oxidation occurred more rapidly in pasteurized than in raw milk, irrespective of the feeds, cottonseed and linseed cake. He attributed the oxidation to light, though there was not a clear definition of the type of flavor experienced.

FEEDS, VITAMINS AND OXIDIZED FLAVOR

Anderson, Hardenberg and Wilson (1) used vitamin A to control oxidized and rancid flavors. Their claim that 8 pounds of carrots in the daily ration was superior to 500,000 U.S.P. units of vitamin A has been observed also under commercial conditions but not reported experimentally. While Whitnah, Martin and Beck (70) concluded that there was no certain relation between feed and oxidized flavor, they did observe that all samples that developed the flavor were below the breed average in color of fat intensity (vitamin A). They also ranked the breeds in order of vitamin C content of the milk--Jersey, Guernsey, Ayshire and

Holstein and found that the incidence of oxidized flavor from these breeds was in reverse order. Other evidence that vitamin A does inhibit or prevent oxidized flavor is presented by Brown, Thurston and Dustman (7) Kende (38) Garrett, Bender and Tucker (27). Prewitt and Parfitt (53) found both soybean oil and unprocessed beans to be protective against the development of oxidized flavor, even when copper had been added to the milk. None of the samples to which copper had not been added showed any oxidized flavor after pasteurization and holding at 4.4° C. for 72 hours. Supplee and Bellis (60) found no difference between the copper content of cow's milk when pasture and stall feeding were compared. They suggested (this was in 1922) that copper "may prove to be significant in connection with the high susceptibility of the antiscorbutic vitamin to oxidation." Since they found cow's milk to contain from 0.2 to 0.8 milligrams of copper per liter, average 0.52 milligrams, there is a basis for the statement made in 1922 by Kende (40) regarding "inner and outer" metal contamination.

HEAT AND DEAERATION

Dahle (15) collected milk samples directly from cows into amber glass bottles and studied the influence of pasteurizing on oxidized flavor development. When heated at 71.1° C. for 30 minutes, cooled and stored at 4.4° C. for 3 days the flavor was decreased or

prevented. There was no evidence of oxidized flavor in the skim-milk so treated and the flavor was more pronounced in whole milk than in cream. Foremilk had less noticeable flavor than middle-drawn or last-drawn milk. Cows that produced milk with the flavor in winter did not produce it in summer. There was no statement made in this report indicating that the raw samples were tasted. McFarland and Burgwald (44) prevented oxidized flavor development in storage cream by heating at 77.7° C. for 5 minutes or by homogenizing. The cooked flavor resulting from the high heat treatment was not noticeable after one month in storage. Kende (40) and Gould and Sommer (30) explained that oxidized flavor is prevented by heating, owing to the liberation at temperatures in the range of 76° to 78° C. of reducing substances. Gould and Sommer (30) specifically named hydrogen sulphide as the protective reducing substance and showed also that the oxidation reduction potential is lowered at these high temperatures. Oleinase, named by Kende (40) as the agent responsible for the oxidized flavor, was apparently prevented. Leeder and Herried (43) evacuated and stored under a vacuum of 23 to 25 inches, milk susceptible to becoming oxidized. Bacteria decreased the oxygen content of milks held at atmospheric pressure and prevented or inhibited flavor development; under vacuum the milk did not become oxidized and bacteria were not considered important as oxygen consumers in this milk. This work showed no relation

between bacteria count and intensity of oxidized flavor or oxygen content. Reed (54) reports that evacuation of milk, when preceded by fortification with ascorbic acid, pasteurizing and homogenizing, prevents or retards oxidized flavor. Low storage temperatures delay the rate of change from ascorbic to dehydro-ascorbic acid; oxidized flavor is delayed simultaneously.

GENERAL UDDER FLORA AND MILK FLAVOR

Little has been done to show that organisms isolated from the udder have any relation on milk flavors. The bacteria counts from healthy udders determined by established methods are usually low; further, it is usually believed that these organisms have no appreciable effect on the properties of fresh milk, and that they do not thrive outside the animal body. However, Evans (20) in 1917 reported a study made on 192 samples from 161 cows in 5 herds in which the organism Bacillus abortus variety lipolyticus was frequently found. It decomposed fat and "imparted undesirable flavors and odors to cream kept under conditions to which cream is frequently subjected." Since the Bacillus abortus strain was isolated from all cows at all 5 dairies it was assumed that it could be isolated from all mixed milk even though the total count were low. In 1917 oxidized flavor in milk was not reported on as such. The "undesirable" flavor reported by Evans was probably rancid. In further work on lipolytic rod shaped bacteria, Evans (21) found as high as 112,000 per milliliter of the Bacillus

abortus strain in the milk of an apparently normal cow. Again the "disagreeable" flavor was noted but it was observed that the organism was destroyed in 25 minutes at 51.7° C. or in 25 seconds at 62.6° C. Tracy, Ramsey and Reube (65) concluded that tallowy flavors could not always be produced to the same degree by adding equal amounts of copper salt to aseptically drawn samples nor to mixed herd samples. They felt that a lack of bacterial metabolism accounted for oxidized flavor in low count raw or pasteurized milk and that leucocytes as well as bacteria functioned as reducing bodies. Udder tissue did not function as a reducing substance.

FLAVORS CAUSED BY SPECIFIC ORGANISMS

When Ruehle (56) inoculated into intermittently heated sterile milk, single species of organisms, and made parallel plantings of each specie in association with Streptococcus lactis, a wide variety of flavors developed at room temperature in 48 hours. While the organisms were originally isolated from butter some of them could have been of udder origin. This is not known, partially because the description of the organisms was limited to morphological characteristics. Oxidized flavors were not mentioned (the work was reported in 1920) but "astringent or metallic," "oily" and "flat" were used to describe some of the flavors that resulted. Associative action was apparently necessary to bring out flavors indicating that bacterial flavors

are chemically complex. The numbers of organisms required to effect the certain flavors were not indicated. Brown, Smith and Ruehle (5) found that tallowy flavors developed more frequently in raw cream butters than in pasteurized cream butters, but in a study of split churnings pastuerized cream butters developed faster in which cream was inoculated with fishy butter and treated with wash waters of varying acidities. Some of the organisms isolated from these butters were able to grow in agar containing 16 percent salt.

LIPOLYTIC AND OXIDIZING ORGANISMS

Jensen and Grettie (36) found in work carried out on butterfat, shortening and lard that these fats serve as substrates for certain strains of organisms, which are responsible both for enzymes that liberate free fatty acids and oxidation products. They called the property "oxidative rancidity" and attribute it in part to the effect of light. Their analysis of the problem of fat hydrolysis or oxidation helps to explain the confusion that exists. They found that moisture-free fats did not support the growth of organisms but that 0.3 percent or more of moisture in an animal fat promoted growth. Stark and Scheib (59) named several species of micrococci as being prominent fat splitters. These were isolated from butter. In view of the explanation by Jensen and Grettie (36) that organisms may be both oxidative and lipolytic, and that Stark and Schieb found micrococci capable of growth at temperatures ranging from 5° to 45° C., it seems possible

that the oxidative processes associated with oxidized flavor could originate in the normal cow's udder.

A quantitative estimation of the relative oxidizing capacities or organisms has been made by Gordon and McLeod (29). They streaked heated blood agar plates with pathogens. After colony development, oxidizers were detected by flooding the plates with p-phenylene-diamine. When this was oxidized to indophenol, colors of the colonies varying from pink through red to black resulted. Mixed cultures gave vivid color contrasts. The method had been used formerly by others to distinguish between myeloid and lymphocytic leucoytes, on the assumption that the reaction in leucoytes was due to an oxidative ferment. When the anthrax bacillus was suspended in the reagent in a hanging drop, blue granules developed in the cell. Ellingworth, McLeod and Gordon (18) could not successfully incorporate p-phenylenediamine into the agar medium. It detected more oxidizing organisms than did other diamines. These authors found also that when the colony turned black, indicating the maximum detectable oxidizing capacity of the enzyme elaborated by the organism, the organism was then dead; no further reduction being possible. Fabian and Trout (22) developed a technique for the isolation of lipolytic organisms. Nile blue sulphate and sterile cream were incorporated into tryptose broth just prior to pouring plates, in a study of frozen cream. Tryptose gave higher counts than standard agar

and lipolytic organisms were easily detected by the blue color of the fatty acids released. Castell and Garrard (10) grouped several important genera according to their oxidizing abilities, using the technique of Gordon (29) except that they used p-aminodimethylaniline hydrochloride as the oxidation indicator. Pseudomonas and Achromobacter were the most strongly oxidative. Alkligenes and Brucella followed, though still strongly positive. Other gram negative genera varied from weakly positive to negative. The bacilli were variable and the cocci and one anaerobe (Clostridium butyricum) were negative. All strong oxidizers were gram negative; those classed as not being strong oxidizers were positive. Carpenter, Suhrland and Morrison (9) claimed to have improved the technique of Castell and Garrard (10) by employing the oxalate rather than the hydrochloride salt of p-aminodimethylaniline. The oxalate was more stable in crystalline than in liquid form. It showed less precipitate as it contacted the colonies and gave more distinct colors, ranging from pink to black.

OXYGEN METABOLISM

Keyes and Gillespie (41) made a study of the oxygen metabolism by Escherichia coli and Clostridium welchii. Wide differences were noted; the respiration by-products CO^2 and H_2 varied much more for Escherichia coli (facultative) than for Clostridium welchii (strict anacrobe). That organisms generally

have a wide range of metabolic activity has been shown also by Mundt and Fabian (47) who were studying the oxidation of corn oil. Some of their test organisms were udder-borne micrococci. The oat derivative Avenex, commonly used as an anti-oxidant in dairy products, was utilized by four species and did not prevent bacterial respiration in the presence of corn oil. Nunheimer and Fabian (48) studied the respiratory properties of the micrococci. Four of the 5 species studied are borne by the udder; these were found to be oxidizers of several sugars, alcohols and amino acids. Micrococcus aurantiacus was most active as a dehydrogenator on lactose and d-galactose. It also was more active on glycerol than Micrococcus flavus, Micrococcus luteus, Micrococcus cinnebareus and Micrococcus freundenreichii in the order of 367-114-56-32-24 respectively, using the dehydrogenation value on glucose as 100. Substrates of particular interest in a cow's udder were not employed. Mattick (46) systematically eliminated bacteria as a possible cause of "oily" flavors, arguing that by their own metabolism of molecular oxygen or by the production of acidity that would carry the electric potential outside the limiting pH, they retard oxidation; in so doing they are indirectly invovlved in preventing "oily" flavor.

BACTERIAL GROWTH AND OXIDATION-REDUCTION

While there is a difference of opinion regarding the ability of organisms to affect the oxidation-reduction potential of milk, most of the evidence points toward their being able to lower it.

Coulter (12) showed that a medium of deaerated sterile culture bouillon drifted from an initial plus 0.250 volts to a minimum of minus 0.060, a marked increase in reducing intensity; the original value was restored upon the re-admission of air, indicating that organisms were not necessary for the change. Using washed yeast suspensions as well as growing cultures of Pseudomonas fluorescens, Bacillus subtilis, Streptococcus lactis, Escherichia coli and Proteus vulgaris in the presence of methylene blue, succinate and glutathione, it was found by Cannon, Cohen and Clark (8) that progressively more negative potentials develop. In some of the earliest studies of the reducing capacity of organisms, Potter (52) measured the E.M.F. produced when platinum electrodes were immersed in two portions of a culture medium separated by a porous membrane, one half cell being inoculated and the other sterile. With yeast and Escherichia coli the inoculated portions were always more reducing than the uninoculated. In a similar study Gillespie (28) compared aerobes to anaerobes, particularly soil types, and found that while the former showed progressively increasing reduction potentials with lapse of time, the latter developed only to a uniform constant.

That the rate of reduction of one organism is affected by another when associative action is involved was shown by Frazier and Whittier (26). Streptococcus lactis for example, was restrained from making a rapid drop in Eh in the presence of gas forming fecal types of organisms. In another study the same

authors (25) concluded that of a wide variety of natural milk contaminats, Streptococcus mastitidis was the only one unable to produce a negative potential. It did not show a reduction in three days. Comparing pH and Eh measurements in this study it was found that between pH4 and 7 the relation to Eh is practically a straight line function, Eh increasing about 0.06 volts for each one point decrease in pH, between 25° and 40° C. If oxygen is diffused into milk at a rate slower than its consumption by a single specie of organism, that organism, according to Hastings, Davenport and Wright (32), would appear to be a non-reducer. The authors felt, however, that in a mixed culture definitely showing reduction, the slow consumer would exert its effect, though slight.

"There is no relationship between oxidized flavors and oxidation reduction potentials in the milk from individual cows. The decreased susceptibility of summer milk to oxidized flavor does not appear to be due to bacteria." This statement by Guthrie and Brueckner (31) was made in view of studies that showed milk with high potentials having oxidized flavor. Summer milk resisted oxidized flavor development even in the presence of a higher potential and its cause was attributed to oleinase not of bacterial origin.

LEUCOCYTES

All milk contains leucocytes (16). Normally a cow has about

100,000 leucocytes per cubic centimeter (19). Horrall (34) examined mastitic udders that showed from 1,200,000 to 9,390,000 leucocytes per cubic centimeter. That high leucocyte counts regularly accompany streptococci infection and high pH, was shown by Plastridge, Anderson and Williams (51). The reason for this according to Baker and Breed (3) is that the infection causes more unmodified blood to enter the udder; blood is alkaline. Fewer leucocytes are held back by filtration. In unhomogenized milk, leucocytes congregate in the cream (2). Trout, Scheid, Peters and Mallmann (67) also observed that leucocytes as well as bacteria accompany fat to the cream layer in unhomogenized milk. In homogenized milk bacteria and fat remained distributed but leucocytes settled to the lower layers in great numbers. Yeasts also settle. Trout and Halloran (66) observed that an increased gravitation of leucocytes occurs in unhomogenized milk that has been heated as high 70° C. This was ascribed to the retarted cream layer formation or to the possible change in charge on the fat globules or leucocytes. Skar (57) has shown that leucocytes may play an important part in bringing about a reducing potential. Peters and Trout (49) (50) studying further the attraction, which they believed to be mutual, between fat and leucocytes, discovered that adding 7.5 grams per pint of washed leucocytes would deepen the cream layer and that 15 grams actually carried some of the fat to the bottom of the bottle. This was well

illustrated by coloring the fat with Sudan III. This attraction was greatest at pH 4.3, the iso-electric point of the fat-serum interface. Tarassuk and Palmer (62) did not specifically attribute the diminished tendency toward oxidized flavor to leucocyte removal in supercentrifuged milk, as they were studying phospholipids in remade milk samples. The work of Peters and Trout (49) would indicate, however, that leucocytes may have been involved.

LECITHIN

In 1940 Brown and Thurston (6) presented an exhaustive literature review on oxidized flavor and stated "the trend of the literature at the present time seems to point to the phospholipid fraction as the source of oxidized flavors in milk and cream." Dahle and Palmer (15) and Swanson and Sommer (61) both reported a reduction of the iodine number of the phospholipid fractions of milk exhibiting oxidized flavor; the latter authors further found no significant difference in the iodine numbers on butterfat from normal and oxidized flavored milks. Whitnah, Martin and Beck (70) concluded that milk, low in lecithin, developed oxidized flavor as readily as that high in lecithin. In order to disengage the phospholipid membrane, sometimes called the hull, from the globule of fat, Thurston, Brown and Dustman (64) employed various homogenizing pressures, violent and prolonged (two and one-half hours) agitation and alternate freezing and thawing on milk. All three practices reduced or eliminated

oxidized flavor development. While still engaged to the fat globules, the phospholipid membrane regularly caused oxidized flavor. This adds weight to another study by the same authors (63) showing that the development of oxidized flavor was a progressive reaction, initiated in the phospholipid fraction and continuing or completed on the butterfat. Whereas in prepared synthetic milks containing only pure butterfat, tallowy flavor was noticed, those containing lecithin had oxidized flavor. The work of Tarassuk and Palmer (62) is confirmatory. Roland and Trebler (55) and Holm, Wright and Deysher (33) partially confirm the work of Thurston, Brown and Dustman (64) by adding that in cream separation there is a change in the distribution of lecithin and related substances between the fat and aqueous phases of the milk system and that this may be responsible for the decreased sensitivity of milk to oxidized flavor. Kurtz and Jamieson (42) demonstrated that the lecithin-cephalin fraction of milk phospholipid is highly oxidizable. After separating true butterfat from phospholipid in 300 pounds of spray process sweet cream buttermilk by repeated acetone precipitations and ether extractions, they analyzed the fatty acid composition of each. The lecithin-cephalin fraction contained none of the lower fatty acids common to true neutral butterfat, but did contain, along with myristic, stearic and arachidic acids, the highly unsaturated dicostretinoic acid in the amount of 6.3 percent and 70.6 percent

of oleic acid. Palmitic acid was not present.

According to Fetzer (23) milk from mastitic cows contains less lecithin than that from normal cows. Horrall (34) reports the opposite. However Dahlberg, Kucera and Hening (13) grouped mastitis into latent and active cases and found that in milk from the former there was no appreciable variation from normal values. In the latter, considerable variations were noted.

ORIGIN OF THE PRESENT STUDY

SPONTANEOUS OXIDIZED FLAVOR IN ASEPTICALLY DRAWN FRESH MILK

During the fall and winter of 1938-39 a study was undertaken to determine if the salty flavored milk from individual quarters of certain cows could be more specifically expressed by chemical analysis than by taste. Individual quarter samples were collected aseptically from 63 cows, milked directly into sterile glass containers. The tasting and salt testing of the warm milk followed immediately. On several occasions 2 or more of the 6 men who were judging flavors, noted that oxidized flavor was present in the milk of some quarters. Though it was known that milk from certain cows was susceptible to becoming oxidized by copper or or iron contamination and by exposure to light, it was not known at the time that strictly fresh uncolled raw milk would have the flavor.

OCCURRENCE OF PIN POINT COLONIES IN AGAR PLATES, FROM SAMPLES OF ASEPTICALLY DRAWN MILK SHOWING AN OXIDIZED FLAVOR

After observing oxidized flavor under the conditions set forth in the salt flavor experiment, it was thought desirable to

study flavors of similar samples of milk before and after pasteurization, and to note also the relationship, if any, between the bacterial counts of the raw and pasteurized samples. Some cows in the herd were known to have mastitis. In fact the salt flavor study had been undertaken partially to determine if a flavor test for salt could be used by the dairymen as an index to infections in the herd. Mastitis cows were excluded in the further study of the oxidized flavor problem and several series of platings were made on raw and pasteurized aseptically drawn samples from non-infected cows. Following the customary 48 hour incubation period for plate counts, one set of plates was permitted to stand at room temperature for one week. Pin point colonies had developed after this period of time in the plates of the raw and pasteurized samples from one cow whose milk had had an oxidized flavor both before and after pasteurization, and in the plates on pasteurized milk only from a cow whose raw milk was normal in flavor but whose pasteurized milk was oxidized. Pin points were not detected in any other plates.

Milk from one of the College herds was pasteurized and bottled separately. It was in this herd that the salt flavor study showed 10 of 63 cows giving milk with an oxidized flavor immediately after milking. Inspection at the Creamery receiving room never showed oxidized flavor in the mixed raw milk but it was uniformly present after pasteurization and after storage at

4.4° C. for 24 to 48 hours. For several years this situation had been casually observed to exist during November and continuing through March.

SUMMARY OF PRELIMINARY OBSERVATIONS

OXIDIZED FLAVOR IN ASEPTICALLY DRAWN FRESH RAW MILK

Cows	Quarters Examined	Oxidized Flavor In Cows	Quarters	Agreement Among Six Judges on Flavor Identity
63	252	10	18	2 or more

PIN POINT COLONIES IN ASEPTICALLY DRAWN MILK

Breed	Oxidized Raw	Past.	Standard Count 48 Hours Raw	Past.	Showing Pin Points After 7 days at 23°C.
Jersey	+	+	320	210	In both raw and pasteurized
Jersey	-	-	280	200	
Jersey	-	-	1230	130	
Holstein	-	+	570	300	In pasteurized only
Holstein	-	-	540	110	
Holstein	-	-	450	200	

THE PROBLEM

A literature survey indicates that other workers do not attribute oxidized flavor to bacterial activity, but rather in most cases to a lack of bacterial activity. The general conclusion of others is that bacteria are unimportant because the total counts in affected samples are uniformly low. When based on conventional standard methods of bacteria enumeration and identification this is

apparently true. In 1939 a new bacteriological medium for the enumeration of bacteria in milk and dairy products became official. This new medium, Tryptone glucose skimmilk extract agar, is responsible for more and larger colonies than is the previously official Standard Nutrient Agar. Even when adopted, however, it was admittedly not a medium adapted to the best development of all the possible types of organisms that might be found in milk and milk products. It has been shown in a number of instances that the intensity of oxidized flavor increases during the normal storage period of market milk; that is, from one day to 4 or 5 days.

Observation of pin point colonies in oxidized flavored milk and the intensifying of the flavor after pasteurization, together with a lack of pertinent data on the bacteriological phases of the problem seemed to justify further investigation in this direction.

SCOPE OF THE INVESTIGATION

The present study was designed to determine:

1. The numbers and types of organisms found in udders of cows whose milk becomes oxidized without the catalyzing effects of metals outside the udder, or by exposure to light.
2. If modifying standard bacteriological practices would result in better growth of pin point colonies.

3. If oxidized flavor can be produced in milk otherwise free by inoculating into normal and multiple-clarified samples, certain udder borne organisms.
4. The efficiency of the oxidase indicator p-phenylenediamine oxalate in classifying organisms associated with oxidized flavor.
5. Dehydrogenase studies of the udder flora.

PROCEDURE

The cows used in these studies were in the two herds maintained at the Agricultural and Mechanical College of Texas. In addition to their milk, mixed patron milk at the College Creamery was used in the work on clarification. Most of the work was done between October 1946 and July 1948. Some of the determinations on the heat resistance of udder borne organisms were made in the laboratories of the Department of Bacteriology and Public Health at Michigan State College during the Spring quarter of 1946.

Samples collected from individual mastitis-free cows were taken after the hind quarters had been brushed and udders washed with cloths soaked in 250 ppm. chlorine solution. The ends of the teats were given a thorough washing with a separate chlorine soaked cloth. The assisting milker dipped his hands into a 250 ppm. chlorine solution and allowed them to air dry before drawing the milk. The fourth stream drawn was placed directly into 9 milliliters of sterile tryptose broth. The tubes were calibrated so that a near 1-10 dilution could be made. There was no practical

difficulty because of foam. Tubes were placed instantly into ice water away from light. Never more than 6 cows were sampled at one milking, and plating or other culture settings were completed within two hours of miling time. Larger samples for laboratory pasteurization, clarification and flavor studies were taken with the same precautions. Sterile one-half pint milk bottles and Erlenmeyer flasks were used to collect them. Laboratory pasteurization was done in the same containers in which the samples were collected. A Precision Scientific Company water bath, equipped with a motor driven agitator, was used.

Plating and counting techniques were those recommended by the American Public Health Association in "Standard Methods For The Examination Of Dairy Products," 1944 (58). Culture media used were those manufactured by the Digestive Ferments Company, Detroit, Michigan. The special media not available from this company were compounded according to their general directions. A Beckman potentiometer was used to adjust all media to pH 6.8.

An udder unfusion broth containing no other nutrient, was prepared by heating six pounds of trimmed udder tissue in distilled water. The filtered broth was brought to three liters volume. The udder used was from a Jersey cow that had passed two lactation periods and had shown no evidence of mastitis. Half of the broth was converted to 1.5 percent agar. Separator slime agar was prepared as a possible source of phospholipid nutrient. No attempt was made to secure pure phospholipid nor to have the

medium completely devoid of other milk constituents except free fat; free fat was removed by alternately washing, agitating and centrifuging hot dilute slime. Agitation was done in a Waring Blendor and centrifuging in an International centrifuge operated 15 minutes at 4000 rpm. After repeating this sequence of treatments four times, a 10 ml. sample of the diluted slime, taken from the top of a centrifuge tube, showed less than 0.0001 grams of fat or less than 0.001 percent, by the Mojonnier fat extraction technique. The resulting liquid, slightly turbid, was used as the sole source of nutrient and was converted to 1.5 percent agar.

One-tenth percent lecithin agar was prepared from soybean lecithin, using tryptose agar (Difco) as a base. The lecithin was first emulsified in one-fourth its weight of Tween 80, the oleic acid ester of a sorbitan derivative, made by the Atlas Powder Company, Wilmington, Delaware. This produced an agar having a slight turbidity, but without a troublesome precipitate after autoclaving.

Oxygen demand of organisms was determined by the usual stab and slant techniques, together with parallel incubation in regular incubators and in carbon dioxide chambers. Preliminary tests showed no difference in effect on growth between a 10 percent and a 25 percent carbon dioxide; 10 percent was used thereafter. It was apparent after a few trials that with the organisms being studied and in consideration of the work of Nunheimer and Fabian (48) on the micrococci, there was no good reason to determine the

need of free versus intramolecular oxygen. Manometric studies were therefore discontinued, but a series of Thunberg dehydrogenation tests were run according to the modification by Umbreit, Burris and Stauffer (69).

Flavor determinations were made ordinarily by three experienced judges. On occasions four or five participated. There was no discussion among the judges until all had made decisions and sample identity was never disclosed until all had finished. The presence and relative intensity or the absence of oxidized flavor was noted, and inidcated by plus or minus signs, according to the scheme of Trout and Sharp (68). By this, +++ indicated a strong to very strong oxidized flavor; ++, distinct to pronounced; +, slight; ?, doubtful and -, no oxidized flavor.

On larger batches of milk, homogenizing and clarifying were done in the Creamery, using a Manton Gaulin two-stage, 200 gallons an hour homogenizer and a DeLaval clarifier of the same capacity. The pasteurizing vat, all connections, all milk contact parts of the machines and the tubular cooler were of stainless steel. Laboratory clarifying was carred out with the International centrifuge on milk pasteurized and homogenized in the Creamery. Organisms inoculated into the homogenized and/or clarified milks were grown in flat bottles and harvested in distilled water. A saline wash was avoided to prevent confusing flavors caused by the organisms; but to avoid the toxic effects of distilled water alone, the organisms were collected and placed into the milks as rapidly as possible. By handling a small number of samples it was uniformly

possible to limit the exposure of organisms to distilled water to less than ten minutes. Control milk samples, uninoculated, indicated that this method was satisfactory.

R E S U L T S

OCCURRENCE OF OXIDIZED FLAVOR IN MILK FROM INDIVIDUAL COWS

To determine if the oxidized flavor of fresh milk from individual cows is a consistent property throughout a lactation period 6 cows were selected for study. Two of these (1039 and 1264) had previously shown it in their milk at once after milking, two (975 and 1083) had not shown it until the milk was held at 4.4° C. for 24 to 48 hours, and the milk of two other (1113 and 1131) did not show it at the end of one week, similarly held. The results shown in table 1 indicate that pasture feeding, previously reported by Brown, Thurston and Dustman (7) Kende (39) and Whitnah, Martin and Beck (70),had an influence. However, the individuality of the cow is a prominent factor. In this connection it should be noted that these 6 cows were selected from a herd then numbering about 95, fewer than 10 percent of which were having oxidized flavored milk before pasteurization. The period of lactation exerts some influence; there were 13 negative judgments during the first half of the study and 9 during the second half. Dahle (14) reported no influence.

Table. 1. The Occurrence of Oxidized Flavor in Milk at Different Stages of the Lactation Period.

Cow No.	Breed	Freshened	Fresh Raw Milk Tested Apr. 28	June 18	Aug. 21	Oct. 12	Dec. 23	Feb. 26
975	Holstein	April 7	-	-	-	-	+	++
1039	Jersey	April 10	+	+	-	-	+	++
1083	Holstein	April 9	-	+	-	+	+	+
1113	Holstein	March 26	-	-	-	-	-	-
1131	Jersey	March 20	-	-	-	-	-	No sample
1264	Holstein	March 8	++	+	-	+	++	Dry

Preparatory to bacteriological studies of individual udders it was deemed advisable to determine the flavors of milk from the same 6 cows before and after pasteurization. The same periods were used as in Table 1 except that no tests were made in February. The testing days were uniformly within one week of those reported in Table 1. Samples were collected directly into glass containers, were cooled at once to 15.5° C. and were protected from light. Pasteurization by the holding method (61.8° C. for 30 minutes) was begun within an hour of milking time. Prompt cooling at 1.7° to 4.4° C. followed in an ice bath. The results are shown in Tables 2a, 2b and 2c.

Table 2a. The Occurrence of Oxidized Flavor in Raw and Pasteurized Milk During One Lactation Period, of Two Cows Whose Milk Had Previously Shown the Flavor Immediately After Milking.*

A, cow 1039; B, cow 1264.

Time	April				June				August				October				December			
	Raw		Past.		Raw		Past.		Raw		Past.		Raw		Past.		Raw		Past.	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
At Once	+	+	+	+	+	?	+	sl.	-	-	-	-	-	-	-	-	+	+	+	+
1 day	+	+	+	++	+	+	+	+	-	-	-	-	+	-	+	sl.	+	+	++	+
2 days	+	++	++	++	+	+	+	+	-	-	-	-	+	+	+	+	++	++	++	++
3 days	+	++	++	+++	+	+	+	++	-	-	-	-	+	+	++	+	++	++	+++	++
4 days	+	+++	++	+++	+	+	++	++	?	-	-	-	+	+	++	+	+++	++	+++	++
5 days	+	+++	+++	+++	+	++	++	++	sl.	-	?	-	+	+	++	++	+++	++	+++	+++

* Observations made April 2-20, 1947

Table 2b. The Occurrence of Oxidized Flavor in Raw and Pasteurized Milk During One Lactation Period of Two Cows Whose Raw Milk Did Not Become Oxidized Until 24 to 48 Hours After Milking, Held at 4.4° C.* C, cow 975; D, cow 1083

Time	April				June				August				October				December			
	Raw		Past.		Raw		Past.		Raw		Past.		Raw		Past.		Raw		Past.	
	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
At Once	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	?
1 Day		-	+	sl.	-	-	-	-	-	-	-	?	-	?	+	+	+	+	+	+
2 Days	+	-	+	+	-	-	-	+	-	-	-	-	+	sl.	+	+	+	+	++	+
3 Days	+	+	+	+	-	-	-	+	-	-	-	-	+	+	+	+	+	+	++	+
4 Days	+	+	+	+	-	-	-	+	-	-	-	-	+	+	+	+	++	+	++	++
5 Days	+	+	++	+	-	-	-	+	-	-	-	-	+	+	+	+	++	+	+++	++

* Observations made April 2-20, 1947

Table 2 c. The Occurrence of Oxidized Flavor in Raw and Pasteurized Milk During One Lactation Period, of Two Cows Whose Raw Milk Had Previously Not Become Oxidized After Holding at 4.4° C. for one Week.* E, cow 1113; F, cow 1131

	April				June				August				October				December			
	Raw		Past.		Raw		Past.		Raw		Past.		Raw		Past.		Raw		Past.	
	E	F	E	F	E	F	E	F	E	F	E	F	E	F	E	F	E	F	E	F
At Once	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 Day		-	-	-		-	-	-	-	-	-	-		-	-	-	-	-	+	-
2 Days		-	-	-		-	-	-		-	-	-		-	-	-	-	-	+	?
3 Days		-	-	-	-	-	-	-	-	-	-	-		-	-	-	?	-	+	+
4 Days	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		?	-	+	+
5 Days	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	sl.	+	+

* Observations made April 2-20, 1947

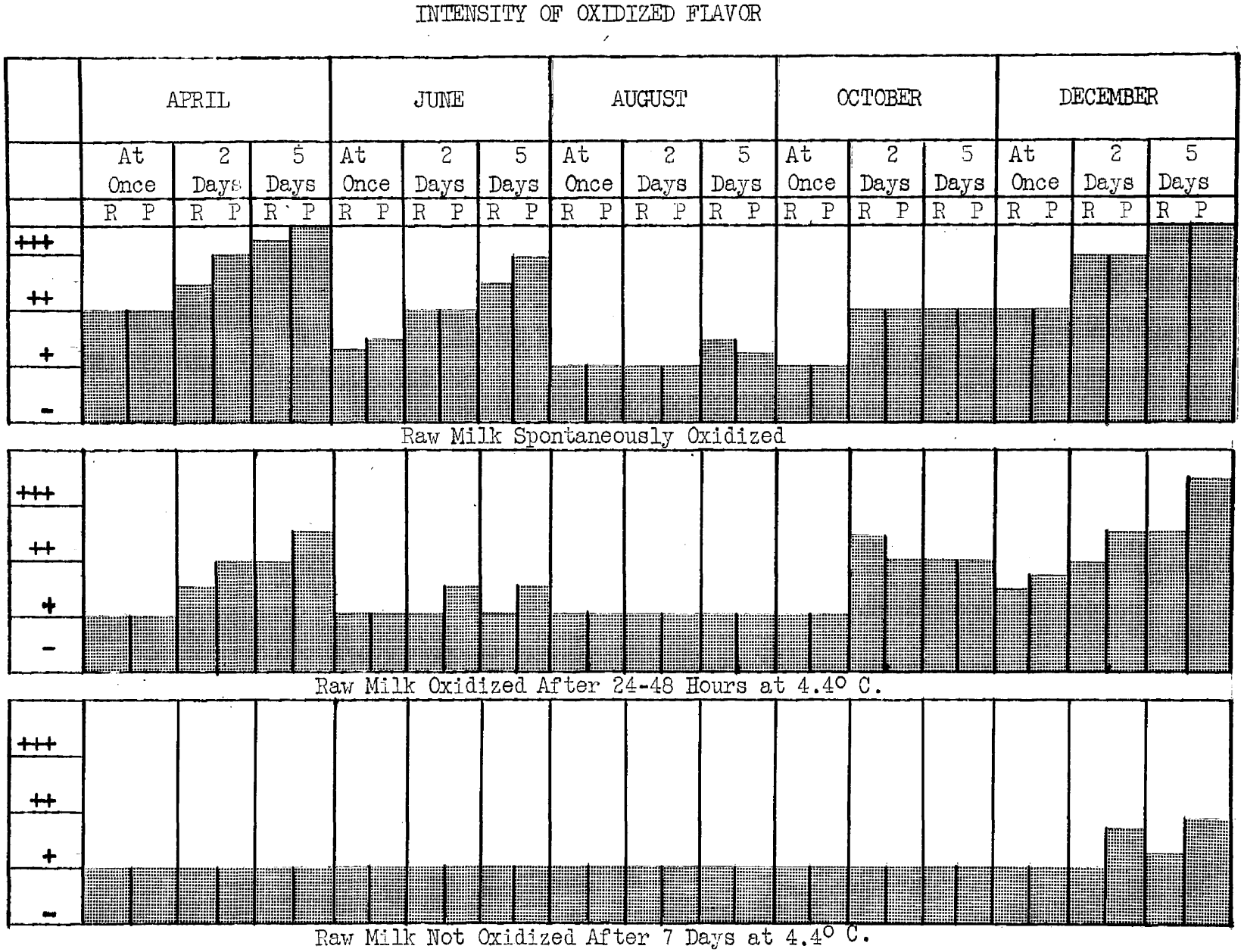

Figure 1. The relation of Oxidized Flavor Intensity in Raw and Pasteurized Milk from Individual Cows, to the Lactation Period and to the Age of the Milk.

The data in Tables 2a, 2b, and 2c further emphasize the influence of oxidized flavor caused by pasture feeding and individuality of the cow. Table 2a indicates that the influence owing to individuality was broken during the pasture season about two months after the cows had passed their peak production. Tables 2a and 2b show that the milk had consistently more intense oxidized flavor with age, both before and after pasteurization. The data in Table 2b strongly indicates the difference in protective action against flavor development, for whatever reason it may have been, between milks that oxidize spontaneously and those that do not show it until 24 to 48 hours after milking. Table 2c again shows the effect of individuality and perhaps a slight weakening (see December) of the protective action.

Figure 1 is a summary of Tables 2a, 2b, and 2c.

Another flavor study was made to find the effect of holding raw for 24 hours, the milk of the same 6 cows, before pasteurizing. The results show the same general pattern for the three groups of cows as was shown by the more extensive study that covered an entire lactation period, but emphasize that there is an increased tendency toward becoming oxidized, caused by the aging period of 24 hours, held at 4.4° C. Table 3 presents a typical trial of four such trials made early in May. It will be noted in Figure 1 that the occurrence of oxidized flavor was pronounced at this time for the first two groups of cows.

Table 3. The Effect on Oxidized Flavor Development in Pasteurized Milk Owing to a 24 Hours Aging Period of the Raw Milk. One of 4 Trials.

Cows	Flavor Intensity													
	Previsouly Fresh After Pasteurization Following 24 Aging at 4.4° C.													
Oxidized Spontaneously	Raw	At once		1 day		2 days		3 days		4 days		5 days		
		R	P	R	P	R	P	R	P	R	P	R	P	
1039	+	+	+	++	++	++	++	+++	+++					
1264	+	+	+	++	+++	+++	+++							
Previously Oxidized after 24 to 48 hours														
975	-	-	?	-	sl. +		+	+	+	+	++	++	+++	
1083	-	-	-	-	+	-	+	+	++	+	++	+	++	
Previously Not Oxidized After 1 week														
1113	-		sl. heat	-	sl. heat	-	-	-	-	-	-	-	**	
1131	-	-	-	-	-	-	-	-	-	-	-	-	*	

*Not Oxidized After 10 Days

** Flat, stale

COLLECTION OF SAMPLES FOR BACTERIAL ANALYSIS

It was noted earlier that in a plating trial, one series of plates stood for one week at room temperature, about 23° C., after the 48 hour incubation period at 37.5° C. Pin point colonies deep in the agar had developed by this time in the plates of both the raw and pasteurized samples from one cow and in the pasteurized sample from another cow.

Other plates were free of pin points and some were too crowded with other growth for accurate detection of pin points. The pasteurized milk from both of these cows was oxidized after holding at 4.4° C. for 2 days. Raw milk from the first cow was oxidized as soon as it was drawn. This chance observation suggested that an investigation into the relationship of bacteria and oxidized flavor might be valuable. These immediate problems were suggested: The possible need for drawing milk samples aseptically directly into a nutrient medium rather than into plain tubes, to permit an unbroken growth cycle; the possible need in the agar medium of nutrients or accessory substances to develop the pin points into larger colonies; incubation temperatures; frequency of occurrence of pin point colonies in milk subject to becoming oxidized; relation of the pin points to other udder borne organisms; identification, if possible, of the pin points.

Owing to its well known ability to develop fastidious organisms, tryptose broth (Difco) plus 0.1 percent glucose was chosen for use in collecting samples. Samples collected into sterile tubes and into tubes of sterile broth were compared. In this way, exact duplication of samples was impossible from a single quarter at one milking but the experiment was repeated 24 hours later, reversing the order of placing the fourth and fifth streams drawn. The use of a broth collection tube did not increase the size of pin point colonies. It did, however, raise the total count; it also gave a higher percentage of the total count after the first 24 hours of the 48 hour incubation period. There was no relation between the

magnitude of the total count and the percentage increase caused by collecting the samples directly into broth. (See Appendix, pp. i, ii) Subsequently all aseptically drawn samples from individual quarters collected for bacteriological examination were collected directly into tryptose broth.

As a preliminary to employing modifications of standard procedures, three plating trials were run, having all conditions standard, except that incubation was continued and plates were re-counted after 4 days and 6 days. Standard conditions require tryptone glucose skimmilk extract agar and incubation at 37.5° C. for 48 hours only. The results in Table 4 confirm the previously held opinion that, except for pin point colonies, the milk from the cows under observation was of good quality, bacteriologically. The pin points that developed were noted after 96 hours incubation.

The influence of holding the raw aseptically drawn milk, on the total standard count and upon the incidence of pin point colonies, was determined. The results are shown in Table 5. There is a general indication that pin points were more numerous in low count samples, though none of the counts could be considered high, and that they occurred in samples showing a declining count as the milk aged. Plating the samples after 1, 2 and 3 days had an inconsistent effect on the total count but it is significant that the two cows whose milk was consistently not oxidized (1113 and 1131), showed slight, steady increases in count as the milk aged. This agrees with the opinion held by Mattick (46) that all bacteria are probably capable of effecting a reducing action. Owing to the low magnitude

of all of the total counts and to the impracticability of counting pin point colonies accurately, it cannot be concluded from the data that there is a positive relationship between the two.

Table 4. Standard Bacteria Counts and the Incidence of Pin Point Colonies in Aseptically Drawn Milk and Counts After Extended Incubation.

Cow	Trial	Bacteria Counts: Standard Count After 48 hrs.	P.P.	96 hrs.	P.P.	144 hrs.	P.P.
1039	1	70	-	180	+	210	++
	2	110	-	230	-	260	+
	3	30	-	40	+	60	++
1264	1	1100	-	1400	-	1200 ?	-
	2	700	-	1000	+	1600	+
	3	200	-	370	-	380	-
		Raw Milk Spontaneously Oxidized					
975	1	310	-	380	-	410	-
	2	170	?	190		190	+
	3	740	-	860	-	900	-
1083	1	250	-	380	-	Spreader	-
	2	900	-	1230	-	1210 ?	-
	3	1200	-	1600	-	2000	-
		Raw Milk Oxidized After 24-48 Hrs. at 40° F.					
1113	1	1350	-	1380	-	1380	-
	2	1600	-	2010	-	2040	-
	3	860	-	860	-	910	-
1131	1	760	-	790	-	830	-
	2	1390	-	1420	-	1420	-
	3	520	-	700	-	740	-

Raw Milk Not oxidized After 7 days at 40° F.

Table 5. The Effect of Holding Aseptically Drawn Raw Milk at 4.4° C. Before Plating on the Standard Plate Count and on the Incidence of Pin Point Colonies

Cow	Time Plated							
	Within an Hr. of Milking		After 24 Hrs.		After 48 Hrs.		After 72 Hrs.	
	Count	P.P.	Count	P.P.	Count	P.P.	Count	P.P.
1039	150	-	170	-	210	+	140	+
1264	2150	-	2020	-	2000	-	2300	-
975	370	-	300	-	330	+	190	+
1083	600	+	520	+	580	+	560	+
1113	600	-	900	-	1280	-	1900	-
1131	1270	-	930	-	1400	-	5180	-

COUNTS EMPLOYING MODIFIED AGARS

An agar medium containing udder infusion as the only source of nutrient was used in a plating trial on samples of milk which had previously contained pin point colonies in T.G.E.M. agar. This infusion agar supported no bacterial and was given no further consideration. Its use was attempted on the assumption that some nutrient substance that supported growth within the udder was lacking in T.G.E.M. agar. If such a nutrient were in the infusion it was appartnely destroyed by autoclaving or could not support growth alone.

PHOSPHOLIPID ENRICHED AGARS

Since the exhaustive literature review reported by Brown and Thurston (6) led them to conclude that oxidized flavor originated in the phospholipid fraction, agars fortified with this material were used for determining the effect on pin point growth. While, as shown previously, there was no definite relation between countable organisms

and the incidence of oxidized flavor, there was a slight indication that in both raw and pasteurized milks from certain cows whose milk was oxidized, that pin point colonies involved.

Throughout the course of the study colonies were picked from plates at various times until a collection of 40 cultures were collected. They were selected on the basis of color principally, according to Hucker (35), and Dorner (17) but particular attention was given to isolating and culturing the gray to white pin points that uniformly grew only deep in the agar plates. All of the organisms that were obviously aerobic grew well on stock culture agar (Difco) slants, some proved to be facultative in stabs but the pin point colonies would not grow under either condition. The use of CO_2 Chambers (10 percent) was of no value in increasing the size of the pin points. This was determined with pour plates and streak plates of tryptose agar prepared in duplicate from broth cultures and incubated at 37.5° and 20° C. The broth cultures of all organisms except the pin points were ready for use after 18 to 24 hours. The pin point broth cultures however did not show sufficient sediment until 48 to 72 hours.

A short study of the effect of adding whole fresh egg yolk to tryptose agar proved that the pin points needed nutrient, or perhaps a combination of nutrients, not supplied in tryptose agar alone. The yolk of one fresh egg was well washed, the yolk sac removed and the yolk only added to 500 ml. of tryptose agar. The heavy precipitate brought down by autoclaving was effectively re-

moved by filtering. Three pin point broth cultures were chosen for study, together with 3 cultures of representative micrococci, and an alkaline milk digesting gram variable rod that grew a pink surface colony on T.G.E.M. There was a definite increase in the size of all colonies except two. The yellow micrococcus colonies were no larger on the yolk-enriched medium. The gram negative rod failed to grow on it.

The egg yolk possibly furnished nutrient other than phospholipid that promoted larger colonial growth of the micrococci. The results are shown in Table 6.

Another similar study using separator slime gave about the same results. The slime was washed, shaken and centrifuged several times until it was essentially freed of free fat. This, however, still left protein, lactose and mineral accompanying the phospholipid.

Pure soybean lecithin was incorporated into tryptose agar by first emulsifying it in one-fourth its weight of Tween 80, the oleic acid ester of a polyoxyethylene derivative of sorbitan. Tween 80 was regarded as the best of the Tween series because the fatty acid content of lecithin is predominately oleic also, 70.6 percent according to Kurtz and Jamieson (42). There was no apparent difference between agars containing 0.1 percent and 0.2 percent lecithin. One-tenth percent was used following this observation.

After the selection of the original six cows used throughout this study, routine tests on a 4 year old Holstein cow, number 1279, showed that milk from her right front and left rear quarters was consistently higher in total count than that in the other two quarters.

Pin point colonies had frequently been noticed in the right rear and left front quarters and raw milk from these two quarters was oxidized after holding at 4.4° C. for 24 hours. Milk from the right front and left rear did not become oxidized when similarly held. (See Plate 3). Four plating trials on milk from the 4 quarters of this cow were run, using tryptose agar, tryptose agar plus 0.15 percent Tween 80, and tryptose agar plus 0.1 percent soybean lecithin emulsified with Tween 80.

Table 6. The Influence of Enriching Tryptose Agar with Egg Yolk on the Colony Size of 7 Selected Udder Borne Organisms, 24 hrs. at 32° C.

Morphology	Colony Color	B. C. P. Milk	Previous Tryptose Agar Growth	Tryptose Agar	Tryptose Agar Plus Egg Yolk
Tetrad	Gray White	-	Deep Pin Points	Deep P.P.	Deep 1 to 2 mm.
Tetrad	White	Sl. acid	Deep Pin Points	Deep P.P.	Deep 1 to 2 mm.
Micro. Packets	Gray White	-	Deep Pin Points	Deep P.P.	Deep 1 to 2 mm.
Micro Packets	White	Acid Digester	Surface 3 - 5 mm.	3-5 mm.	4 to 6 mm.
Micro Single & Prs.	Yellow	Sl. acid	Surface 2 - 4 mm.	2-4 mm.	2 to 4 mm.
Micro Single & Prs.	Orange Red	Sl. alkaline	Surface 4 - 5 mm.	4-5 mm.	5 mm.
Short Rod	Pink	Alkaline Digester	Surface 2 - 3 mm.	2-3 mm	No Growth

Table 7. The Effect of Soybean Lecithin on the Development of Organisms in Aseptically Drawn Milk from One Cow. Four Trials.

Quarter	Trial	Tryptose Agar		Tryptose Agar plus 15% Tween 80		Tryptose Agar plus .1% Lecithin with Tween 80	
		Distinct Col.	P.P.	Distinct Col.	P.P.	Distinct Col.	P.P.
R F	1	4960	-	5300	-	6200	-
	2	4020	-	4600	-	5300	-
	3	8400	-	7500	-	8700	-
	4	15000	-	167000	-	17200	-
R R	1	0	+	50	few	60	few
	2	0	+	110	+	80	few
	3	230	-	200	-	270	-
	4	90	+	40	-	300	-
L R	1	2880	-	3500	-	5100	-
	2	2090	-	2200	-	3600	
	3	1860	-	2700		4600	-
	4	3500	-	3300	-	5800	-
L F	1	0	+	0	+	30	+
	2	0	+	20?	+	70	few
	3	120	-	50	-	140	-
	4	30	+	0	few	120	few
Average		2699		2829		3598	

+ = too numerous to count

few = scattered but estimate difficult

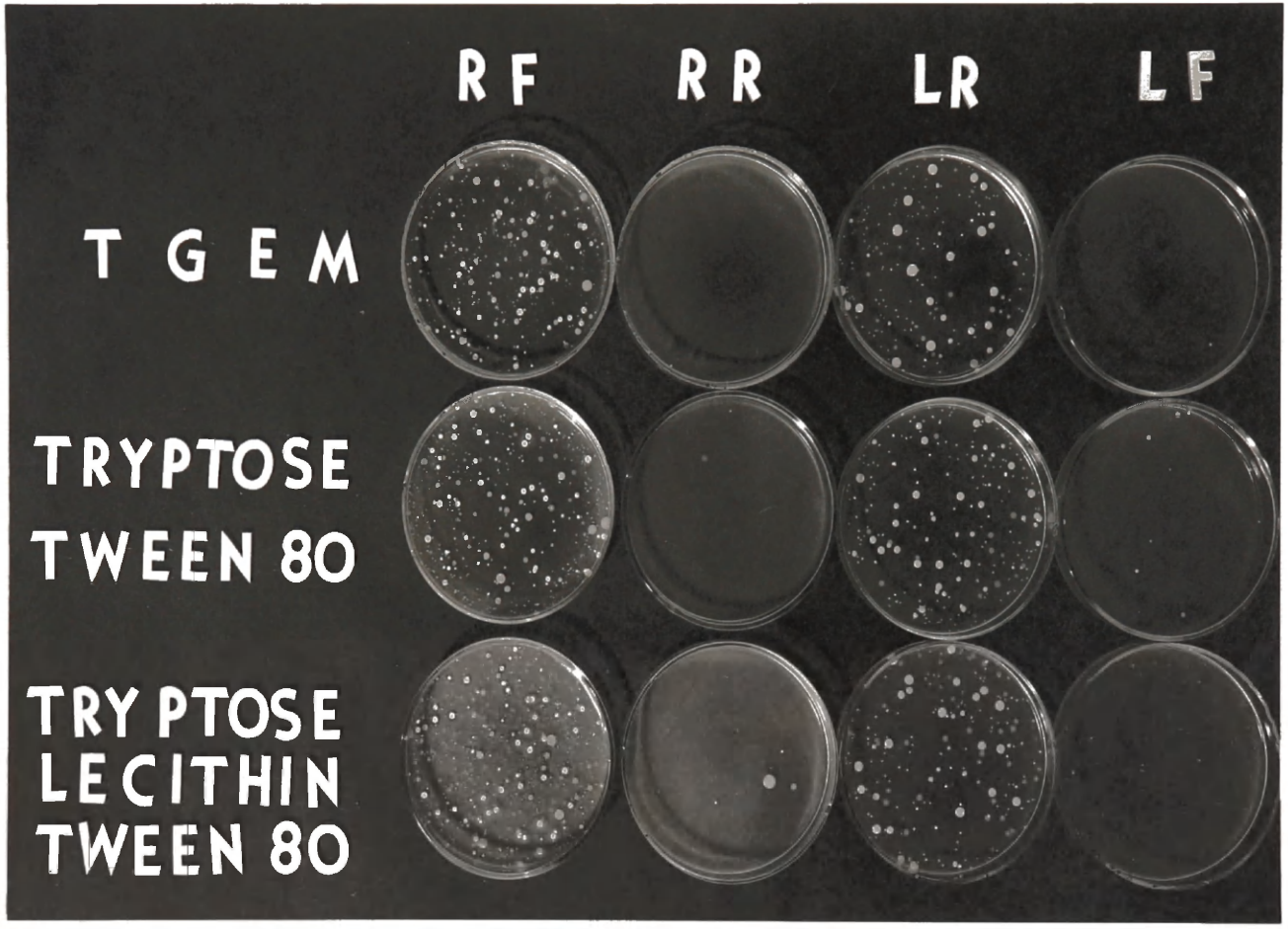

Plate 3. Showing the distribution by quarters of organisms in aseptically drawn milk from a 4 year old Holstein. Fresh raw milk from the left front quarter was regularly oxidized in flavor. While Tween 80 (oleic acid) grew a few larger colonies there was a much higher count in the Tween 80 - lecithin agar in this quarter. The large white and yellow micrococci common to the right front and left rear quarters were completely absent in the left front quarter. This unusual distribution shown was typical of several platings made on this cow. All total counts are highest in lecithin agar.

Lecithin provided better growth than Tween 80 in 14 of 16 cases. Tween 80 provided better growth than the basic Tryptose agar in 9 of 16 cases. Six of these 9 cases occurred in the right front and left rear quarters, both of which were free of pin points. It has been shown by Williams, Brequist and Snell (71) that oleic acid favors certain organisms and may be toxic to others, depending somewhat on the concentration used. This suggests that organisms from the right rear and left front quarters may have been inhibited by oleic acid but that this inhibition was overcome by some substance in the whole lecithin. Even though this possible explanation is based on results obtained from the milk of one cow, it is supported by the fact that in the herd as a whole, the variety of organisms from individual quarters was distinctly limited. Frequently not more than two different organisms, as could be identified macroscopically, were seen. Occasionally as many as four were seen. The variety from all four quarters of one cow, however, reached 6 - 10 frequently and 12 to 14 rarely. One particular cow not used in this study consistently showed very high counts of Micrococcus caseolyticus in all four quarters. Rarely was any other organism seen.

The work of Dorner (17) substantiates this view, in part, though he grouped together the "white cocci", "yellow cocci", "streptococci of the long chain type" and "rods of the Bacterium lipolyticum type". Differentiation of white and yellow cocci particularly can be made further by colonial characteristics other than color. Because of the careful selection of cows used in the present study streptococci were

not encountered. Very few rods of any kind were ever encountered and none of these were fat splitting. Consistent with Dorner's results, however, as well as those of Copeland and Olson (11) the micrococci predominated (see appendix p. iii).

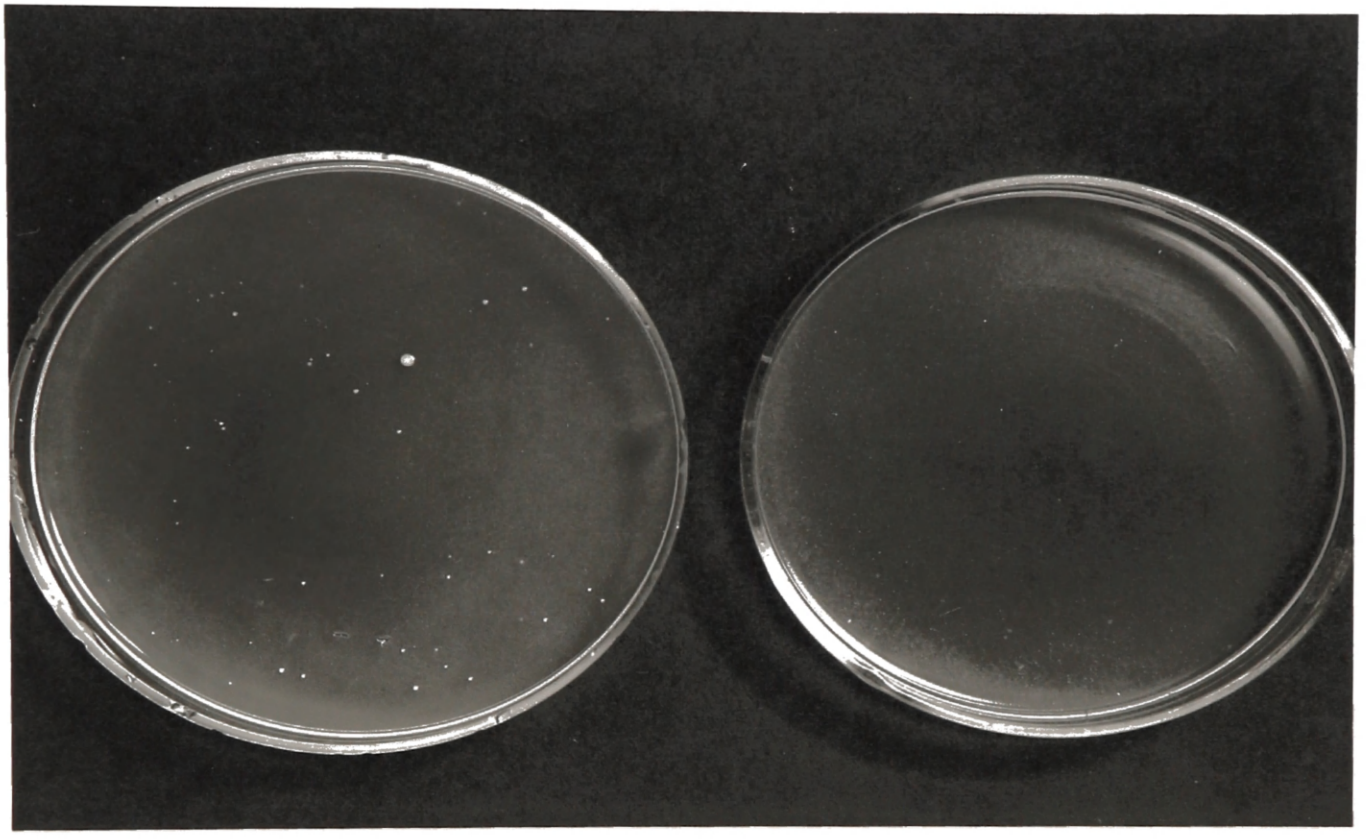

Plate 1. Comparing growth of an organism similar to Gaffkya tardissima on lecithin-fortified tryptose agar (left) and on tryptone glucose skimmilk agar (right). On the former some colonies are on the surface while on the latter all (pin points) are sub-surface.

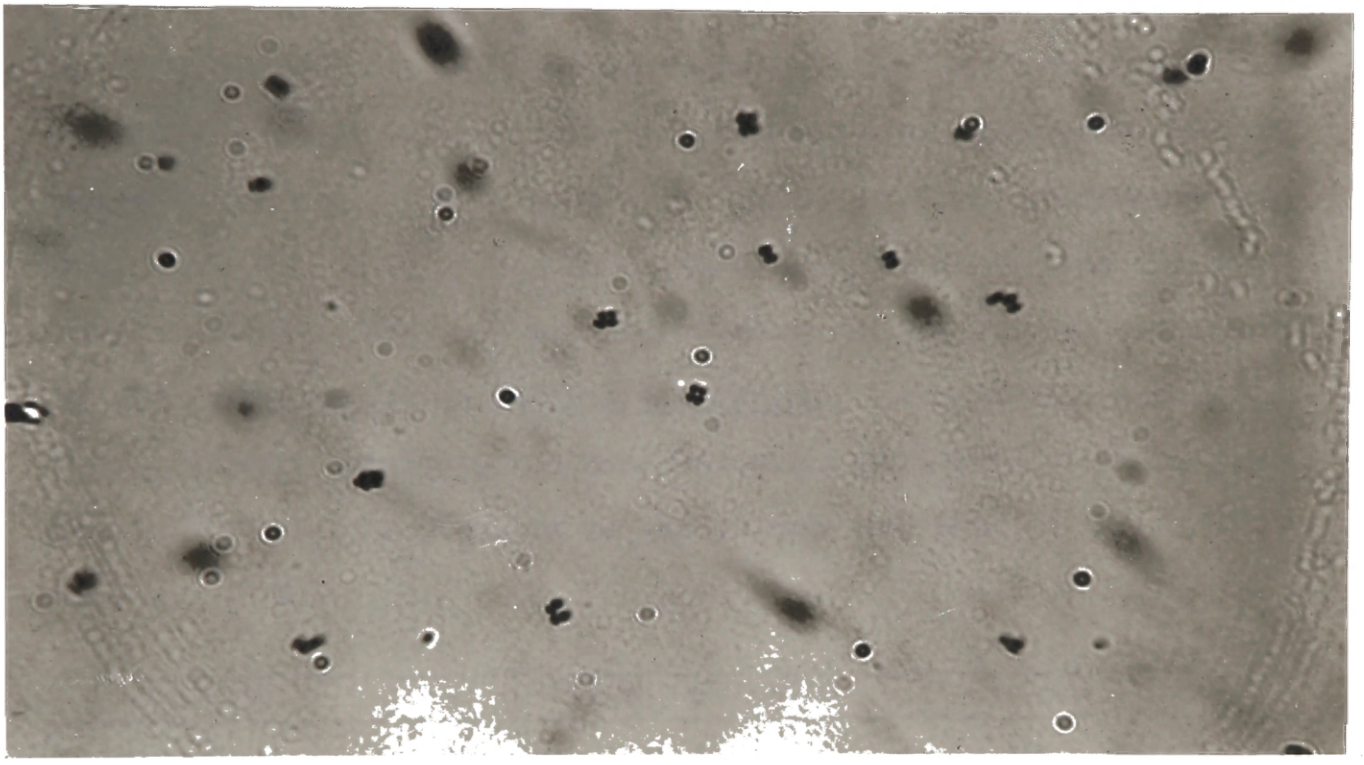

Plate 2. Showing the tetrad similar to the Bergey Manual description of Micrococcus Gaffkya tardissima. The two best focused are near the center.

INOCULATION OF MILK AND MILK FRACTIONS WITH AN ORGANISM SIMILAR TO GAFFKYA TARDISSIMA

Bergey's Manual (4) was consulted for a description of the organism that had formed pin point colonies deep in T.G.E.M. medium and Tryptose agar culture dishes but which proved to be facultative when grown in lecithin agar (see Plate 1). Its properties did not completely match those of any described in the Manual but were closest to those of Micrococcus Gaffkya tardissima. It resembled also Micrococcus Gaffkya anaerobia but was distinctly different in two outstanding properties: It produced no gas in agar and grew better at 20° C. than at 37° C.

Property	Gaffkya tardissima	Isolated Pinpoint
Shape	Small oval	Small oval
Arrangement	Grouped in fours	Grouped in fours
Capsulated	Yes	Yes
Gram	Positive	Positive
Gelatin colonies	Very small, circular brownish by reflected light; coarsely granular under microscope	No growth on gelatin
Gelatin stab	Very slow and poor development, no liquefaction.	Very slow and poor development
Agar colonies	Very small, white, granular circular, entire	Very small, white granular, erose
Broth	Fine, granular sediment	Fine granular sediment
Litmus milk	Unchanged	Unchanged
Potato	No visible growth	No visible growth
Indol	Not formed	Not formed
Dextrose	Not fermented	Not fermented
Aerobe or Anaerobe	Aerobic, facultative	Anaerobic
Temperature	Optimum 37° C.	Optimum 20° C.
Habitat	Natural infection in guinea pigs	Cow's Udder

Large quantities of an aqueous suspension of the pin point organism were prepared from rinses in flat bottles. The organisms could not be harvested from the surface of the agar, but by shaking and subsequent aseptic filtering through a loose sterile cotton pad, the agar particles were removed. The turbidity of the filtrate was adjusted with sterile distilled water so that the addition of 5 ml. to one-half pint of product amounted to an increase of about 10,000,000 organisms per ml. of product.

Samples of Winter and Summer milk were used to prepare raw and pasteurized 25 percent cream, 4 percent milk, skimmilk, 4 percent milk clarified once, 4 percent milk clarified twice and 4 percent milk clarified three times. For each trial one set of samples was made from milk known to be susceptible to becoming oxidized in flavor; another set was made from milk known to be non-susceptible to becoming oxidized. Table 8 is a summary of the results of inoculating the various products and examining them for the occurrence and intensity of oxidized flavor after holding at 4.4° C. for 72 hours. The details for all trials, 5 on susceptible and 3 on non-susceptible milk are shown in pages IV and V of the Appendix.

Table 8. The Occurrence and Relative Intensity of Oxidized Flavor in Milk and Milk Fractions Inoculated with Organism Similar to Micrococcus Gaffkya tardissima; Summary of 8 Trials.

Product	Summer				Winter			
	Susceptible		Non-Suscep.		Susceptible		Non-Suscep.	
	Raw	Past.	Raw	Past.	Raw	Past	Raw	Past.
25% Cream	-	-	-	-	-	+	-	-
4% Milk	-	-	-	-	+	++	-	-
Skimmilk	-	-	-	-	-	-	-	-
4% Milk Clarified Once	-	-	-	-	+	++	-	-
4% Milk Clarified Twice	-	-	-	-	+	++	-	-
4% Milk Clarified 3 Times	-	-	-	-	+	++	-	-

These results indicate that the organism, though originally isolated from quarters of cows whose milk became oxidized, was unable to initiate the flavor in any of the samples inoculated. It did however increase the intensity of the flavor in those milks that were already oxidized or subject to becoming so. The fact that clarified raw susceptible milk became somewhat more oxidized than the non-clarified, can possibly be explained by the removal of leucocytes. There was no apparent advantage of multiple clarification over single clarification, if the removal of leucocytes was the controlling factor.

It was never possible to produce oxidized flavor in pasteurized homogenized milk as prepared commercially in the creamery, with inoculations of the organism described above. Several trials on this milk, both clarified and non-clarified were made during the winter only. When inoculated and uninoculated samples were exposed to direct sunlight for one hour, only the typical sunlight flavor developed.

HEAT RESISTANCE OF UDDER BORNE ORGANISMS

Page vi in the Appendix shows the numbers of organisms per ml. surviving pasteurization in 8 random aseptically drawn milk samples. While the average counts at both incubation temperatures 20° C. and 37° C. were lower than for corresponding counts in raw milk samples similarly collected (appendix page vii) the percentage reduction of counts caused by pasteurization was very low. This suggested that udder borne organisms were thermoduric, that the milk medium served as a protectant against heat, or that high percentage reduction was not realized on samples kept free of contamination outside the udder. By heating separately several isolated species of organisms in broth and in milk it was found that none of the organisms was thermoduric and that milk did not serve as a protectant. Apparently, pasteurization of the original milk samples within an hour of the time the cows were milked came at a time when the growth of organisms was more or less static. At this stage they are more difficult to kill. Table 9 shows the effect of heating separately several selected organisms in the presence of tryptose broth and in sterile skimmilk. Broth cultures

of each organism were transferred every other day while this study was in progress. Because of the slow growth of some of the micrococci in broth, cultures were not transferred to the tubes of milk and broth until after 36 to 48 hours of incubation. The holding temperature of pasteurization, 61.8°C., was used and inoculations made at 5 minute intervals into tryptose broth. Final judgment was not passed on the presence or absence of growth in the broth tubes inoculated from heated cultures of broth and milk until after 36 to 48 hours incubation. This extended incubation period was necessary because of the property of several of the micrococci to exhibit growth in the form of a viscous or granular sediment, while showing a clear broth above.

All of the udder borne organisms shown in Table 9 were gram-positive except the short rod that formed a pale white colony; it was gram-variable. No gram-negative organisms were found in the isolations. These heating tests on single organisms are at variance with those on fresh milk. This was probably caused by the different stages of the life cycle at which the organisms in fresh milk and in broth cultures were heated.

Even though the organisms were subjected to heat after a 36 to 48 hour incubation period, most of them were probably still in the logarithmic growth phase. The slow development of turbidity and/or sediment evidences this.

Table 9. Time Resistance to 61.8°C. of Udder Borne Organisms in Milk and Broth. Figures Indicate the Number of Instances Among 8 Trials that the Organism Survived.

Organism	Heated in Milk						Heated in Tryptose Broth					
	Minutes						Minutes					
	5	10	15	20	25	30	5	10	15	20	25	30
White micrococcus	8	2	0	0	0	0	8	3	1	0	0	0
White tetrad (surface)	0	0	0	0	0	0	0	0	0	0	0	0
Gray tetrad (deep pin p.)	8	4	2	1	0	0	8	2	1	1	0	0
Pale white short rod	8	3	0	0	0	0	8	2	0	0	0	0
Buff micrococcus, ropy	5	3	1	0	0	0	6	2	0	0	0	0
Yellow micrococcus	0	0	0	0	0	0	2	0	0	0	0	0
Yellow short paired rods	0	0	0	0	0	0	1	0	0	0	0	0
Orange micrococcus large	0	0	0	0	0	0	0	0	0	0	0	0
Orange micrococcus small	3	0	0	0	0	0	2	1	0	0	0	0
Pink short rod	2	1	1	0	0	0	1	1	1	0	0	0

To determine if any of the organisms heated (Table 9) and surviving 10 or more minutes were made more heat resistant, the longest heated broth tubes showing sediment and/or turbidity after 72 hours incubation were again heated similarly. Table 10 shows that the initial heating did not develop a thermal resistance in any of the organisms. All of them withstood 61.8°C. for the same time or less during the second heating. Since, in the first experiment on heat resistance a milk

medium appeared to offer no protection, only tryptose broth was used in the second experiment.

Table 10. Time Resistance to 61.8°C. of Organisms Previously Withstanding the Same Temperature for 10 or More Minutes in Milk or Tryptose Broth.

Organisms Which Previously Withstood 61.8°C. for Minutes in			Resistance to 61.8°C. on Second Heating for Minutes in Tryptose Broth					
	Tryp. Broth	Milk	5	10	15	20	25	30
White Micrococcus	15	10	+	+	+	-	-	-
Gray tetrad (deep Pin P.)	20	20	+	+	+	+	-	-
Pale white, short rod	10	10	+	-	-	-	-	-
Buff Micrococcus, ropy	10	15	+	+	+	-	-	-
Orange Micrococcus, small	10	5	-	-	-	-	-	-
Pink, short rod	15	15	+	+	+	-	-	-

USE OF THE OXIDASE INDICATOR P-PHENYLENEDIAMINE OXALATE

On the strength of the recommendations made for detecting oxidizing organisms by Castell and Garrard (10) and Carpenter, Suhrland and Morrison (9), tests on all isolated udder borne organisms were run. For reference, plates of E. coli were prepared, in order to contrast a typical gram negative organism with the gram positive and gram variable udder organisms. Pure culture plates were flooded with a 1 percent aqueous solution of p-phenylenediamine oxalate and were observed over a period of several hours. This confirmed the opinion of Castell and Garrard (10) that gram positive organisms are poor oxidizers. Whereas

the E. coli colonies quickly turned pink and progressively purple to black in 30 to 40 minutes, the udder borne organisms changed slightly or not at all during 4 to 5 hours. It was observed that the indicator solution did not wet the surface of some colonies. To overcome this, aqueous and saline suspensions were prepared and the indicator was added to the suspensions in test tubes. Slight degrees of variation in shades of pink color were noted immediately after mixing but within a few minutes the differences were too slight to detect. When these tubes were compared in a Klett Summerson photoelectric colorimeter it was observed that the deeping of pink color with time, was more a function of the instability of the indicator than a measure of oxidation by the organisms. Since the indicator was prepared immediately before use, in double distilled water (glass), its use as a measure of the variation in oxidizing activities of udder borne organisms can not be recommended.

DEHYDROGENATION BY UDDER BORNE ORGANISMS

Nunheimer and Fabian (48) have shown that the micrococci vary widely in their ability to dehydrogenate methylene blue when in the presence of different substrates. While their work included the use of specific carbohydrates, alcohols and amino acids, substrates of particular interest in this study were whole lecithin and oleic acid, owing to the increase in growth in agar plates on these materials. It seemed to be relatively unimportant whether any fractional part of lecithin other than oleic acid was the activating substance, particularly for the organisms that were difficult to cultivate (pin points). In

a series of tests on methylene blue reduction, however, choline was used as a substrate also to determine if the rate of reduction were increased after its liberation from lecithin. The modified Thunberg technique recommended by Umbreit, Burris and Stauffer (69) was used. This differs from the original in that the cell suspension is added directly to the substrate and methylene blue mixture in an ordinary test tube. Oxygen diffusion is prevented except in the upper layers of the tube by the addition of a buffered 2 per cent agar. The agar solidifies the entire mass just prior to incubation. Reduction is noted in the lower parts of the tube.

Table 11. A Comparison of the Methylene Blue Reducing Ability of Udder Borne Organisms in the Presence of Lecithin, Oleic Acid and Choline at 35°C. Average of 5 Trials

Organism	Reduction Time Minutes			
	No Substrate	Lecithin	Oleic Acid	Choline
1. White, Micrococcus	420	205	185	530
2. White tetrad (surface)	206	215	191	224
3. Gray, tetrad (deep, pin point)	40	26	47	72
4. Pale white, short rod	35	30	20	43
5. Buff, Micrococcus, ropy	140	108	115	130
6. Rose, Micrococcus, uncommon	432	462	335	588
7. Yellow, short paired rods	260	135	121	278
8. Orange, Micrococcus, large	39	36	33	47
9. Orange Micrococcus, small	170	190	163	254
10. Pink, short rod	152	90	95	176

Of the 10 representative organisms studied, 7, including the unclassified tetrad (No. 3), reduced methylene blue in the presence of lecithin faster than they did with no substrate. Seven of them were more active on oleic acid than on lecithin; the unclassified tetrad was not in this group. All but one organism, reduced more slowly in the presence of choline than with no substrate, and in all cases the entire group was slower in the presence of choline than in lecithin or oleic acid. Hastings, Davenport and Wright (32) felt that all organisms in milk were reducing and that even a very slow reducer contributed its effect to the total reaction. It was pointed out earlier that there were very few species of organisms found in the separate quarters of the cows used in this study, and further (see Plate 3) that the pin point colony of the tetrad similar to Gaffyka tardissima was at times the only organism found in quarters showing milk with an oxidized flavor. Because of a lowered Eh in milk having a mixed flora and a high leucocyte count as well, it is conceivable that the effect of a single organism would be unnoticed. In this case, the ability of the tetrad to utilize lecithin, or a component of lecithin, would be inhibited or prevented.

DISCUSSION

Market milk without an oxidized flavor can be uniformly produced. Regardless of the breed of cows, their feed, the season, stage of lactation, copper or iron content of the milk, the character or numbers of bacteria, enzymes of non-bacterial origin or the chemical composition

of the milk, the trade practice of homogenizing and in some cases the use of antioxidants or high heat treatment prevents oxidized flavor. Even so, there is every reason to believe that a market for raw milk and non-homogenized pasteurized milk will continue. As sanitary practices have improved and as the federal, state and city governmental control agencies have striven more intently to prevent the sale of milk from diseased cows, oxidized flavor has become more of a problem. For the distributor who is compelled to market all or part of his supply as raw or non-homogenized pasteurized, or for whom the high temperature short time method of pasteurization is impractical, or the use of antioxidants illegal, the solution to the oxidized flavor problem may be a matter of selecting milk on an individual cow basis. This has been necessary before in order to produce milk within certain bacteria count limits, to maintain a minimum fat or total solids percentage, to control color, and in some cases to avoid off flavors.

SUMMARY

The occurrence and intensity of oxidized flavor in the milk of 3 pairs of cows was established. The pair whose milk was oxidized immediately after being drawn exhibited the defect, as has been shown by several previous investigators, more prominently in winter than in summer, more prominently in pasteurized than in raw milk and more prominently when their milk was pasteurized after an aging period of 24 hours.

The second pair of cows, chosen for study because their milk did not become oxidized until it was held for 24 hours or more, showed the same tendencies as the first pair, though the rate of flavor development was slower.

The third pair of cows, a control pair, had not previously shown oxidized flavor in their milk after it was held for one week. This pair stood apart, distinctly, from the other two throughout the entire study in that their milk was oxidized for a short period only during the winter and then, not as intensely so, before or after pasteurization.

Of primary interest was the bacteriological picture of the milk from these 3 pairs of cows because standard plate counts on milk of three of them in the first two pairs had shown pin point colony development when the plates were incubated beyond the standard 48 hour period. The pin points did not develop at all when an udder infusion was used as the sole source of nutrient. Agars fortified with egg yolk and with separator slime were used to provide a source of phospholipid. Larger total counts resulted on these agars but the pin points did not grow appreciably larger. Finally, pure soybean lecithin was used as an enrichment in Difco Tryptose Agar. This not only developed the pin points into larger colonies but let them develop on the surface of the agar as well as in the deeper layers. None of them had ever developed on the surface of tryptose glucose skim-milk extract agar nor on any of the other enriched media.

When suspensions of the pin point colony organism were inoculated into milk and milk fractions in the amount of about 10,000,000 per ml., oxidized flavor was not initiated in samples that were normally non-susceptible. This was true in both winter and summer milk. However, when inoculated into susceptible milk and milk fractions, the flavor was more intense in the inoculated than in the uninoculated controls. There was a tendency toward a greater increase in intensity of flavor in raw clarified milks than in non-clarified whole milk, cream or skimmilk. Leucocyte removal in the clarified milks may have been responsible for this. Triple clarification effected no difference over double or single clarification. Inoculated homogenized pasteurized milk was completely immune to a change in flavor as a result of similar inoculations.

Organisms borne by the normal udder, under the conditions which they were studied, were not resistant to pasteurization by the holding method and did not develop a resistance during the first heating. The facts that pasteurization efficiencies on aseptically drawn milk were low and that the udder organisms in pure culture were never able to withstand over 20 minutes at 61.7°C. seem to be contradictory; this can be explained on the basis of the phase of growth that the organisms were in at the two different times. Since all total counts on the fresh milk were low, comparatively, the organisms were probably subject to the germicidal action within the udder and were not developing. If this had not been true, higher counts could have been expected.

The fourth drawn stream was consistently taken for examination. According to Dorner (17) such samples should have shown much higher counts. His samples were taken near the end of the milking as an extra precaution against outside contamination, but his counts were higher than those obtained in this study. The low heat resistance of the organisms in pure culture was probably owing to their being in the logarithmic growth stage when heating began.

As Copeland and Olson (11), Hucker (35) and Dorner (17) have shown, the micrococci are the most numerous of udder borne organisms. The one that showed an association with, but which was unable to initiate oxidized flavor in milk, was tetrad meeting the Bergey Manual description of Gaffkya tardissima, except for differences with respect to growth on gelatin, colony description as grown in agar, and habitat. These differences are admittedly slight. Except for its ability to grow at 20°C. and that it did not produce gas in agar, the pin-point-tetrad found also resembles the Bergey Manual description of Gaffkya anaerobia.

The micrococci of the udder showed a wide range of dehydrogenatinn power against methylene blue when in the presence of lecithin, oleic acid and choline. In most cases oleic acid was activated at a faster rate than was lecithin, but choline was activated more slowly. Seven of 10 selected udder borne organisms including the tetrad similar to Gaffkya tardissima, were able to activate lecithin faster than they did without an added substrate. This lends weight to the conclusion of Brown and Thurston (5) who in 1940 said "the trend of the literature

at the present time seems to point to the phospholipid fraction as the source of oxidized flavors in milk and cream".

CONCLUSIONS

Standard procedure is inadequate for the determination of the organisms in aseptically drawn milk, particularly freshly drawn milk showing an oxidized flavor.

An organism similar, if not identical to Micrococcus Gaffkya tardissima is associated with the development of oxidized flavor inside the udder of certain cows.

While actively growing pure cultures of this organism in broth or skimmilk are destroyed in 20 minutes at 61.6°C., it survives 30 minutes at the same temperature when heated in fresh aseptically drawn milk in which it occurs naturally.

Udder borne organisms develop better in lecithin fortified agar media.

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A P P E N D I X

A comparison of standard bacteria counts in aseptically drawn milk from individual quarters of normal cows when samples were collected into sterile test tubes and into tubes of sterile tryptose broth (Difco) plus .1 percent glucose. Series I and II collected and plated 24 hours apart.

Series I
Fourth Stream Drawn Into Tube; Fifth Stream Into Broth.

	Standard Bacteria Count							
	No Broth				Broth			
			Pin Points				Pin Points	
Sample	24 hrs.	48 hrs.	48 hrs.	120 hrs.	24 hrs.	48 hrs.	48 hrs.	120 hrs.
1	0	0			0	0		
2	0	0	-	+	30	40	-	+
3	0	10			20	20		
4	20	50			30	40		
5	80	100			90	90		
6	120	170			220	220		
7	200	220	-	+	210	230	-	+
8	400	460			450	490		
9	460	500			490	500		
10	620	710	-	+	660	690	-	+
11	1470	1620			1700	1740		
12	2500	2530	-	+	2700 ?	2600	-	+
13	5300	6000			5000	5900		
14	7600	8500			8200	9100		
15	12100	13800			13300	14600		
16	17200	19000			20000	21500		
Average	3004	3354			3319	3610		

Series II
Fourth Stream Drawn Into Broth; Fifth Stream Into Tube

	Standard Bacteria Count							
	No Broth				Broth			
			Pin Points				Pin Points	
Sample	24 hrs.	48 hrs.	48 hrs.	120 hrs.	24 hrs.	48 hrs.	48 hrs.	120 hrs.
1a	30	60			100	110		
2a	0	0	-	+	40	60	-	+
3a	50	90			200	200		
4a	0	20			10	20		
5a	100	90	?		150	190		
6a	200	320			240	280		
7a	350	410	-	+	480	500	-	+
8a	100	100			160	160		
9a	670	880	-	+	990	1100	-	+
10a	1000	1300			1130	1200		
11a	920	1150			1200	1320		
12a	4100	4900	-	+	4200	6000	-	+
13a	2000	2300			2000	2000		
14a	10600	11100			12600	13100		
15a	17000	17200			17000	17700		
16a	26800	25900	? -	+	27000	27300	-	+
Average	3995	4114			4219	4452		

Frequency of occurrence of pigmented colonies was recorded during the study. A total of 2150 plates made on asceptically drawn milk were examined. Since there was no apparent selectivity for certain pigmented colonies by any particular agar medium, the percentage distribution shown below is for all agars used. Approximately 85 percent of the plates, however, were poured with Tryptose, Tryptose plus Tween 80, Tryptose plus lecithin and Tween 80, and T.G.E.M.

SUMMARY OF OCCURRENCE OF PIGMENTED COLONIES ON 2150 PLATES OF ASEPTICALLY DRAWN MILK

Colony Color	Plates	Percentage
White	1554	72.3
Yellow	176	8.2
Orange	166	7.7
Buff	155	7.2
Pink	56	2.6
Gray	37	1.75
Rose	6	.25

The occurrence of oxidized flavor, as a result of inoculating an organism similar to Gaffkya tardissima into susceptible raw cream, milk, skimmilk and clarified milks; flavor observations made after 72 hours holding at 4.4° C.

	Inoculated to Susceptible Raw											
	25% Cream		4% Milk		Skimmilk		4% Milk Clar. Once		4% Milk Clar. Twice		4% Milk Clar. 3 times	
January	Con.	Inoc.	Con.	Inoc.	Con.	Inoc.	Con.	Inoc.	Con.	Inoc.	Con.	Inoc.
Trial 1	-	-	+	++	-	-	+	++	+	++	+	++
2		-	+	++		-	+	++	++	+	+	+
3	+	+	+	+	-	-	+	+	+	+	+	+
4	-	-	-	-	-	-	-	-	-	-	-	?
5	-	-	+	+	-	-	+	++	+	+	+	+
July												
Trial 1	-	-	-	-	-	-	-	-	-		-	-
2	-	-	-	-	-	-	-		-	-	-	-
3	-	-	-		-	-	-	-	-	-	-	-
4	-		-	-	-	-	-	-	-	-	-	
5	-	-	-	-	-	-	-	-	-	-	-	-

The occurrence of Oxidized flavor, as a result of inoculating an organism similar to Gaffkya tardissima into non-susceptible raw cream, milk, skimmilk and clarified milks; flavor observations made after 72 hours holding at 4.4° C.

	Inoculated to Non-Susceptible Raw					
	25% Cream	4% Milk	Skimmilk	4% Milk Clar. Once	4% Milk Clar. Twice	4% Milk Clar. 3 times
December						
Trial 1	-	-	-	-	-	-
2	-	-	-		-	-
3	-	-	-	-	-	-
August						
Trial 1	-		-	-	-	-
2	-	-	-	-	-	-
3	-	-	-	-	-	-

The occurrence of oxidized flavor, as a result of inoculating an organism similar to Gaffkya tardissima into susceptible pasteurized cream, milk, skimmilk and clarified milks; observations made after 72 hours at 4.4° C.

	Inoculated to Susceptible Pasteurized											
	25% Cream		4% Milk		Skimmilk		4% Milk Clar. Once		4% Milk Clar. Twice		4% Milk Clar. 3 times	
January	Con.	Inoc.	Con.	Inoc.	Con.	Inoc.	Con.	Inoc.	Con.	Inoc.	Con.	Inoc.
Trial 1	+	+	+	++	-	-	+	++	+	++	+	++
2	+	+	+	+	-	?	+	+	+	+	+	+
3	+	+	+	++	-	-	+	+	+	++	+	++
4	+	+	+	++	-	-	+	++	+	+	+	++
5	+	++ ?	+	+	-	-	+	++	+	++	+	++
July												
Trial 1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	+	+	-	-	+	+	+	+	+	-
3	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-

The occurrence of oxidized flavor, as a result of inoculating an organism similar to Gaffkya tardissima into non-susceptible pasteurized cream, milk, skimmilk and clarified milks; observations made after 72 hours at 4.4° C.

	Inoculated into Non-Susceptible Pasteurized					
December	25% Cream	4% Milk	Skimmilk	4% Milk Clar. Once	4% Milk Clar. Twice	4% Milk Clar. 3 times
Trial 1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	-	-	-	-	-	-
August						
Trial 1	-	-	-	-	-	
2	-	-	-	-	-	-
3	-	-	-	-	-	-

A comparison of Tryptone glucose skimmilk agar and Tryptose agar, with standard Nutrient Agar (Official for milk before 1939) as reference, on aseptically drawn individual quarter pasteurized random samples. This study was made prior to the one employing Agars fortified with phospholipid.

SAMPLE	TRYPTOSE 20° C.	TRYPTOSE 37° C.	T. G. E. M. 20° C.	T. G. E. M. 37° C.	S. N. A. 20° C.	S. N. A. 37° C.
1	80	590	10	210	70	460
2	700	900	1500	1700	210	160 ?
3	100	300	270	520	270	320
4	400	200 ?	260	490	10	60
5 ##	60	170	40	760	0	50
6	460*	580*	500	540	100	180
7 # ##	500*	800	340*	250	100	200
8	200*	270	350	200	90*	180
Average	313	476	409	584	106	201

* Pin points # Oxidized when drawn ## Oxidized after pasteurization

Colonies of apparently the same species were the largest on Tryptose agar, next largest on T.G.E.M., and the smallest on S.N.A. This was true in both the plating of raw and pasteurized random samples shown in the two tables above. It was also observed that proteolytic colonies were more easily distinguished in T.G.E.M. than in the two other agars. This has been a well recognized observation, however, owing to the clearing of turbidity of the skimmilk.

A comparison of Tryptone glucose skimmilk agar and tryptose agar, with standard Nutrient Agar (Official for milk before 1939) as reference, on aseptically drawn individual quarter raw milk random samples. This comparison and the one on pasteurized samples, preceding, was made preliminary to the study of Agars fortified with phospholipid.

SAMPLE	TRYPTOSE		T. G. E. M.		S. N. A.	
	20° C.	37° C.	20° C.	37° C.	20° C.	37° C.
1 #	50	150*	200	900	100	200
2	1300	1400	1800	1300	1200	1200
3	600*	920	200	470	200	510
4	400	790*	100	300	0	200
5	2600	2900	1600	3300	500	1900
6 #	300*	4900*	460	540	170	Spreader
7 #	800*	6000*	300	1000*	0	400
8	650	900	400	820	350	610
9	590	2000	360	900	230	500
10	3000	6500	2100	4500	1800	3000
Average	1029	2554	752	1356	455	801

* Pinpoints

Oxidized Flavor

Leucocyte Counts of Six Mastitis-Free Cows During One Lactation Period.

			000 Omitted					
Cow	Breed	Freshened	April	June	Aug.	Oct.	Dec.	Feb.
1039	Jersey	4-10-47	72	105	127	166	200	175
1264	Holstein	3- 8-47	67	79	83	101	129	Dry
		Raw Milk Spontaneously Oxidized						
975	Holstein	4- 7-47	276	181	232	250	400	600
1083	Holstein	4- 9-47	125	165	300	280	290	520
		Raw Milk Oxidized After 24-48 hrs. at 4.4° C.						
1113	Holstein	3-26-47	164	300	210	400	526	700
1131	Jersey	3-20-47	324	262	360	700	721	890
		Raw Milk Not Oxidized After 7 days at 4.4° C.						