A STUDY OF SOME FACTORS AFFECTING THE MOLD CONTENT OF CREAM

by

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INTRODUCTION

Within recent years a number of studies have been made regarding the occurrence and significance of mold in cream and butter. The presence of mold in these products is not a new discovery, in fact, it has been known for a long time that certain types of fungi are almost always associated with milk and related dairy foods. Not until lately, however, has serious consideration been given to this problem of mold contamination except as a criterion of sanitation in creameries during the manufacture of butter. This interest on the part of the industry has largely been brought about by the activities of the Federal Food and Drug Administration. Research carried on by that department has resulted in the development of quality tests based upon the mold content of cream and butter. These tests are now being widely used in connection with the quality improvement program which is sponsored by the Food and Drug Administration.

It has been definitely established by numerous investigators that a marked correlation exists between high mold content in cream and poor production methods. Furthermore, the presence of excessive numbers of mold hyphae in the finished butter is generally considered to indicate conclusively that cream having a high mold content has been used in the manufacture of that butter. It follows logically that emphasis may be placed upon the presence of mold in cream and butter as a criterion of the conditions under which these products have been produced and handled.

The use of mold content as an index of quality immediately brings forward the problem of controlling the entrance into and growth of mold in the products concerned. There are many ramifications involved upon which adequate information is lacking. Most of the investigational work has been carried out upon the manufacturer's side of the problem. There appears to be a need for data on those phases of the problem in which the producer is primarily concerned.

The purpose of this study was to determine the relationship of a number of factors including age, atmospheric temperature, atmospheric humidity, season of the year, butterfat test, size of delivery, titratable acidity, grade and production methods on the farm to the mold content of cream produced by a selected group of dairymen. Since Oospora lactis (Fresenius) is the only mold normally found in cream in significant numbers, this work was limited primarily to that organism. The effect of holding cream in the buying station prior to shipment to the creamery was investigated to observe changes in mold content and other factors associated with cream quality during the storage period. Some study was also made of the sources of O. lactis found in cream, with the object of determining at what points in the process of producing cream that control measures might be most effectively employed.

REVIEW OF LITERATURE

Molds Found in Cream

Occurrence of Oospora lactis. As early as 1851, Fresenius (17) indicated that O. lactis was commonly associated with sour cream and milk. Numerous studies since have confirmed his observations. Müller (48) reported that O. lactis was always present in freshly drawn milk from normal cows. Thom (71) said O. lactis is practically always associated with the handling of milk and its products and that its presence in raw materials must be assumed. Milk and cream appear to provide a a more suitable substratum for the growth of O. lactis than any other agricultural product (66). In the study of hundreds of samples of raw cream Macy and Combs (42) were unable to find a single sample that was free from mold. Hammer (22) indicated that O. lactis occurs in any sample of raw milk or cream under normal conditions. He stated it is "the common mold of milk which from the standpoint of the group of molds, stands in about the same position as Streptococcus lactis (Lister) does among the bacteria." Seasonal variations in numbers do occur (42, 43, 55, 79), but the presence of O. lactis in raw milk and cream may be expected during all seasons of the year.

Other fungi found in cream. Many molds other than O. lactis are commonly encountered in raw milk and cream. Cummins, Kennelly, and Grimes (12) made a study of fungi found in milk during the course of a clean milk competition. The survey covered a period of five months in 1929 during which 21 species were isolated. Some of them had not previously been reported as having been found in milk. The species most commonly found were O. lactis, Acrostalagmus cinnabarinus and Phoma. Lund (36) has reported finding Penicillium practically always present in collected cream. Thom (71) stated that Cladosporium and the racemose Mucors are abundant in many samples of cream in addition to O. lactis which is found most often. In referring to kinds of molds found in milk and cream Hammer (22) said "the number of species that can be found in dairy products is limited only by the time that one wants to spend."

Methods of Estimating the Mold Content of Cream and Butter

Cultural methods. The plating method has been used for many years for the isolation of molds. It is necessary to use a medium which is favorable to the growth of molds but inhibitory to bacteria. Henrici (26) found that such a medium may be readily prepared by adding sugar to ordinary nutrient agar and acidifying the mixture rather strongly. A wide variety of media have been used including those devised by Waksman (76), Sabouraud (32), and Czapek (73). Wort agar has also been widely used. One of the most commonly used media in this country for examination of cream is acidified potato dextrose agar, the plating procedure being carried out according to the method outlined in Standard Methods for the Examination of

Dairy Products (4). By means of these cultural methods it is possible to determine the approximate numbers of viable spores or fragments of living mycelia present in the cream.

Microscopic methods. Among the early microscopic methods for the estimation of mold in cream was the procedure suggested by Redfield (60) in 1922. It was an adaptation of the Breed method for counting bacteria in milk (3). This procedure differed from the Breed method in that the 0.01 cc. sample of cream may be spread on the slide over an area of 1, 2, or 4 square c. according to the quality of the cream being examined. Distilled water, as necessary, may be added to facilitate the spreading of the cream. The method of spreading over a large area was used as equivalent to dilution. The yeasts and oidia were counted separately.

Other microscopic methods have been proposed by Ruehle (64), Hammer and Nelson (24, and Fay (15). All of these procedures may be used to estimate the numbers of both viable and non-viable organisms which are present in a given sample of cream. With some slight modifications they may also be applied to butter.

Another microscopic test has more recently been devised by Wildman (77) for the examination of butter. It is commonly designated as the "mold mycelia test" although it is actually used to determine the number of mold hyphae present. The mold mycelia test differs from the other microscopic tests both in method and in purpose. In Wildman's technique the butter sample is mixed with a hot gum solution, then a portion of the mixture is mounted on a Howard counting chamber and the mold hyphae are estimated according to the Howard method (27). Results are expressed as the percent of fields examined which show mycelia fragments. A field is recorded as positive if the combined lengths of the two longest mold filaments exceed one sixth the diameter of the field. The mold mycelia test was tentatively adopted in 1939 by the Food and Drug Administration (78) and is being widely used as a means of detecting butter made from unfit cream.

Macroscopic or "visual demonstration methods" of detecting the presence of mold in cream have been developed since 1934. In that year Greene (20) observed, while making sediment tests on butter, that the use of a hot borax solution caused the formation of clumps of mold in certain samples. Further study demonstrated that mold tends to clump in an alkaline solution, pH 9.2 being the most favorable reaction. It was upon this observation that Wildman's (97) methylene-blue-borax (MBB) method of mold detection in cream was based. The test was advanced in 1937 following studies which he had begun in 1934. The cream sample was mixed with a hot borax solution containing methylene blue dye and agitated for a short time to facilitate the dispersion of the casein, and the clumping and staining of the mold mycelia, then the clumped mold was collected in a conical seive for quantitative estimation.

In 1940, Parsons (57) proposed a modification of the Wildman (77, 78) test for mold in cream. The principle differences were in the solution used and in the methods of filtering the mixture. Parsons' solution contained crystalline disodium phosphate and sodium hexameta phosphate in addition to the ingredients used by Wildman, better dispersion and filtration being claimed under these conditions. The mixture was filtered through an American organdy cloth disc thus collecting the clumped mold in a manner similar to that used in making sediment tests on cream. The discs were then compared with standards and classified as good, fair, doubtful, or excessive.

The visual demonstration methods may be used to estimate quantitatively the amount of mold mycelia present in a given lot of cream (57, 77). The extent of the mold growth which has taken place may be determined, rather than the number of viable or non-viable spores present.

Numbers of Oospora lactis Found in Cream

No definite statement can be made regarding the number of O. lactis found in cream except that they will vary from practically zero to several million per cubic centimeter (43, 49, 55). North and Reddish (49) in their studies of yeasts and oidia in high grade experimental butter found no sample of either high grade butter or cream having 5000 oidia per ml. Cultural methods were used in counting the oidia. Macy, Coulter and Combs (43) examined 45 vats of raw sweet cream at periods throughout one year and found the number of all types of molds present to vary between 2 and 32000. Twenty six counts were below 100. Parfitt and Galema (55) made counts on 48 batches of 4-day old cream before neutralization and found an average mold count of 325,000 per ml. Sorensen (68) plated 148 samples of cream and reported counts ranging from 100 to 1,5000,000 per ml. These counts were made on individual can shipments of 4-day old cream. Oospora lactis was the dominant mold encountered and most frequently a single variety was present in a single sample.

Relationship of mold content of cream to the mold mycelia content of butter made from that cream. A limited number of studies have been made to determine the amount of mold which is carried over from the cream into the finished butter. Results indicate that the percent of mold fragments retained in the butter is quite variable. Wildman (77) reported that the mold was actually concentrated in the butter when the cream was churned out by hand under laboratory conditions. These results were based upon the study of a limited number of samples. The microscopic method of estimating mold content was used. Wildman (77) also made mold mycelia counts on 44 individual samples of butter churned from cream upon which he had previously made MBB tests. The average mold count on the butter was found to increase as the mold content of the cream increased. A number of exceptions to this relationship were observed, however. The results reported by Vandaveer and Wildman (75) in 1940 supported the findings of this study. On the other hand, Adams and Parfitt (2) examined 121 samples and found no relationship to exist between mold content of cream and mold content of resultant butter. The samples of cream were obtained from commercial plants just before churning and samples of butter made from the cream were also collected. The cream was examined for mold mycelia by a microscopic method, and the butter was analyzed by the Wildman mold mycelia test. The percentage of mold retention in the butter was found to vary from less than 10 percent to 70 percent. The retention was below 31 percent in 66.2 percent of the samples, and, in general, it was between 20 and 30 percent. There appeared to be no correlation between the amount of mold in the cream and the percent retained in the butter. Similar results were obtained by Slatter (67). More extensive study is necessary before conclusions can be drawn regarding this relationship. The fact that a high mold mycelia count on a butter sample was nearly always associated with a high mold content in the cream from which it was made is significant here. The reverse of this statement was not found to be true in all cases.

Studies on factors affecting mold retention in butter. In view of the finding by Grimes (21) that the size of butter granules were important in determining numbers of bacteria retained in butter, Adams and Parfitt (2) studied this factor as related to molds. They secured samples from the churn when the granules were one-half the size of wheat grains and again when they were twice as large as wheat grains. These were tested by plating and by microscopic examination. No marked difference was found by either method between underchurned and overchurned butter. These investigators also studied the effect of cream neutralization upon the mold mycelia content of butter and found that the limits to which cream is normally neutralized under commercial conditions do not effect the amount of mold fragments retained in the butter. The procedures employed in the manufacture of butter apparently do not influence the mycelia content of the butter (2, 54). The work of Adams and Parfitt (2) and Wildman (77) led those workers to the conclusion that the mold filaments as carried over into the butter are uniformly distributed throughout the mass.

Effect of Oospora lactis upon the Quality of Cream

There is some lack of agreement in the reports regarding the action of O. lactis upon cream. These differences may be partially accounted for by the lack of a systematic classification of the various strains (66).

The work of Kirchner (31) and Orla-Jensen (52) indicated that O. lactis was able to hydrolyze fat in butter producing an intensely rancid flavor. These findings were supported by the findings of other investigators (6, 9, 29, 44, 55). It was later suggested by Lewkowitsch (33) that the rancidity was caused by hydrolytic action of enzymes produced by the mold rather than due to direct action on the fat.

In 1901 O'callaghan (50) published the first of a series of articles (50, 51) showing that O. lactis causes fishiness in butter. He stated that when O. lactis was grown in conjunction with Streptococcus lactis in cream, fishy flavor resulted invariably. Rogers (63) was not able to verify these results. In 1939, Davies (13) offered an explanation for fishiness caused by molds. He stated that they have the property of liberating oleic acid which may catalyze the oxidation of the lecithin.

O. lactis has been credited with the production of acid in milk by Schnell (66) and Thom (70), but this observation was not comfirmed by Macy and Gibson (44). Numerous workers agree, however, that the organism is capable of using up acid sometime during its normal growth period, resulting finally in a reaction alkaline to litmus (34, 66, 70).

Stokoe (69) reported that O. lactis produced a strong cheesy odor when grown on a butterfat medium for seven days at 68°F. Certain other investigators (6, 37, 44) have also indicated that a cheesy flavor is sometimes produced. This organism either alone or with other types has been said to produce many other flavors including putrid, yeasty, bitter, sour, stale, unclean, old cream and fruity (33, 65, 66). Many of the off flavors have been attributed to the action of enzymes secreted by the mold (5, 10, 69, 70).

Combs and Eckles (10) observed that pure cultures of O. lactis grown upon samples of sweet cream produced effects detrimental to the quality of the cream, but when the same cultures were grown on sour cream no ill effects were noted. The resulting butter, however, went off flavor in both cases. This was attributed to enzymes produced by the mold while growing in the cream. Ause and Macy (5) found that O. lactis, whether grown in sweet or sour cream, was not a major factor in any deterioration of the butter made from such cream.

After an exhaustive review (7, 16, 22, 23, 39, 53) of the relation of _O. lactis_ to the quality of dairy products a group of dairy scientists concluded that this mold was not as important in its affect upon quality as some other organisms frequently found in cream and butter. Parfitt and Galema (55) had reviewed the literature four years previously and concluded that molds associated with sour cream decompose proteins and often fats, producing products undesirable in the manufacture of butter. The strain of _O. lactis_ present in cream is an important factor in determining its importance from a cream quality standpoint (68).

Relationship of mold content of cream to quality of the cream and resultant butter. As early as 1921, North and Reddish (49) reported a correlation between the presence of oidia in appreciable numbers (500,000 per ml.) in butter and the use of old, stale or deteriorated cream in the manufacture of the butter. Redfield (60) published results in 1922 which substantiated the earlier study. Parfitt and Galema (55) made mold counts by the plate method on route cream delivered twice a week at the Purdue University creamery during the summer of 1930. They found the average count of first grade cream to be 275,000 per ml. and that of second grade cream to be 380,000 per ml. Contrary to this, Sorenson (68) studied 78 shipments of cream during the first fifteen days of April and found no relation to exist between grade of cream and _O. lactis_ content. When Wildman (77) published his methylene-blue-borax method for demonstrating mold in cream, he reported finding off flavors frequently to be associated with the presence of mold.

He pointed out, however, that a low MBB test did not necessarily indicate good cream. Subsequent studies verified these results. Recently, Campbell (8) stated that, "Studies completed in 1939," by the Food and Drug Department, "show conclusively that a high mold mycelia count in finished butter never occurs unless some of the cream was, to a material degree, filthy, putrid or decomposed."

These reports (8, 49, 55, 75, 77, 79) may appear to be exactly opposite to some of those cited in reference to the effect of O. lactis upon the quality of cream (5, 16, 22, 23, 39, 53). The apparent difference may be explained by the fact that the researches regarding the correlation of mold content of cream with the quality of the cream and resultant butter are not concerned fundamentally with the effect of O. lactis upon the cream but more specifically with changes which take place in the cream when it is handled under conditions favorable for the growth of O. lactis. A number of investigators (5, 16, 22, 23, 39) have shown that O. lactis is not as important in bringing about the deterioration of cream as certain other factors, but these factors bring about changes most quickly under conditions which favor rapid growth of O. lactis. The studies of the correlation between the extent of O. lactis growth in cream and the quality is based upon this observation.

Control of the Mold Content of Cream

Sources of Oospora lactis found in cream. The reports of numerous investigators indicate that the common milk mold

O. lactis is almost universally distributed throughout nature. Schnell (66) reported that it occurs on all products of agriculture. It may be found on field and garden soil which has been fertilized with barn manure, on fermenting masses of hay, on acidified corn fodder and beets, on damp grain, potatoes, turnips cabbage leaves, cucumbers and a wide variety of other products. Schnell says that "Oidia get into milk mainly through the barn manure, or through the feeds, or out of the soil itself, or while milking from the cows' teats, where it is supposed to occur parasitically." The udder has been shown to be free of all molds except in special cases when it may become infected with a mycotic disease (71). The more probable sources during the milking process would be the hands of the milker, the skin of the cow, contaminated surfaces in the utensils used, and the air (40). Hansen (25) reported that O. lactis could live throughout the winter in the soil. He was able to demonstrate its presence in the air only during the months of July and August. Other workers have also found that the air was more frequently contaminated with molds during the summer months (43). Adametz (1) believed that O. lactis could be transferred from the soil into the milk by feeding potatoes to the cows, but no confirmation of this was found. Wildman (79) reported that moldy utensils were the most likely source of contamination of cream.

Factors affecting the growth of Oospora lactis in cream and other dairy products. It is generally agreed that cream and other dairy products contain the substances necessary to satisfy the food demands of O. lactis (5, 6, 22, 23, 28, 37, 42, 45, 66, 70, 71). In addition to these food materials, however, certain other requirements must be fulfilled to provide optimum growth conditions for this organism.

Oxygen relationships. O. lactis is an aerobic organism, therefore the oxygen supply may be a limiting factor in its growth in and upon dairy products. In his studies of factors influencing mold growth in butter Macy (37) found that a number of molds including O. lactis were able to grow under a vacuum of 25 inches, but none of them were able to grow in the complete absence of oxygen. He also observed that partial removal of carbon dioxide did not effect growth on butter. Thom (71) indicated that Oospora is one of a few forms which can penetrate rather deeply into semisolid substrate. This is due to its ability to grow with a decreased oxygen supply. Sorenson (68) noted that daily agitation of a set of pure cultures of eight varieties of O. lactis in cream produced higher counts in all but two cultures than was the case with undisturbed cream. Some investigators (2, 6) have found similar results, but Wildman (79) has suggested the addition of fresh cream with thorough stirring twice daily to prevent development of the spores in cream. The work of Parfitt and Galema (55) indicated that mold growth was inhibited by a decreased air supply. Adams and Parfitt (2) and others (6, 68) agree that the area of cream surface exposed to the air markedly effects the amount of growth.

Moisture. Under normal conditions any one of the dairy products contains sufficient moisture to support the growth of

O. lactis. Low humidity will inhibit its growth, however. Thom and Shaw (72) state that mold will not grow on the surface of a piece of butter at humidities of 70 percent or lower. When grown in cream, Macy (37) found that the humidity had little effect upon their development.

Temperature. The average optimum growth temperature for O. lactis as reported by Schnell (66) was 23° to 28°C., depending upon the variety. In general, the results of other workers confirm these data (2, 28, 41, 44, 61, 68, 71). Thom (70) stated that O. lactis, when inoculated into slanted tubes of gelatin, grew and fruited well at 5° to 10°C. Macy and Gibson (44) reported that most strains were restricted by a temperature of 10°C. They also observed that certain cultures developed normally at 30°C. while others were practically dormant at that temperature. The studies made by Macy and Steele (45) have shown that molds do not grow on inoculated, unsalted butter during storage for twenty weeks at -18°C., and that some molds are checked at 5°C. for two weeks. These low storage temperatures did not, however, prevent the development of the molds after cold storage when the butter was placed under conditions favorable for their growth. Time was considered to be a factor by Macy (37) who found that O. lactis grew at 0°C. but not so extensively as at 10° to 20°C. Macy and Anderson (41) reported that O. lactis grew well on all media used when held at 20°C., less luxuriantly at 10°C. and 2°C. and was entirely checked at -23°C. Sorenson (68) studied eight cultures of O. lactis and found that all grew well at 70°F. and all but one culture showed slight increase at 40°F. in eight days. Two cultures showed slight growth at 98°F. and one produced highest counts at 98°F., while the others failed to grow.

Time relationships. The length of the holding period is important in its effect upon the extent of mold growth (37, 43, 46, 47, 61, 68, 75, 79). The growth of the mold is so closely dependent upon such factors as variety of mold, humidity, temperature, available oxygen, and acidity, it is evident that the amount of growth in any given length of time must be evaluated in relation to the existing conditions. In general, it may be stated that from four to eight days usually are required for significant amounts of growth to take place in cream (68, 75, 79).

Acidity. O. lactis grows best in a rather acid medium (21, 41, 44, 68). In 1923, Grimes (21) stated that the acidity of ripened cream did not favor deterioration by certain bacteria, yeasts, and molds (including O. lactis). Macy and Anderson (41) concluded from their study of seven cultures of O. lactis that the degree of acidity alone did not have a marked effect upon the growth. Sorenson (68) reported that O. lactis grew in sterile acidulated cream over an acidity range from sweet cream to that containing 1.3 percent lactic acid. Highest counts were noted between 0.4 percent and 0.7 percent acidity. Studies on 61 cultures by Macy and Gibson (44) indicated that growth was meager at pH 3.0 or lower, best at pH 4.0 and somewhat abnormal at pH 7.0 or higher.

Fat test of cream. A correlation between the fat content of cream and the amount of mold growth has been reported by some workers (2, 54, 77). Those lots of cream containing about 45 percent fat were found to show a little less mold growth than those samples containing a low percentage of fat.

Surface area. It is generally agreed that the area of the surface of cream exposed to the air is an important factor in determining the amount of mold growth in cream (2, 6, 23, 37, 55, 58, 68). Adams and Parfitt (2) found that the amount of mold in a given sample of cream was almost directly proportional to the amount of cream surface exposed to the air. The work of Parfitt and Galema (55) showed that the use of air tight containers for cream gave a marked reduction in mold count in a 72 hour period. This work has been corroborated by several workers and has led to the proposal of methods to control the mold growth in cream. These methods will be discussed at a later point in this paper.

Salt. A concentration of 2.5 to 3.0 percent of salt in butter or 7.5 to 10 percent in cream will markedly inhibit and often eliminate growth of molds in these products (37, 38, 41, 44, 72, 74, 81). Thom and Shaw (72) found that O. lactis, Alternaria, and Cladosporium could not develop in butter containing 2.5 percent of salt. The addition of 2.5 to 3.0 percent salt to butter eliminated mold growth or reduced it to a very small amount. A salt concentration of 7.5 percent to 10 percent in the medium is generally considered to be effective in controlling the growth of those strains of O. lactis which have been studied, there being only a few strains which show any sign of growth at a salt concentration of 10 percent (37, 41, 44, 74). Macy (37) pointed out that the effect of salt on molds depends upon the species, amount of moisture and temperature.

Nutritional requirements of Oospora lactis. Very little information was found in the literature which concerns specifically the nutritional requirements of this mold. Thom (70) in 1906 was among the first investigators to show that O. lactis was capable of reducing the acidity of a medium, and to suggest enzymes as the causative agents. The use of media containing no protein resulted in poor growth, while pure casein supported luxuriant growth. When it was grown in sterilized milk, a large proportion of the colonies sank below the surface, the milk becoming filled with mycelium, although the chains of spores were produced at or just below the surface. Milk and curd were digested slowly; a reaction acid to litmus was produced at first, then a strong and continued alkaline reaction. Schnell (66) reported that O. lactis produced the enzymes lipase, occasionally catalase, and a protease which was capable of decomposing protein substances to the amino acids aspartic, laucine, and tyrosine. Many other workers have postulated the presence of various enzymes in explaining the results observed (10, 33, 35, 37, 68, 70, 72).

Ten species of molds (including O. lactis) isolated from butter have been studied at the Minnesota Station (37). Purified butterfat was not readily utilized unless water was present, and even then the growth was moderate. Lecithin and hydrolysis products of fat including glycerol, palmitic acid and oleic acid were capable of supporting fair growth. Nitrogen containing compounds such as peptone, curd from butter, and serums from cream were found to be excellent sources of food. Lactose and lactic acid in 1.0 percent solutions were not satisfactory. The molds did not grow satisfactorily upon solutions of milk ash unless it was neutralized. Combinations of fats; lecithin and water; fat and ash; fat, ash and water; and lactone and peptone were more satisfactory than single substances. The best growth was observed on the surface of media containing nitrogen bearing compounds. Growth upon substrate containing fats, fatty acids, glycerol, lactose, or lactic acid appeared to be largely below the surface of the medium. Macy and Gibson (44) studied 61 strains of <u>O. lactis</u> all of which were able to produce indole, liquify gelatin to some extent, and to cause lipolysis of cottonseed oil and butterfat. None were able to hydrolyze starch or produce an acid reaction in litmus milk. Acid was formed in glucose broth by all cultures, but only nine produced gas.

Associative action and symbiosis with certain other organisms has been suggested by some investigators in accounting for changes in growth relationships of this organism when grown in the presence of certain bacteria (34, 50, 51, 72). There is diversity of opinion in regard to the nature of this growth relationship. Ause and Macy (5) studied the effect of <u>O. lactis</u> upon neutralized sour cream and reported no apparent associative action of <u>O. lactis</u> with the natural microflora of the cream.

Methods of limiting entrance and growth of Oospora lactis in cream. Hunziker (28) has stated that "it is commercially impossible to prevent the presence of mold in milk and cream at the time of delivery to the creamery." Practically all investigators agree that some mold spores are nearly always present in raw cream (6, 16, 22, 23, 28, 35, 36, 42, 43, 53, 66, 68, 71). The sources of contamination are so numerous and varied that any attempt to eliminate all spores from raw cream would be impractical under average farm production conditions. It is, however, feasible that the amount of contamination may be appreciably reduced by careful control of the most important sources. Furthermore, the growth of those spores which do gain entrance to the cream may be inhibited by proper methods of handling during the storage period.

In 1930, Bouska (6) suggested the exclusion of air from the cream as a method for reducing mold growth. To accomplish this he proposed the use of a float, parchment paper, paraffin or an inert gas such as carbon dioxide. He also questioned the advisability of stirring cream frequently during the storage period. Since that time, the work of Sorenson (68), and Parsons (58) and others (55, 68) has confirmed the desirability of leaving the cream undisturbed during storage. These investigators found that fresh cream should be added in such a manner that it forms a layer on top of the older cream.

Williams (81) has advanced a new idea for preserving cream quality during storage. This involves the addition of salt to the cream in sufficient concentration to inhibit the growth of microorganisms. About 7.0 percent was recommended. A number of workers (44, 74) have studied the effectiveness and practicability of such a method and reported favorable results when 7.5 percent to 10 percent concentrations of salt were used.

Other methods which have been suggested for the preservation of cream quality during farm storage include the use of lactic cultures (59) and the addition of lactic acid fungi (14).

Control of the holding temperature has been found to be one of the most effective methods of inhibiting mold growth (45, 46, 47, 79). Martin, Fay, and Caulfield (46) reported that practically no yeasts or molds developed in cream held for 16 days at 50°F. but at 70°F. they grew rapidly. The initial contamination of the cream with mold is believed to be a factor in determining the amount of mold growth in cream (53, 79).

Parsons (58) has suggested the use of a straining pad over the top of the pail while milking as a method for reducing mold contamination of milk from such sources as the air and the coat of the cow.

Sanitation, cooling, and frequent delivery have often been emphasized as the three most important factors in the production of cream low in mold content (40, 42). Recent advances in sanitation include the development of an acidified steam rinse in the cleaning of milk cans (56, 62). Gluconic acid has been used for this purpose and appears to result in an improvement in the bacterial flora of the cans.

There is a lack of data in the literature upon the importance of such factors as atmospheric temperature, atmospheric humidity, age of cream, and acidity in their effect on mold content of cream actually produced and handled under average farm conditions. The activities of the Food and Drug Administration in quality improvement work and the use of the visual mold test as a criterion of the fitness of cream for buttermaking makes it desirable to have more information on the value of the visual mold test and the conditions in which it may be used as a reliable indication of the mold content and the fitness of cream for manufacture of butter.

Creameries are now using the visual mold test on cream which has been collected in cream buying stations, and held for periods of from one to three or more days before shipment to the central plant. During this period of holding in the cream stations the cream is often held under essentially anaerobic conditions. Since mold requires oxygen for growth the question rises as to what effect this may have upon the mold content and whether or not it effects the reliability of the visual mold test as a gauge of cream quality.

There is also a need for information on the relation of the mold count by the plate method and the visual mold test. Only by such comparison can the visual mold test be placed on a sound basis.

Other problems upon which data is lacking include sources of the common milk mold found in cream and the effect of methods used by individual cream producers upon the mold content and general quality of their product.

The study reported here was undertaken for the purpose of collecting information pertinent to these problems.

EXPERIMENTAL METHODS

Samples

The samples of cream examined in this work were secured from deliveries made by a selected group of producers to local cream buying stations. The selections were made upon the basis of such factors as frequency of delivery, size of delivery, average fat test, and average quality of cream for the purpose of obtaining cream fairly representative of the type of product marketed through the Manhattan cream stations. In all, 136 producers were selected. Samples were taken, so far as possible, of each delivery of cream made by these producers over approximately 14 day periods at intervals throughout the year from September 1940 until the following May 31. The samples were collected in sterile jars and held at a temperature near 40°F. until they could be analyzed.

Examination

Yeast and mold counts. The yeast and mold contents were determined by plating the cream on acidified potato dextrose agar according to the procedure outlined in Standard Methods for Examination of Dairy Products (4). Yeasts and molds were counted separately.

Visual mold determinations. The cream was examined by the Parsons' (57) modification of the Wildman (77) methylene-blue-borax method for estimation of the mold content.

<u>Acidity</u>. The acidity of the cream was determined by titration of a 9 gm. sample, diluted with 9 ml. of distilled water, with 0.1 N sodium hydroxide, using phenolphthalein as the indicator.

<u>Atmospheric temperature and humidity</u>. These data were secured from the Department of Physics of Kansas State College. Temperature and humidity readings were obtained at two hour intervals throughout the periods in which studies were made. The maximum, minimum and mean temperature and humidities were then determined for each day of the period. From these data the maximum, minimum, and mean temperatures and humidities of the interval during which each lot of cream was being accumulated on the farm was calculated.

<u>General</u>. The cream samples were examined organoleptically and classified as sweet, first, second, and third grades as defined in the Kansas State Dairy law (30). The weight of cream in each delivery, fat test, and age were ascertained from the cream station records. In some cases the station operator, or producer was consulted regarding age of the cream. The information on production methods at the farm was obtained by personal interviews with the producers, either at the farm or at the cream station.

Equipment

The mechanical agitator which was used for the visual mold test was quite similar to the one described by Wildman (79) except that a metal water bath fitted with a rack to hold six bottles was substituted for the wooden block suggested by Wildman. A filtering apparatus constructed to hold six organdy cloth discs was used to filter the cream and mold reagent mixture following agitation. The organdy discs were then mounted on sheets of cardboard, size 8 by 11 inches, by means of paper cement and the entire sheet covered with cellophane.

Standards

The visual mold standards used for evaluating the discs were somewhat different from those proposed by the American Butter Institute. Seven standard discs were employed instead of four in order to obtain more detailed classification to facilitate comparison of this test with the plating method and other criteria. The relationship between these two sets of standards is shown in Table 1.

Table 1. Comparison of visual mold standards used in this study with those proposed by the American Butter Institute

Class	Standards used in this study	Corresponding American Butter Institute Standards
Good	1	1
	2	-
Fair	3	2
Doubtful	4	3
Excessive	5	4
	6	-
	7	-

Studies on Cream Storage

The studies on the affect of holding cream in cream buying stations were carried out during the month of May, 1941. The station under observation held the cream in ten gallon cream cans. Samples were taken from each can at the close of the days buying activities, and again from the same cans just before they were shipped to the creamery. The cream was thoroughly agitated and the samples taken in sterile jars. These samples were immediately cooled and held at 40°F. until analysis could be made. The temperature of the cream and the amount of air space between surface of the cream and the closed lid were observed in each case after the samples were secured. In the laboratory the samples were examined in the same manner as those secured from individual producers.

Studies on Sources of Mold

A modification of Wildman's (80) agar slice method usually was used in determining the presence of O. lactis on equipment. The acidified potato dextrose agar slices contained 3 percent of agar and were 2.5 inches in diameter instead of test tube size as proposed by Wildman. Some sources were examined by means of a sterile water rinse, followed by plating of the water on acidified potato dextrose agar. The sterile water rinse method was particularly well adapted to examination of utensils. The mold counts on such materials as grain, silage, hay and manure were made by weighing out one gram portions of the materials and placing them in 99 ml. of sterile water. The resultant mixtures were plated on acidified potato dextrose agar, using 5 ml., 1 ml., and 0.1 ml. quantities in different plates. The rumen samples were obtained by means of a chlorine-treated stomach pump and were plated on wort agar adjusted to a reaction of pH 4.5.

EXPERIMENTAL RESULTS

Relation of the Mold Count (Plate Method) to the Visual Mold Score

The visual mold test is being widely used in the dairy industry for the estimation of the mold mycelia content of cream and as an indication of the conditions under which the cream has been produced and handled. The quantity of mold mycelia present as measured by the visual mold test is considered to be an index of the methods used during production in regard to sanitation, holding temperatures, and age of cream. The important question arises as to what relationship exists between the mold content of cream as shown by the visual mold test and the mold count as determined by the plate method.

An attempt was made in this phase of the study to answer this question. Plate counts and visual mold determinations were made on 1031 samples of cream collected from 136 producers from September, 1940, until May 31, 1941. The results of these tests are shown in Table 2. In this table the number of samples have been grouped according to the results of the plate count and the visual mold test.

A close relationship is shown to exist between these two tests. Table 2 shows that no sample having a plate count of 29,000 per ml. or less was placed in the excessive visual mold class and that no sample having a mold count by the plate method of 500,000 or more per ml. was placed in No. 1 or good visual mold class. All samples classified as good by the visual test are shown to have a plate count of 499,000 or less per ml. while all samples classed as No. 7 may be observed to have a plate count of 500,000 or more per ml.

Table 2. Relationship of the mold count (plate method) to the visual mold score. (1031 samples)

Visual mold score	Mold count per ml. by the plate method: Less than 10M*	10M to 29M	30M to 99M	100M to 199M	200M to 499M	500M to 999M	1000M to 1999M	2000M to 2999M	3000M and over	Total No. of Samples
	No. of Samples									
1	508	35	8	1	1					553
2	65	36	45	13	11	2		2		174
3	12	12	33	41	28	12	4		1	143
4	3	3	10	10	26	25	8	3	3	91
5			3	1	4	11	16	5	1	41
6				1	1	2	5	5	2	16
7						4	4	3	2	13
Total No. Samples	588	86	99	67	71	56	37	18	9	1031

*M = one thousand

The relationship between the visual mold test and the plate count is shown on a percentage basis in Table 3. Certain points regarding the relationship are brought out more clearly.

Table 3. Relationship of visual mold score to the mold count by the plate method. (1031 samples)

Mold count per ml. by the plate method	Visual Mold Score: 1 % of class	2 % of class	3 % of class	4 % of class	5 % of class	6 % of class	7 % of class	Total No. of Samples
Less than 10M*	91.9	37.4	8.4	3.3				588
10M to 29M	6.3	20.7	8.4	3.3				86
30M to 99M	1.4	25.9	23.1	11.0	7.3			99
100M to 199M	.2	7.5	28.6	11.0	2.5	6.3		67
200M to 499M	.2	6.3	19.6	28.5	9.8	6.2		71
500M to 999M		1.1	8.4	27.5	26.8	12.5	30.77	56
1000M to 1999M			2.8	8.8	39.0	31.3	30.8	37
2000M to 2999M		1.1		3.3	12.2	31.2	23.1	18
3000M and over			.7	3.3	2.4	12.5	15.4	9
Total No. Samples	553	174	143	91	41	16	13	1031

*M = one thousand.

Of all samples having a No. 1 visual mold score 91.9 per cent contained a mold count of less than 10,000 per ml., and all samples in visual classes of 5, 6 and 7 (excessive) are shown to have a mold count of 30,000 or more per ml. the greatest percentage of them having counts in excess of 500,000 per ml. The greatest variation in mold count occurs among the samples in visual mold classes 3 and 4. (fair and doubtful). However, in the No. 3 visual mold class over 70 percent of the samples had a mold count of 30,000 to 499,000 per ml. and in No. 4 visual mold class over 70 percent of the samples had a mold count of 30,000 to 999,000 per ml.

Since the visual mold standards used in this study were somewhat different from those proposed by the American Butter Institute, the data shown in Table 2 has been grouped according to the four Butter Institute standards to show the relationship on that basis. This is shown in Table 4.

Table 4. Relationship of mold count (plate method) to the visual mold score on basis of American Butter Institute Standards (1031 samples)

Visual mold score	Mold count per ml. by the plate method (No. of samples of cream): Less than 10M*	10M to 29M	30M to 99M	100M to 199M	200M to 499M	500M to 999M	1000M to 1999M	2000M to 2999M	3000M and over	Total No. of Samples
1	573	71	53	14	12	2		2		727
2	12	12	33	41	28	12	4		1	143
3	3	3	10	10	26	25	8	3	3	91
4			3	2	5	17	26	13	4	70
Total No. of samples	588	86	99	67	71	56	38	18	8	1031

*M = one thousand.

This table shows the result of combining visual mold classes 1 and 2, and 5, 6 and 7 of Table 2 thus presenting the distribution of the samples according to the classification in general use throughout the industry.

The results in this phase of the work have revealed that a close relationship exists between the visual mold test and the cultural method. The visual mold test appears to be a satisfactory method for determining whether conditions associated with the production of cream on the farm have been favorable for the growth of mold in the cream.

The Relation of the Mean Atmospheric Temperature to the Visual Mold Score

Since many cream producers follow the practice of cooling their cream in the air under atmospheric conditions, it is desirable to have information on the relationship of atmospheric temperature to the visual mold content of cream. Temperature is one of the most important factors affecting the growth of mold in cream, therefore if the temperature of cream is allowed to fluctuate with changes in atmospheric temperature a close relationship might be expected.

In this study of the relation of atmospheric temperature to the visual mold score the mean atmospheric temperature was selected as most representative of the temperature conditions prevailing during the period in which the cream was being accumulated on the farms. The mean atmospheric temperature during accumulation period and the visual mold score were determined for 1173 samples of cream. The results grouped in Table 5 show the percent of samples falling in each visual mold class.

Table 5. Relationship of mean atmospheric temperature during the accumulation period to the visual mold score (1173 samples)

Visual mold score	Mean atmospheric temperature - °F.							Total No. of Samples
	10-19: % of group	20-29: % of group	30-39: % of group	40-49: % of group	50-59: % of group	60-69: % of group	70-79: % of group	
1	60.0	87.3	74.4	76.9	51.3	40.0	36.1	614
2		5.6	8.8	17.3	18.0	18.9	22.8	193
3	20.0	3.9	6.9	3.9	14.7	17.4	23.4	171
4	20.0	1.6	6.9	1.9	10.9	13.0	12.2	118
5		1.6	1.2		3.2	6.5	2.0	44
6			1.2		1.3	2.7	0.5	18
7			0.6		0.6	1.5	3.0	15
Total No. of samples	5	126	160	52	156	477	197	1173

Table 5 shows clearly that a rather close relationship existed between the mean atmospheric temperature during the accumulation period and the mold content as determined by the visual mold score. Of all samples accumulated during periods in which the mean atmospheric temperature was 20° to 29°F., 87.3 percent had a No. 1 mold score, while of those samples accumulated in the range of 70° to 79°F. only 36.1 percent fell in the No. 1 visual mold class. The greatest decrease in per cent of samples falling in the No. 1 visual mold class is shown to have occurred at the mean atmospheric temperature of 50°F. where the percent dropped from 76.9 percent in the temperature range of 40° to 49°F. to 51.3 percent in the range of 50° to 59°F. A considerable increase in percent of samples in visual mold classes 5, 6 and 7 (excessive) also occurred at mean temperatures above 50°F. The fact that a number of samples contained excessive quantities of mold even though accumulated in periods in which the mean atmospheric temperature was 20° to 29°F. and 30° to 39°F. indicates that some producers have held their cream under temperatures other than atmospheric. Keeping cream inside to prevent freezing in the winter season and providing cooling, either by cold water, or refrigeration during the summer would of course reduce the relationship between mean atmospheric temperature and visual mold content.

The results of this study show that the mean atmospheric temperature prevailing during the period in which the cream was accumulated on the farm was an important factor in determining the mold content.

The Relation of Mean Atmospheric Humidity to the Visual Mold Score

The growth of mold in butter and cheese is known to require relatively high humidities (70 percent or above), but in the case of cream the presence of excessive moisture in the cream itself would be expected to eliminate the necessity for a large amount of atmospheric moisture to be available.

A total of 1173 samples of cream were examined and the mean atmospheric humidity of the accumulation periods calculated in making this study. The data is shown in Table 6 grouped according to the number of samples falling in each visual mold class.

There appears to be a negative relationship between the mean atmospheric humidity and the visual mold score, for as the humidity increases the proportion of lots of cream in the fair and good visual mold classes also increases, figures for periods below 50 percent being the exceptions to the general rule. This may be explained by the fact that high humidities are usually associated with low temperature. Table 6 shows that with an increase in atmospheric humidity from 50 percent to 79 percent there is a tendency for the proportion of samples falling in the visual mold classes 5, 6 and 7 first to increase, then to decrease again as humidity approached 79 percent.

Table 6. Relationship of mean atmospheric humidity during accumulation of cream on the farm to the visual mold score.

Visual mold score	Mean atmospheric humidity during accumulation period						Total No. of Samples
	40-49%	50-59%	60-69%	70-79%	80-89%	90-99%	
	No. of samples of cream						
1	47	152	196	140	79		614
2	15	74	53	41	9	1	193
3	9	64	68	21	9		171
4	11	37	48	20	2		118
5	1	15	23	4	1		44
6	2	8	6	2			18
7	1	4	7	3			15
Total No. Samples	86	354	401	231	100	1	1173

These data confirm Macy's (37) report that humidity has little effect upon the growth of mold in cream.

Relation of the Age of Cream to the Visual Mold Score and to the Grade

Relation of age of cream to the visual mold score. The length of time required for marked changes to take place in the quality of cream is dependent upon a number of factors, the most important of which are the bacterial flora of the cream and the temperature of holding. The mold content of cream of a given age, like the quality, is generally considered to depend to a large degree upon initial flora and temperature at which the cream is held. The effect of temperature has already been shown in Table 5.

The purpose of this phase of the work was to determine the relationship of age to the visual mold content of cream. In all, 1173 samples of cream were studied. These data were placed in four age groups and are shown in Table 7 as percent of samples falling in each visual mold class.

Table 7. Relationship of age of cream to the visual mold score. (1173 samples)

Visual mold score	Age of cream in days: 1 to 3 % of age group	4 to 6 % of age group	7 to 9 % of age group	10 and over % of age group	Total No. of Samples
1	65.5	56.2	40.4	29.6	614
2	18.8	16.0	15.0	14.8	193
3	10.0	14.0	18.2	22.2	171
4	4.6	10.3	13.9	14.8	118
5		2.6	7.4	7.4	44
6	0.8	0.3	2.6	7.4	18
7	0.3	0.6	2.5	3.7	15
Total No. of Samples	351	349	446	27	1173

This table shows clearly that a close relationship exists between the age of the cream and the visual mold content. Of the total number of lots of cream falling in the one to three day age group 65.5 percent were classed as No. 1 by means of the visual mold test, while only 29.6 percent of the lots of cream in the 10 day and over age group had a visual mold score of 1. In the one to three day age group only 0.3 percent of the samples fell in No. 7 visual mold class, but in the 10 day and over age group 3.7 percent of the samples were classed as No. 7 in mold content.

The age of the cream was found to be an important factor in determining the mold content of the cream. The visual mold content increased quite regularly with an increase in age of the cream.

Relation of the age of cream to the grade. The age of cream was shown in Table 7 to be related to mold content. If the mold content is an index of cream quality, then the age of cream should also be closely related to the grade. The purpose in this phase was to study this relationship.

The age and grade of 1183 samples of cream were determined and placed in four age groups. These data are shown in Table 8. In the one to three day age group 74.7 percent of the samples were first grade cream and 25.3 percent second grade and in the 10 day and over age group only 29.6 percent of the samples were first grade cream and 70.4 percent were second grade. No samples were graded as sweet or third grade.

Table 8. Relationship of age of cream to the grade (1183 samples)

Grade of Cream	Age of cream in days				Total
	1 to 3	4 to 6	7 to 9	10 and over	No. of
	% of group	% of group	% of group	% of group	Samples
1st	74.7	60.7	43.6	29.6	682
2nd	25.3	39.3	56.4	70.4	501
Total No. of samples	352	354	450	27	1183

The data from this study indicate that the grade of the cream is controlled to a considerable degree by the length of time it is held before being marketed.

The Relation of the Season of the Year to the Visual Mold Score

The samples of cream examined in this study were collected during five different seasonal periods, the first beginning in September and the last ending on May 31 of the following year. A total of 1173 samples were examined the results of which are summarized in Table 9. The effect of the season of the year

Table 9. Seasonal distribution of the lots of cream in relation to the visual mold score (1173 samples)

Period	Visual Mold Score							Total No. of Samples	Mean atmospheric temperature °F.
	1	2	3	4	5	6	7		
	Percent of Samples								
Sept.-Oct.	43.8	16.1	14.3	14.9	6.5	3.4	0.9	322	64
December	76.9	10.0	6.8	4.7	1.1		0.5	190	33
February	84.1	5.1	5.1	3.8	.7	0.6	0.6	157	31
Apr.-May	42.4	22.8	15.6	12.0	4.0	1.4	1.8	224	59
May	35.7	22.5	24.6	10.0	3.9	1.1	2.2	280	70
Total No. of samples	614	193	171	118	44	18	15	1173	

and the corresponding mean temperature upon the visual mold score of the cream is shown in a rather striking manner. In the September - October period in which the mean atmospheric temperature was 64°F., only 43.8 percent of the samples collected were placed in the No. 1 visual mold class, while in the December period when the mean atmospheric temperature was 33°F., 76.9 per cent of the samples collected were classed as No. 1 by means of the visual mold test. The percentage was up to 84.1 percent in the February period when the mean atmospheric temperature was 31°F., and dropped to a low of 35.7 percent of the samples collected in the May period when the mean atmospheric temperature was 70°F. Table 9 also shows that the percentage of samples placed in the doubtful to excessive classes was significantly greater in the high temperature periods than in the low temperature periods.

The relationships observed in this portion of the work show clearly that during the seasons of the year in which temperatures are highest the cream tends to contain the greatest amount of mold.

The Relation of the Butterfat Test of the Cream to the Visual Mold Score

In order to determine the effect of the butterfat content of cream upon the visual mold content a study was made of 1173 samples of cream. The data which are summarized on a percentage basis in Table 10 shows that a slight relationship exists between the fat test of the cream and the mold content.

Table 10 reveals that 31.6 percent of the samples having a butterfat test of 20 to 24 percent were in No. 1 visual mold class, while 61.1 percent of the samples with a fat test of 45 to 50 percent were in No. 1 visual mold class. The situation is just reversed in the high mold content classes. For example 5.3 percent of those samples testing 20 and 24 percent fat were placed in No. 7 visual mold class and 0.0 percent of samples testing 45 to 50 percent fell in the No. 7 visual mold class. The tendency for a larger percentage of the low testing samples (below 30 percent) to contain excessive quantities of mold may be partially explained by the fact that some of the farmers producing low testing cream were skimming their milk by hand.

Table 10. Relationship of butterfat test of cream to the visual mold score (1173 samples)

Visual mold score	Butterfat test of cream - Percent								
	15-19 % of group	20-24 % of group	25-29 % of group	30-34 % of group	35-39 % of group	40-44 % of group	45-50 % of group	50-54 % of group	Total No. of Samples
1	40	31.6	44.1	45.8	51.7	59.8	61.1	40.0	614
2		10.5	11.8	19.6	16.7	15.8	14.9	13.3	193
3		21.0	16.2	17.7	14.1	12.4	11.6	26.7	171
4	60	10.5	14.7	10.7	11.0	7.2	8.3	6.7	118
5		5.3	8.8	2.2	3.7	3.5	4.1	13.3	44
6		15.8		1.8	1.8	1.0			18
7		5.3	4.4	2.2	1.0	0.3			15
Total No. of samples	5	19	68	271	383	291	121	15	1173

The results of this study show a slight tendency for the mold content of cream to decrease as the butterfat test increases.

The Relation of the Size of Delivery to the Visual Mold Score

An attempt was made in this portion of the study to determine the relative importance of the quantity of cream produced by each of a selected group of dairymen in determining the amount of mold mycelia in the cream.

The weight of each of 1173 lots of cream delivered at three cream buying stations **was** secured from the station records and the effect of this factor upon the usual mold content was studied. These data are shown in Table 11 distributed according to percent of samples falling in each visual mold class.

The relationship of size of delivery to visual mold content of cream is not marked, and the increase in percent of samples falling in the No. 1 visual mold class does not follow regularly the increase in weight of deliveries. Perhaps the most striking point brought by Table 11 is the fact that a disproportionately large number of lots of cream delivered in quantities weighing less than 20 pounds contained excessive quantities of mold. A similar though less pronounced tendency was apparent among deliveries weighing 20 to 40 pounds. Of the deliveries containing one to nine pounds of cream 6.0 percent fell in visual mold class No. 5, 6.9 percent in No. 6 and 5.2 percent in No. 7, giving a total of 18.0 percent having an excessive mold content. In contrast to this, only 2.6 percent of deliveries containing 40 to 49 pounds of cream contained excessive quantities of mold.

The high mold content of the lots of cream weighing less than 40 pounds may be due in part to the existence of a higher surface to volume ratio, with the result that more oxygen is available for mold growth per unit volume of cream. Another point of some importance is the fact that some producers having small quantities of cream use the gravity creaming method of separation.

Table 11. Relationship of size of delivery to the visual mold score (1173 samples)

Visual mold score	Pounds of cream in each delivery											Total
	1-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-99	100 & over	
	% of group	% of group	% of group	% of group	% of group	% of group	% of group	% of group	% of group	% of group	% of group	No. of Samples
1	48.3	50.0	57.3	44.7	61.0	43.6	51.0	48.2	56.1	33.3	70.6	614
2	11.2	16.7	12.6	13.7	19.5	21.8	26.5	18.5	24.6	33.3	11.8	193
3	15.5	12.4	12.1	20.5	10.8	20.0	18.4	18.5	14.0	33.4	11.8	171
4	6.9	10.5	13.6	14.7	6.1	10.9	4.1	11.1	5.3		5.8	118
5	6.0	5.4	3.9	3.7	2.6	3.7		3.7				44
6	6.9	2.3	0.5	1.6								18
7	5.2	2.7		1.1								15
Total No. of samples	116	258	206	190	195	55	49	27	57	3	17	1173

The size of delivery, while of some significance with respect to excessive mold scores, was not a major factor in determining the mold content of the cream studied.

The Relation of the Titratable Acidity of the Cream to the Visual Mold Score

The titratable acidity of cream has been widely used as a quality test in grading cream for buttermaking. Since the visual mold test is now coming into general use for the detection of unfit cream, it seemed desirable to find out whether the acid test is supplementary or complementary in its relationship to the visual mold test.

The titratable acidities of 1166 samples of cream were determined and compared with the visual mold scores of those creams in this work. The results grouped in Table 12 show that a close relationship exists between acidity of cream and the mold content.

Table 12. Relationship of titratable acidity to the visual mold score (1166 samples)

Visual mold score	Percent titratable acidity of cream: Less than 0.20 % of group	.20 to .49 % of group	.50 to .69 % of group	.70 to .89 % of group	.90 to 1.09 % of group	1.1 & over % of group	Total No. of Samples
1	100.0	79.1	46.8	14.5	5.5		614
2		12.1	19.4	13.7	22.2	25.0	193
3		6.2	16.1	27.4	16.7	50.0	167
4		2.3	11.1	23.9	11.1	25.00	114
5		0.3	4.7	11.1	5.6		47
6			1.3	3.4	22.2		17
7			0.6	6.0	16.7		14
Total No. of samples		340	677	117	18	4	1166

This table shows that 79.1 percent of the samples containing .20 to .49 percent acid had a No. 1 visual mold score, while only 14.5 percent of the samples containing .70 to .89 percent acid scored No. 1. A pronounced increase in the percentage of samples having a high mold content occurs at .50 percent acidity and continues through the acidity range of .90 to 1.09. Above this range adequate data is lacking because of the small number of samples. In the .20 to .49 percent acidity range 0.3 percent of the samples were classed as No. 5 or over (excessive) in mold content, while in the acidity range of .70 to .89 this figure increased to 20.5 percent.

A close relationship was found between the acidity of the cream and the visual mold content. Cream having a titratable acidity of .50 percent or more contained excessive quantities of mold much more frequently than cream containing less than .50 percent acid. These results corroborate the basic assumption of the mold mycelia test; e. g., that if conditions are such that molds can develop, other microorganisms which may cause much more serious changes also could have developed.

The Relation of the Grade of the Cream to the Mold and Yeast Content

<u>Relation of the grade of the cream to the visual mold score</u>. If the visual mold test is to be a satisfactory method of segregating unfit cream from the good cream, it must give results closely related to the organoleptic grade of the cream.

The grades and visual mold scores on 1173 lots of cream were used in studying this relationship. There were 665 samples of grade No. 1 cream and 508 samples of grade No. 2 cream. The data were grouped according to percentage of samples falling in each visual mold class and are shown in Table 13.

Table 13. Relationship of the grade of cream to the visual mold score (1173 samples)

Grade of Cream	Visual mold score 1	2	3	4	5	6	7	Total No. of Samples
	Percent of samples							
1st	66.8	15.4	10.5	6.0	1.0		0.3	665
2nd	33.5	16.5	19.5	16.5	7.5	3.9	2.6	508
Total No. of samples	614	186	169	124	45	20	15	1173

These results show that 66.8 percent of all first grade samples fell in the No. 1 visual mold class, while only 33.5 percent of all second grade samples were classed under visual mold score No. 1. Only 1.3 percent of the samples of cream graded as first were found to contain excessive amounts of mold, while 14.0 percent of second grade samples were placed in mold classes No. 5, 6 and 7 (excessive). The mold alone is not generally considered to be the important cause of change in cream quality, therefore these data emphasize the fact that where mold has developed other organisms will also have had favorable growth conditions.

The percent of samples of first and second grade cream falling in the mold classes No. 2 and No. 3 is shown in Table 13 to be relatively close together. This brings out the fact that a lack of mold growth does not necessarily indicate that other organisms have not developed, since conditions may have been favorable for such organisms and not for the mold.

The visual mold content of the cream studied was not closely related to the grade of the cream, but may be used as supplementary to organoleptic grading of the cream.

The relation of grade of cream to the mold count (plate method). Since the visual mold content of the cream was shown in Table 13 to be related to some extent to the grade of the cream, it might be expected that the plate count should show a similar relationship. Plate counts were made on 605 samples of first grade cream and 434 samples of second grade cream in this phase of the work. The results were grouped on basis of percent of samples falling in each mold count range in Table 14.

Table 14. Relationship of the grade of the cream to the mold count by the plate method. (1039 samples)

Grade of Cream	Mold count by the plate method									Total No. of Samples
	Less than 10M*	10M to 20M	30M to 99M	100M to 199M	200M to 499M	500M to 999M	1000M to 1999M	2000M to 2999M	3000M and over	
	Percent of samples									
1st	69.9	8.3	9.9	4.5	3.8	1.5	1.3	0.3	0.5	605
2nd	38.0	7.6	10.8	8.8	11.3	11.3	6.9	3.9	1.4	434
Total No. of samples	588	83	107	65	71	58	38	19	9	1039

*M = one thousand.

This table shows that the relationship between the mold count by the plate method and the grade of the cream is not marked, and is quite similar to the relation brought out in Table 13. In Table 14, 69.9 percent of the first grade samples are shown to have contained a mold count of less than 10,000 per ml. and 0.5 percent of the first grade contained a mold count of over 3,000,000 per ml. Of the second grade samples 38.0 percent had a mold count of less than 10,000 per ml. and 1.4 percent showed a mold count of over 3,000,000 per ml.

From these results it appears that the mold count by the plate method is no more closely related to the grade of the cream than is the visual mold content. Neither, however, can be considered as being closely related to the grade of the cream.

Relation of the grade of the cream to the yeast count (plate method). The yeast counts on the samples of cream plated on acidified potato dextrose agar were found to vary from less than 100 per ml. to approximately 26,000,000 per ml. The yeast count was almost always found to be higher than the mold count on a given sample of cream. The purpose of this phase of the work was to find what relationship existed between the yeast count of the cream and the grade. The yeast counts on 1035 samples of cream were available for this study. Table 15 shows the percentage distribution of the samples according to the yeast counts.

This table shows that 51.5 percent of the grade No. 1 samples had a yeast count of less than 10,000 per ml. and 0.6 per cent had a yeast count of over 3,000,000 per ml., while 30.1 percent of the grade No. 2 samples had a yeast count of less than 10,000 per ml. and 5.2 percent had a count of over 3,000,000 per ml.

A fair relationship was found between the yeast count and the grade of the cream samples studied. Indications were that the yeast count was almost as satisfactory as an indication of the grade of the cream as was the mold count (plate method).

Table 15. Relationship of grade of cream to the yeast count by the plate method (1035 samples)

Grade of Cream	Yeast count by the plate method: Less than 10M*	10M to 29M	30M to 99M	100M to 199M	200M to 499M	500M to 999M	1000M to 1999M	2000M to 2999M	3000M and over	Total No. of Samples
1st	51.5	14.1	13.1	6.6	6.6	2.5	3.8	1.2	0.6	610
2nd	30.1	14.6	13.9	6.1	9.4	8.7	8.5	3.5	5.2	425
Total No. of samples	442	148	140	66	80	52	59	22	26	1035

*M = one thousand

<u>The relation of the mold count (plate method) to the yeast count (plate method).</u> In counting the yeast and mold colonies following plating of the cream samples on acidified potato dextrose agar, the question arose as to whether a relationship existed between the mold content and the yeast content of the cream. The mold counts and yeast counts on 1035 samples of cream are shown in Table 16 grouped according to the number of samples in each count range.

This table shows that there is little if any relationship between the mold and yeast count of average producer's cream. It does, however, reveal the tendency for the yeast count of a given sample of cream to be higher than the mold count. For example, 67 samples of cream having a mold count of less than 10,000 per ml. had a yeast count of 30,000 to 99,000 per ml., while only 19 samples of cream with a yeast count of less than 10,000 per ml. had a mold count of 30,000 to 99,000 per ml.

Table 16. Relationship of the mold count to the yeast count (1035 samples)

Yeast count Plate method	Mold count per ml. by the plate method: Less than 10M*	10M to 29M	30M to 99M	100M to 199M	200M to 499M	500M to 999M	1000M to 1999M	2000M to 2999M	3000M and over	Total No. of Samples
Less than 10M	352	20	19	16	13	9	8	3	2	442
10M to 29M	87	16	10	8	7	8	6	5		147
30M to 99M	67	15	20	11	12	5	3	3	2	138
100M to 199M	27	10	9	5	4	7	2		2	66
200M to 499M	33	5	11	11	6	9	7	3		85
500M to 999M	7	10	3	7	9	6	5	1	1	49
1000M to 1999M	11	5	11	6	16	3	8	2		62
2000M to 2999M	6	2	3		3	5	1			20
3000M and over	7		7	3	2	3	1	1	2	26
Total No. of Samples	597	83	93	67	72	55	41	18	9	1035

*M = one thousand.

A Study of the Relationship of Farm Production Methods to Cream Quality

In examining samples of cream being sold at the cream stations, marked differences in quality of cream produced by different dairymen were noted in all seasons of the year. To obtain information which might clarify some of the variations observed, 51 producers were interviewed in regard to the methods used in the production of their cream. A standard questionnaire was employed. Some of the practices followed by each of these dairymen and a record of the quality of cream produced is shown in Table 17.

In this table the average visual mold scores and the average grades of the cream have been calculated for the high temperature periods and the low temperature periods. The warm periods included the months of September, October, April and May while the cold periods were December and February. The average age of the cream marketed by each dairyman is also shown. The effect of atmospheric temperature upon the visual mold score of the cream is very clearly shown. During the period in which the mean atmospheric temperature was 64°F. the average visual mold content of the cream was 2.7, while the average score during the period having a mean temperature of 32°F. was 1.4. There was no significant difference in the average grades of the cream produced in the two temperature periods.

Table 17 also brings out certain other points of interest. A limited relationship is shown to exist between the methods

Table 17. Record of production methods and quality of cream produced by 51 dairymen.

Patron Number	Number of cows	No. of times separator was washed each day	Method of straining	Method of cooling	Place of holding	No. of deliveries	Average weight of delivery lbs.	Average visual mold score Mean temperature 64°F.	32°F.	Average grade Mean temperature 64°F.	32°F.	Average age in days
1A	12	1	cloth	water	cooling tank	27	50	1.2	1	1	1.1	2.8
2A	4	1	wire screen	water	cooling tank	11	25.2	2.9	1	1.3	1.3	4.8
3A	9	1	cloth	water in summer	cellar	20	35	2.2	1	1.3	1.2	5.2
4A	8	1	cloth	air	cellar	16	36	3.5	2.7	1.5	1.7	6.2
6A	3	2	wire screen	air	cellar	9	24	2.9	1.25	1.25	1.00	5.3
8A	2	1	wire screen	air	cave	11	13	3.7	1.00	2.0	2.0	6.3
9A	2	2	wire screen	air	porch	13	12	2.2	1.25	1.4	1.5	5.9
14A	5	1	wire screen	air	house, cellar	7	18	1.75	1.00	1.25	1.5	6.5
16A	6	1	cloth	air	porch, cellar	8	36	3.00	2.00	1.8	1.5	6.9
17A	3	1	wire screen	air	cellar	8	31.5	4.2	3.5	1.8	2.0	6.9
18A	4	1	cloth	water	cellar	11	15	2.1	1.0	1.7	1.5	5.8
19A	16	1	cotton pad	air	milk house	32	44	1.2	1.0	1.15	1.1	2.0
21A	7	1	cloth	air	cellar	12	43	1.9	1.0	1.0	1.0	6.1
28A	5	1	wire screen	air	house	32	14	1.2	1.0	1.2	1.1	2.9
32A	3	1	wire screen	air	cave	9	32	2.5	1.0	1.5	1.0	5.2
40A	20	2	cloth	water	porch, cellar	11	44	3.4	3.0	1.9	2.0	6.6
42A	10	2	wire screen	air	house, cellar	9	46	3.1	2.0	1.3	1.5	5.6
44A	5	1	cloth	water in summer	cooling tank, porch	11	22	3.7	1.0	1.8	1.25	6.6
45A	3	2	cloth	air	ice box	7	29	3.0	1.0	2.0	1.00	7.1
46A	5	1	wire screen	water	cellar	9	31	3.3	1.0	1.7	1.00	5.9
50A	8	1	wire screen	water	house, cellar	18	48	2.2	1.0	1.3	1.4	4.9
54A	3	1	not strained	air	porch	2	36.5	---	1.5	---	2.0	7.0
55A	5	1	wire screen	air	cellar	10	37	1.3	1.3	1.8	1.0	5.9
58A	4	1	cloth	air	porch, cellar	9	34	2.4	1.0	1.2	1.25	5.4
59A	2	1	wire screen	air	porch	6	22	5.7	1.0	1.7	1.3	7.0
61A	8	1	cotton pad	air	cellar	8	48	3.2	2.7	1.6	1.2	7.0
65A	3	1	wire screen	air	cellar	4	28	---	1.4	---	1.2	6.2
66A	1		wire screen	air	cellar	10	5.6	5.7	4.3	1.9	2.0	6.3
67A	2	2	wire screen	air	refrigerator	7	10.9	4.5	1.0	1.7	1.3	4.7
68A	16	1	cloth	air	house, cellar	12	103.0	1.9	1.0	1.2	1.0	4.0
70A	6	1	cloth	air	porch	2	17.0	---	2.5	---	2.0	6.0
71A	9	1	cloth	air	cellar	11	48.9	2.1	1.0	1.3	1.4	4.5
73A	2	1	cloth	air	cellar	4	11.2	5	1.3	2	1.0	3.3
74A	5	1	wire screen	air	house	3	28.0	---	1.0	---	1.3	7.0
75A	2	2	cloth	air	house, cellar	2	15.5	---	1.0	---	1.0	4.5
76A	2	1	cotton pad	air	cellar	2	31.5	4.0	1.0	2.0	1.0	5.0
77A	2	1	cotton pad	water	refrigerator	1	11.0	---	2.5	---	2.0	7.0
78A	5	1	cotton pad	air	cellar	11	36.7	3.3	2.7	1.9	2.0	5.0
80A	4	1	cotton pad	air	cellar	10	27.9	1.9	1.0	1.5	2.0	5.7
82A	2	1	cloth	air	porch	14	17.2	1.8	1.3	1.5	1.0	4.0

Table 17 (Continued)

Patron Number	Number of cows	No. of times separator was washed each day	Method of straining	Method of cooling	Place of holding	No. of deliveries	Average weight of delivery lbs.	Average visual mold score, Mean temperature 64°F.	Average visual mold score, Mean temperature 32°F.	Average grade, Mean temperature 64°F.	Average grade, Mean temperature 32°F.	Average age in days
87A	5	1	cotton pad	water	house	7	22.7	4.3	1.5	2.0	2.0	7.0
88A	1	2	cloth	air	refrigerator	8	9.1	1.6	1.0	1.7	1.0	5.9
89A	4	1	wire screen	air	porch	2	40.5	1.0	1.0	1.0	2.0	7.0
90A	8	1	wire screen	air	cellar	6	18.0	3.2	1.0	1.6	2.0	4.8
91A	5	1	cloth	air	cellar	2	40.0	1.0	1.0	1.0	2.0	7.0
92A	4	2	wire screen	air	cave	5	40.0	1.0	1.0	1.5	2.0	7.8
93A	10	1	cloth	air	cellar	11	62.7	1.4	1.0	1.2	1.5	3.0
94A	2	1	cloth	water	porch	2	13.5	3.5	---	1.5	---	7.5
96A	8	1	wire screen	air	cave	5	70.2	3.2	---	1.8	---	5.4
101A	8	2	wire screen	air	cellar	6	50.5	2.7	---	1.7	---	2.5
29B	9	2	cotton pad	refrigerator	refrigerator	10	21.4	1.2	---	1.1	---	2.4

used in production, as related by the producers, and the quality of the cream. If properly handled, cream which has been cooled in water should be lower in mold content than cream which has been cooled in air. In this study it was found that the cream which was cooled in water often had a higher mold content than other cream. The explanation for this fact seems to be that in almost every instance where water was used for cooling the producer was making deliveries only about once each week. In some cases the cream was held in the house or some similar place after the cream had been cooled in water, thus allowing the temperature to rise again. These points are clearly illustrated in the case of patron No. 87 who cooled the cream in water, then held it in the house under atmospheric conditions. Deliveries were made once each week. The result was grade No. 2 cream and an average visual mold score of 4.3.

The number of times which the cream separators were washed each day appeared to have no significant effect on the mold content of the cream, at least it was overshadowed by such more important factors as temperature and age. Such lack of relationship between methods and the quality of the cream suggests that the personal factor has been very important.

The variations in quality of cream produced by three representative dairymen are shown in Table 18. The records on individual lots of cream marketed by patrons 1A, 44A, and 66A are shown. By referring to Table 17 to obtain the information on methods of production used by these dairymen and comparing this with the results in Table 18 an interesting picture may be

Table 18. Records of certain cream producers showing variations in quality factors during this study.

Date		Lbs. of Cream	Age in days	Grade	Mold Score	Percent Acidity	Mean temperature during collection - °F.
Patron - 1-A							
1940							
Sept.	28	79	4	1	1	.59	56
Oct.	2	41	2	1	1	.53	64
	7	104	5	2	1	.59	68
	9	40	2	1	2	.52	57
	11	40	2	1	1	.56	63
	14	40	3	1	1	.56	69
	17	40	3	1	1	.60	54
	21	40	3	1	1	.59	65
	24	64	3	1	1	.66	71
Dec.	10	41	2	1	1	.40	43
	14	65	4	1	1	.35	27
1941							
Feb.	15	66	4	1	1	.44	38
	20	41	5	1	1	.46	33
	24	65	4	2		.45	24
	25	41	1	1	1	.34	28
	28	64	3	1	1	.27	25
Apr.	19	41	2	1	1	.47	65
	21	41	2	1	1	.40	46
	23	65	2	1	1	.48	56
	28	13	1	1	1	.50	63
May	2	40	4	1	1	.47	61
	17	81	2	1	1	.52	69
	19	40	2	2	1	.44	70
	22	64	3	1	2	.53	71
	24	41	2	1	1	.47	62
	29	83	5	1	2	.45	73
	31	40	2	1	1	.46	76
Patron - 44-A							
1940							
Oct.	5	25	7	2	5	.65	66
	26	12	7	2	3	.74	70
Dec.	7	17	7	1	1	.50	37
	14	20	7	1	1	.26	37
	21	19	7	1	1	.40	25
1941							
Feb.	26	23	7	2	1	.49	25
Apr.	26	32	7	2	2	.53	55
May	3	32	7	2	2	.50	62
	17	16	3	1	1	.49	73
	24	29	7	2	4	.50	64
	31	30	7	2	7	.58	74

Table 18 (Continued)

Date		Lbs. of Cream	Age in days	Grade	Mold Score	Percent Acidity	Mean temperature during collection - °F.
				Patron - 66-A			
1940							
Dec.	7	7	7	2	7	.99	37
	14	5	7	2	5	.81	37
1941							
Feb.	22	7	7	2	1	.48	28
Apr.	19	7	7	1	7	.69	60
	26	6	7	2	6	.70	55
May	3	6	7	2	6	.72	62
	17	2	7	2	3	.78	68
	23	6	6	2	7	.70	70
	28	6	5	2	7	.76	69
	31	4	3	2	4	.71	77

obtained. For example patron 1A cooled and held his cream in water and made deliveries in an average of about every three days. As a result the mold content and the grade of his cream is quite constant throughout the changing temperature periods. On the other hand patron 66A cooled his cream in the air and delivered it only about once each week. Low grade, high mold content and high acidity resulted. The high mold content even during low temperature periods indicate that the cream must have been held inside during cold weather.

In the study of production methods among this group of producers it was found that few had knowledge of sanitary production methods or even of satisfactory cooling procedures. Production of cream was not a major interest with the majority of these dairymen, and cream delivery was often neglected. Most of the producers were willing to cooperate in improving cream quality. More frequent delivery or regular pick up by routes appeared to be the most feasible method for improving the quality of the cream.

Effect of Holding Cream in the Buying Station upon Some Quality Factors Including Mold Content

Since the visual mold test is used by many creameries for segregating cream which is unfit for buttermaking due to high mold content, it is desirable to determine what changes, if any, take place in the mold content of cream from the time it is purchased from the producer until it is shipped to the creamery.

In the first portion of this work 75 cans of cream were examined before and after holding in 10 gallon quantities under atmospheric temperature conditions in the cream station for 24 to 48 hours. During this period there was an average increase in the visual mold score of only 0.03 indicating no significant change had occurred. The titratable acidities were run on only 46 of these lots of cream and an average increase of .15 percent was noted.

Following these preliminary studies a more detailed examination was made of 38 cans of cream. Storage temperature, grade, titratable acidities, mold and yeast counts by plate method, air space in cans and the visual mold scores were determined on each lot of cream before and after holding in the cream station. The air space was found to be fairly constant at 2 to 3 inches and the variation was not large enough to have noticeable affect upon results. Table 19 shows a detailed report of the studies made on each sample while Tables 20 and

Table 19. Changes taking place during storage of cream in ten gallon cans in the cream buying stations.

Sample Number	Days in storage	Temperature in °F.	Temperature out °F.	Grade in	Grade out	Criticism in	Criticism out	Percent Titratable acidity in	Percent Titratable acidity out	Percent Titratable acidity increase	Mold count - plate method in	Mold count - plate method out	Mold count - plate method Percent change	Yeast count - plate method in	Yeast count - plate method out	Yeast count - plate method Percent change	Visual mold score in	Visual mold score out
1	2	70	--	2	2	H.A*., feed, slight bitter	H. A., musty, metallic	.62	.91	.29	115,000	100,000	-13	850,000	420,000	-51	3	2
2	2	70	75	2	3	H.A., feed, metallic	H. A., cheesy	.64	.83	.19	1,650,000	1,450,000	-12	2,950,000	2,500,000	-15	5	4
3	2	70	76	2	2	Metallic, stale	H. A., stale, unclean	.64	.79	.15	950,000	1,000,000	+5	1,565,000	2,650,000	+69	4	3
4	2	70	--	1-	2	Musty	Musty, metallic	.54	.93	.39	125,000	65,000	-48	1,030,000	690,000	-33	3	2
5	2	70	75	1	1	Slight stale	Stale	.54	.66	.12	16,500	100	-99	15,500	2,200	-86	1	1
6	2	70	74	1	1-	Feed, stale	Stale bitter	.58	.70	.12	113,000	81,000	-28	140,000	159,500	+14	2	2
7	2	69	--	1	1-	Stale	Slight unclean	.56	.67	.11	165,000	265,000	+61	55,000	31,500	-43	3	2
8	2	70	74	1	1-	Feed	H. A., weeds	.55	.64	.09	135,000	120,000	-11	50,000	65,000	+30	3	2
9	2	68	74	1	2	H. A., feed	H. A., unclean	.62	.89	.27	150,000	215,000	+43	305,000	1,000,000	+228	3	3
10	2	68	80	2	3	H. A., metallic	H. A., yeasty	.86	1.04	.18	40,000	45,000	+12	5,700,000	8,900,000	+56	4	3
11	2	70	75	2	2	H. A., stale, metallic	H. A., metallic	.65	1.0	.35	495,000	360,000	-27	40,000	21,000	-47	3	3
12	2	70	76	2	2	Slight unclean, stale	H. A., unclean	.66	.96	.30	200,000	140,000	-30	2,000	13,500	+575	3	3
13	2	70	--	1	2	H. A., stale	H. A., stale	.66	1.04	.38	140,000	145,000	+11	14,500	19,000	+31	3	3
14	2	72	74	1	2	H. A., stale	H. A., metallic, unclean	.56	1.05	.49	145,000	145,000	none	11,000	20,000	+82	3	3
15	2	72	74	1	2	H. A., thin	H. A., stale	.59	.88	.29	205,000	100,000	-51	2,150,000	2,250,000	+5	3	3
16	2	68	74	1-	2	Slight musty, slight unclean	Unclean	.57	.76	.19	90,000	40,000	-56	1,570,000	830,000	-47	3	2
1c	2	75	76	1	2	Musty	Very musty, metallic	.52	.65	.13	700	73,000	+10,329	14,000	55,500	+296	1	1
2c	2	74	76	1	2	Stale	H. A., metallic, unclean	.53	.78	.25	30,000	70,000	+133	1,090,000	715,000	-344	2	2
3c	2	76	76	1	2	Slight unclean	Stale, metallic, unclean	.54	.66	.12	13,500	180,000	+1233	32,000	275,000	+759	1	1
4c	2	76	76	1	1	Stale	Stale, slight metallic	.59	.68	.09	36,000	135,000	+275	540,000	1,275,000	+136	2	3
5c	2	76	74	1	1	Slight unclean	Slight unclean	.55	.66	.11	80,000	127,500	+59	30,000	48,500	+62	3	3
6c	2	76	76	1	2	H. A., slight cheesy	H. A., musty metallic	.68	1.04	.36	280,000	260,000	-7	525,000	750,000	+45	3	3
7c	2	76	76	1	2	H. A.	H. A., slight unclean	.72	1.35	.63	130,000	73,500	-43	8,500	19,000	+124	3	3
8c	2	100	77	1-	2	H. A., bitter	H. A., stale	.53	1.13	.60	60,000	25,000	-58	500,000	595,000	+19	4	4
9c	2	78	76	1	1-	Stale	Musty, bitter	.48	.69	.21	500	90,000	+1790	18,000	17,000	-6	1	2
10c	2	72	76	1-	2	H. A.	H. A., bitter	.57	.78	.21	18,000	46,000	+156	73,000	110,000	+51	1	2
1h	1	82	78	1	1	Stale, slight unclean	Stale, slight unclean	.65	.77	.12	21,000	36,500	+74	340,000	645,000	+90	1	2
2h	1	78	78	1	1	Stale	H. A., stale	.56	.94	.38	1,005,000	530,000	-47	80,000	200,000	+150	4	3
3h	1	80	78	1	1	H. A.	H. A., musty	.57	.77	.20	55,000	315,000	+473	550,000	40,000	-93	2	3
4h	1	78	78	1	1	Slight Stale	Stale	.57	.63	.06	30,000	435,000	+1350	135,000	425,000	+215	1	1
5h	1	78	78	2	2	H. A, metallic, unclean	H. A., metallic, yeasty	.74	1.08	.34	110,000	190,000	+73	755,000	1,425,000	+89	2	2
6h	1	80	78	1-	1-	Metallic, stale	Metallic, stale	.65	.74	.09	30,000	36,500	+22	680,000	650,000	-4	2	2
7h	1	80	78	1	2	H. A., Stale, Slight metallic	H. A., stale, metallic	.67	.96	.29	70,000	36,000	-49	145,000	365,000	+152	2	2
8h	1	80	78	1	1	Slight metallic	H. A., Slight metallic	.59	.98	.39	58,500	120,000	+105	166,500	475,000	+185	2	3
9h	1	78	80	1	1-	Slight unclean, H. A.	H. A., unclean	.64	.76	.12	113,000	140,000	+24	90,000	265,000	+194	2	3
10h	1	76	78	1	2	Stale	H. A., stale, metallic	.55	1.05	.50	420,000	485,000	+15	155,000	190,000	+23	3	3
11h	1	78	78	2	2	Musty, metallic	H. A., musty, metallic	.66	.73	.07	75,000	105,000	+44	280,000	880,000	+214	3	3
12h	1	78	78	1	2	Slight bitter	H. A., metallic	.56	.70	.14	9,000	100,000	+1011	44,000	85,000	+93	1	2

*High acid.

21 show a summary of the changes which took place in visual mold score, grade, and titratable acidity.

Table 20. Change in visual mold score of cream during holding period in the cream buying station (38 cans).

No. of cans	Visual mold score						
	1	2	3	4	5	6	7
in	8	9	16	4	1		
out	4	14	18	2	0		

Table 21. Changes in grade of cream and titratable acidity during holding period in the cream buying station (38 cans).

Grade of Cream	No. of cans of cream in	No. of cans of cream out	Average increase in titratable acidity during holding period
1st	25	8	.20%
1-	5	6	.12%
2nd	8	22	.31%
3rd	0	2	.18%

These data show that a rapid increase in acidity took place in nearly every instance, the average increase amounting to .24 percent. A rapid change in grade of cream also occurred. At the beginning of the holding period 25 of the cans of cream were graded as first, 5 cans as No. 1-, and 8 cans as second, while at the close of the holding period 8 cans were graded first, 6 as No. 1-, 22 as second, and 2 as third grade. During this time the number of lots of cream classed as doubtful to excessive by the visual mold test was found to have decreased from 5 to 2. Both lots of cream which were third grade at the end of the holding period dropped one point in vusual mold score during storage.

The mold counts showed that out of 19 cans having a mold count of 100,000 or more per ml. at the beginning of the holding period, 11 decreased in count. Of the 22 lots of cream having a yeast count of 100,000 or more per ml. only five showed a decrease during the storage period.

These studies show that the quality of the cream dropped very rapidly during the storage period, while the mold content did not change appreciably. This situation caused the visual mold test to be an unsatisfactory method for detecting fitness of cream for buttermaking at the time the cream was shipped to the creamery from the station. However, as shown in earlier sections, the method is of considerable value when used as an aid in grading cream as it comes from the farms.

Some Observations on the Sources of *Oospora lactis*

The presence of numerous *O. lactis* in almost every sample of cream examined led to this partial study of the sources of that organism.

O. lactis was isolated from a variety of sources including hands of dairymen, separator discs, cream cans, milk pails, flanks of cows, air of stable, silage, manure, sorgo fodder, and rumens of cows. The organism was found most frequently and in largest numbers in fresh manure. Plate counts in the range of 30,000 per gram were noted in four out of 12 trials, while counts of approximately 15,000 per gm. were obtained in six instances. No *O. lactis* were found in the other two samples. The samples were collected and handled with sterile equipment to reduce possible contamination and they were plated on acidified potato dextrose agar within a short time after collection.

Six samples of rumen contents were collected from six cows on two occasions and plated on wort agar. The first series of six samples showed the presence of O. lactis in each case. The mold counts ranged from 200 to 5,000 per ml. of rumen contents. Plating of the second series failed to show the presence of any O. lactis in 0.1 ml. quantities of rumen contents. The reason for the absence of O. lactis in the second series is not known. The cows were fed the same ration throughout both periods in which the samples were taken.

O. lactis was found infrequently on separator discs, cream cans, milk pails, flanks of cows and silage. When the organism did appear, it was in very limited numbers, often only one or two colonies developing on an agar disc 2.5 inches in diameter, or on a plate containing 5 ml. of a 100 ml. water rinse. The organism was isolated from stable air only once out of 13 trials by exposing acidified potato dextrose plates for five minutes. No O. lactis were isolated from soil, grass, or fore milk from cows teats.

The results of this study showed that the mold O. lactis was frequently present in large numbers in fresh manure, but was seldom present in large numbers on utensils used in the production of cream. A number of examinations were made of utensils used on farms producing cream high in mold content with the result that few if any organisms were isolated. These observations indicate that the initial contamination of the cream with mold need not be large, provided the conditions for growth are favorable during the period of holding on the farm.

DISCUSSION

The study of the relation of the Parsons' visual mold test to the plating method revealed a good correlation, but it was found that samples having a low plate count were sometimes classed as doubtful or excessive by the visual test. This occurred more frequently than the classification of a high plate count sample in the good visual mold class. Recent studies by Garrison and Gholson (18, 19) show that the presence of a large number of body cells in some samples of cream may account for a doubtful or excessive visual mold score when the mold count is low. They state that this condition may have been due to infected udders or cows late in lactation.

The most important factors effecting the mold content of the cream were found to be temperature and age. A close relationship also existed between acidity and visual mold score indicating that the development of acid in the cream may have aided the growth of the mold. Sorenson (68) and Macy and Gibson (44) have reported that O. lactis grew over a wide range of acidities, but made best growth in a rather acid medium. The results in this study suggest that acidities of .50 to 1.0 percent may have aided the mold growth.

The results of this study agree with the others (2, 54, 64 77) in showing some relationship between butterfat test of cream and mold content. The relation is not significant, however, and it is clearly evident that the fat test alone is not very important. The serum content of cream is sufficient to supply abundant protein material for mold growth even though the fat test may approach 80 percent.

Gravity separation has recently been shown to result in a high visual mold test more often than when a centrifugal separator is used (17). The centrifugal separator is believed to remove many of the body cells and much of the mucous protein present in the milk. This material tends to be concentrated in the cream layer when gravity creaming is used and may result in a doubtful or excessive visual mold test even though the actual mold content is low (17, 18). This may partially explain the tendency for samples low in fat content to fall in the higher visual mold classes.

Although a close relationship was found between some of the factors studied in relation to visual mold score and mold content by the plate method, in all instances there was evidence to show that no single factor was responsible for the variations observed in mold content of the cream.

The study of production methods brought out the lack of sanitation and of cooling facilities in handling the cream. The results obtained from studying the sources of O. lactis indicate the possibility that a very small initial contamination may result in high mold content under favorable temperature conditions. The important consideration from the standpoint of sanitation, however, is to destroy by careful cleaning and sterilizing not only O. lactis but also those bacteria which attack the cream and damage quality much more rapidly than the mold O. lactis. The mold itself is not the important factor in bringing about rapid changes in cream quality.

No previous reports have been made regarding the effect of holding cream in the buying station on the mold content. It is well known that the common milk mold O. lactis requires oxygen for growth, therefore when the cream is held under essentially anaerobic conditions in ten gallon cans growth might be expected to be checked. During holding in the station the low count lots of cream often showed an increase in mold count by the plate method while those having a mold count above 100,000 per ml. at the beginning of the holding period tended to decrease in count during one and two day storage. The air supplied by the space in the top of the can probably accounts for the increases in mold counts observed. In a few instances a light felt of O. lactis was observed on the surface of the cream following the holding period. The bacterial flora of the cream undoubtedly was of importance in establishing anaerobic conditions beneath the surface of the cream.

The results obtained from the studies on effect of holding cream in the buying stations indicate that the use of the visual mold test as an index of the fitness of cream is limited to the examination of cream as received from the farms. If, however, the cream is cooled in the station and held at low temperatures until shipment is made, then the visual mold test probably may still be used with satisfactory results.

O. lactis was found to be present in practically all samples of cream examined in this study. Other genera of molds were encountered, however. Those most frequently observed were

Penicillium, *Aspergillus*, and *Mucor*, the latter being the most important type other than *O. lactis*. The appearance of mold clumps on the organdy discs was influenced somewhat by the type of mold which predominated the flora of the cream. Molds belonging to the family mucoraceae did not appear to stain quite as dark as did *O. lactis* and the mycelia appeared somewhat more filamentous on the disc.

Farm production methods were subject to criticism in almost all instances observed due to inefficient sterilizing and cooling practices. A large proportion of the producers did not have enough cream to pay them to make deliveries more frequently than once each week, therefore the most effective method of improving the cream quality would appear to be more frequent pick up of the cream by the use of trucks operated by the cream buying agencies.

SUMMARY AND CONCLUSIONS

1. A total of 1183 samples of cream was secured from deliveries made by a selected group of 136 producers to Manhattan cream stations. These samples were studied to determine the relationship of a variety of factors such as age, atmospheric temperature, acidity, fat content, and size of delivery to the mold content of the cream.
2. A standard questionnaire was used in interviewing 51 cream producers and an attempt was made to find the relation between the information on farm methods and quality of cream produced.
3. The effect of holding 113 cans of cream in 10 gallon cans in a cream station for 24 to 48 hours at atmospheric temperatures on the quality of the cream was studied.
4. A limited amount of information was collected relative to the sources of the milk mold _O. lactis_.
5. A close relationship existed between results obtained by the mold count (plate method) and the Parsons' visual mold test.
6. The atmospheric temperature was an important factor in controlling the mold content of the cream studied.
7. Atmospheric humidity had no significant effect upon the growth of mold in the cream.

8. A close relationship was found between the age of the cream and the visual mold score, also between the age of the cream and the grade.

9. The mold content of the cream was usually higher during the Fall and Spring months than during the winter months probably because of temperature.

10. The butterfat test of the cream was not closely related to the visual mold content.

11. The size of delivery was not important in its effect upon the visual mold content of the cream.

12. A close relationship was observed between the acidity of the cream and the visual mold content.

13. A fair relationship was found to exist between the grade of the cream and the visual mold score, also between the grade of the cream and the mold count by the plate method.

14. A fair relationship was also revealed between the yeast count by the plate method and the grade of the cream.

15. There was no apparent relationship between the mold count by the plate method and the yeast count.

16. The farm production methods observed were usually subject to criticism due to inefficient sterilization procedures, poor cooling methods, and failure to market cream frequently.

17. Cream with a mold content of 100,000 or more per ml. often showed a decrease in plate count during one to two day holding in 10 gallon cans at temperatures of 70°F.

or above. The visual mold score of cream held under similar conditions usually shows little change.

18. The visual mold test was not a satisfactory method for detecting fitness of cream for buttermaking after storage of the cream for two days in 10 gallon cans at temperatures of 70°F. or above in the cream station.

19. *Oospora lactis* was isolated most frequently and in largest numbers from fresh manure. Utensils used in production of cream seldom contained large numbers of this organism.

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