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by

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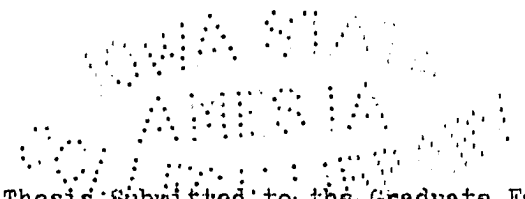

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INTRODUCTION

Coliform bacteria have demonstrated their value as indices of post-pasteurization contamination in the quality control of market milk and ice cream. Their usefulness has been recognized as a check on city and creamery water supplies and on various foods and food products. The literature contains very little information as to whether or not the coliform test can be used effectively to predict either the sanitary condition or the keeping quality of butter. Conditions in butter differ greatly from those in market milk or ice cream. The quality of the raw material used in the manufacture of butter often is poorer. The nature of the equipment used in its manufacture makes sterilization in the true sense impossible. Presence of salt, distribution of moisture and various other factors also play important roles.

It is not the number of organisms in butter that is important but their types. Butter of undoubtedly good keeping quality may give high bacterial and yeast and mold counts, while samples with very few bacteria may show serious spoilage. Failing the establishment of quick and accurate methods for the identification of specific bacterial types which cause defects or disease or indicate sanitation, recourse must be had to total counts and group counts for routine analysis. However, the limitations of such procedures must be appreciated.

The present studies were undertaken to establish whether coliform organisms and/or gram-negative bacteria can be used as an index of butter quality. An attempt was made to separate the microbial flora of commer-

cial butter into important groups as coliform, gram-negative, yeast and mold and total cultivatable population. The investigations were carried on to study the sources of organisms concerned, the effects of the organisms on the score of fresh butter and on its keeping quality, and also the numerical relationship of the various tests which have been applied to commercial butter. The changes in the population of coliform bacteria during storage were studied especially to throw more light on the use of these bacteria as a test for sanitary quality and/or probable keeping quality.

LITERATURE REVIEW

A considerable literature has accumulated dealing with various aspects of the coliform flora of milk and ice cream, but only a few investigations of the significance of these bacteria in other dairy products appear to be recorded. Some attention has been devoted to defective products, but few systematic investigations of the normal incidence of coliform types have appeared in the scientific literature.

Butter is a comparatively poor medium for the multiplication of bacteria. The low temperature of storage, the presence of salt, shortage of food material and a finer distribution of moisture are some of the important factors that govern the growth of micro-organisms in butter. An exhaustive review on the subject has been made by Hammer and Long (1941) and therefore no attempt will be made to repeat the review of literature here, only a few of the more relevant reports being cited as needed.

Hammer and Yale (1932) added identified cultures of coliform organisms isolated from a dairy product to laboratory pasteurized cream, which they churned into butter and held at 7 and 18°C. One portion was salted, the other unsalted. From 25 samples of off-flavored butter they isolated various species of Aerobacter but did not find any Escherichia species. Escherichia organisms were present in other series. In general they found that at 7°C. salt had a definite restraining action on the organisms of the Escherichia-Aerobacter group. In salted butter the Escherichia species did not grow in the 10-day period, and commonly

there was a decrease in numbers. With the Aerobacter species there was usually a decrease and then an increase in numbers. The increase in some instances resulted in counts higher than the original ones. In the unsalted butter, some of the Escherichia and all of the Aerobacter species studied grew, with the latter showing more growth than the former.

At about 18°C. salt had a restraining action on the organisms of the Escherichia-Aerobacter group, but growth occurred with all the cultures studied, in both the salted and the unsalted butter. The Aerobacter species grew more rapidly and reached higher numbers in butter than did the Escherichia species.

Yale (1933) studied 25 cultures of coliform organisms isolated by Hammer from defective butter and found that 88 percent belonged to various species of Aerobacter group and 12 percent to the intermediate group. He stated that absence of the Escherichia group indicates that conditions in butter are not favourable for growth or survival of this group at the temperatures at which butter ordinarily is held. The presence of species belonging to the genus Aerobacter in defective butter indicates that they may be responsible for certain defects. He found the most common species in defective butter was A. aerogenes.

Parfitt (1936) studied the frequency of occurrence of Escherichia-Aerobacter species in commercial butter and found the highest percentage of samples containing these organisms occurred in July and August and the lowest in January, although no definite seasonal trend could be

established. He did not find any relationship between the keeping quality of butter and the presence or absence of Escherichia-Aerobacter group. There was no relationship between yeast and mold count and coliform count.

Rice (1933) studied 181 samples of butter and found that 61 contained coliform organisms. Larger numbers of coliform organisms were present in samples that had higher total counts. There was no significant difference in score between samples highly contaminated with coliform organisms and those with fewer coliform bacteria; the same was true with respect to total counts.

Sjöstrom (1942) found that strains of B. aerogenes could grow better than B. coli in water and whey media with 2 per cent salt concentration.

Long, Hedrick and Hammer (1944) encountered heat-resistant coliform organisms in 1.0 or 0.1 ml. quantities of butter serum. They found that all resistant cultures were of the Escherichia type. Of the 220 samples from 77 plants, 143 from 65 plants contained E. coli. Most samples contained Escherichia or both Escherichia and Aerobacter types. Aerobacter types rarely were encountered alone.

Crossley (1944) examined factory plant surfaces by swab methods after treatment of these surfaces by hot water, steam and hypochlorite rinses or combinations of these. Coliform organisms were found on some swabs and milk souring organisms on 90 per cent of the surfaces in contact with raw milk, 50 per cent of hot milk surfaces and 25 per cent of post pasteurization equipment. The churn was the most important source of butter contamination. This investigator found that cream with no coliform bacteria in 1 cc. of material when placed in the churn

showed coliform bacteria in 0.01 cc. after six to eight revolutions. He also found these organisms in larger numbers in finished worked butter than in washed butter granules.

Crossley (1946) analyzed line run samples for coliform bacteria and found that the percentage of positive samples increased as the pasteurized cream proceeded ahead during the process of butter manufacture. Washed butter granules had the highest percentage of positive samples, and the number of such samples decreased when the butter was salted. He found that 57.0 per cent of 126 samples of English butter gave a positive coli test in varying quantities of material and concluded that coliform organisms were of common general occurrence in butter flora. The prevention of coliform contamination was rendered difficult by the very extensive use of wooden equipment which could not be sterilized by steam and also due to some inevitable manual contact. On the other hand this work also showed that it was practicable to maintain essential freedom from coliform contamination throughout the whole butter making process.

Crossley (1946) after studying a large number of samples of commercial butter stated that during prolonged cold storage a slow decline of the coliform population occurs, the extent depending on the degree of salting.

Robinton and Genung (1945) found that A. aerogenes increased to a greater extent in cream than did E. coli at a refrigeration temperature of 8°C.

The literature contains very few references regarding the effect of gram-negative organisms as a group on the fresh score or keeping quality

of butter. Nelson (1932) studied the keeping quality of 303 samples of commercial butter by holding them at 21°C. for 7 days. He made microscopic examinations both before and after holding to determine the types and numbers of organisms and tried to predict the keeping quality on the basis of his original slides. Large numbers of gram-negative rods commonly were associated with poor keeping quality. Keeping quality could be predicted in 96.4 per cent of commercial salted butter, 79.6 per cent of unsalted and 84.9 per cent of exhibition samples.

A good many gram-negative organisms have been shown to be responsible for various butter defects. Hammer (1948) has listed the Escherichia-Aerobacter group, Flavobacterium maloloris, Proteus ichthyosmius, Pseudomonas fragi, Pseudomonas mephitica, Pseudomonas nigrifaciens, Pseudomonas putrefaciens, Serratia marcescens as among the gram-negative bacteria which have been associated with various kinds of butter defects.

There is a great deal in the literature on the relationship of yeasts and molds to the quality of butter. Only a few of the more pertinent papers will be cited. Bouska and Brown (1921) found that number of yeasts and oidia was not a reliable index of the keeping quality of storage butter. However, other conditions being favourable, butter with fewer yeast and oidia would keep longer in storage than would butter with higher numbers of these microorganisms.

Parfitt (1926) analyzed 434 samples of butter for yeast and mold counts. He found no relationship between the number of molds per gram and the score of the butter.

Macy and Richie (1929) examined 597 samples of butter for yeasts

and molds and keeping quality. No consistent relationship was apparent between the mold or yeast counts and the quality of the fresh butter. Neither mold, yeast or total count of individual samples served as a reliable index of the keeping quality of the butter. Considered as a group, the samples of butter with the lower mold, yeast and total counts showed a tendency toward slightly better keeping quality than those with higher counts.

Grimes (1931) studied the total count and yeast and mold count of a large number of samples and found mold counts the best index of the flavor score of fresh butter. He drew up standards for the total microbiological counts for butter manufactured from sweet cream which are as follows:

	Excellent	Good	Fair	Bad
Total count per ml.	< 50,000	50,100-500,000	501,000-1,000,000	>1,000,000
yeasts " "	-	< 50	51-500	>500
molds " "	-	< 10	11- 50	>50

However, the samples in any one grade group actually showed a wide variation in the numbers of organisms of the various types, and no corrolation could be found between the results of microbiological analysis and the flavor score of butter two weeks old. It was recognized that these organisms were of significance as a guide to the sanitary conditions prevailing during manufacture.

The relationship between the total count, the score of butter and its keeping quality has been studied by many workers. Factors affecting

the total count of butter and those that govern the keeping quality are not always the same and hence lack of a definite relationship is not surprising. However, there is a greater probability of having a larger number of spoilage organisms in butter when the total count is high. It is natural therefore to expect a tendency in samples of butter with high total count towards deterioration, although low count samples will not insure high keeping quality.

Demeter and Maier (1931) noted that the total count on lactose agar demonstrated in a general way that the higher the total bacterial content the lower the grade. However, these general trends did not hold for individual samples. Working with yeast, mold, acidifying, non-acidifying, caseolytic and total counts, they concluded that the most useful determination for predicting the keeping quality of butter was the total count on the casein agar of Frazier and Rupp.

Nelson (1932) did not find any apparent correlation between the plate counts of the butter and the keeping quality. Some of his samples with high plate counts kept well and some with low counts deteriorated a great deal.

The ability of any given lot of butter to retain its desirable flavor and odor characteristics through regular trade channels and in the hands of the consumer is one of the most important requirements. Various workers have used holding tests to predict this quality. Bouska and Brown (1921) found that holding butter at 15.6 to 21°C. was very satisfactory in predicting the keeping quality. They reported that butter with poor keeping quality developed a bad flavor within 3 days,

while that with good keeping quality kept well for 2 weeks.

Jacobsen (1937) used the 7- to 10-day holding test at room temperature as an indication of keeping quality of butter in storage. He found that flavor deterioration in unsalted butter within 7 or 10 days at room temperature frequently indicated flavor deterioration in the corresponding butter held at lower temperatures, but a failure to show flavor deterioration at room temperature did not insure good keeping quality in the butter at lower temperature.

Jacobsen (1939) tried to determine the cause for lack of agreement between the 7-day holding test at 21°C. and the keeping quality of butter at 4.4°C. He found that salt content affected the activity of the organisms and salt above 1 per cent level allowed very little microbial deterioration. The proportion of lipolytic or proteolytic organisms was higher at lower temperatures. He concluded that lack of agreement between the holding test at room temperature and keeping quality at lower temperatures was due to the differences in the active bacterial flora at these temperatures. This was particularly true with butter containing less than 1 per cent salt. Similar conclusions were reached in a later article (Jacobsen, 1941).

Parsons (1933) found a 14-day holding test at 60°F. or a 7-day holding test at 70°F. useful in detection of butter of certain handling quality.

Naylor and Guthrie (1940) incubated salted and unsalted samples at 32 and 60°F. for 10 and 14 days. They found that the test at 60°F. was not perfect for predicting the keeping quality of high-grade butter,

but it did give a fairly good indication of what will happen. They did not find any difference in the keeping quality of unsalted and mildly salted butter made in the laboratory from highly pasteurized cream; but in commercial butter they found that unsalted samples deteriorated badly in all cases during the incubation test, while salted butter showed much better keeping quality.

Sorenson (1940) held 22,060 parchment-wrapped samples of butter at 68 to 70°F. for 7 days for keeping quality tests. He states, "A surprisingly close correlation between keeping quality tests and subsequent difficultywith the churnings tested was noted." He indicated the value of the test in locating contaminated water supplies or insanitary plant conditions.

Recently a number of laboratories have been using a 1 to 2 day's test at 98°F. for the evaluation of the keeping quality of butter. The results of properly controlled experiments with this procedure are not available in the literature.

Hammer (1943) suggested that temperature has a definite effect on the growth of organisms in butter and close correlation between deterioration at various temperatures cannot be expected. However, he states that keeping quality tests are helpful in detecting faulty methods of production, and their use is advisable until more adequate methods are devised.

Only pasteurized cream ordinarily is used in the manufacture of commercial butter and butter with a very low microbial count can be produced. Various workers have shown that pasteurization as is practiced

in the manufacture of commercial butter destroys the coliform organisms, yeast and mold and a marked portion of the total microflora. However, it is well known that finished butter often contains these microorganisms. A number of workers have tried to locate the sources of these organisms, where the cream or the butter picks up the contamination during the process of manufacture. Stiritz (1922) analysed raw cream, pasteurized cream, buttermilk and butter from the same churnings for yeast and mold. He fixed a standard of 30 colonies per ml. of finished butter and considered this test as an index of sanitary efficiency for the entire butter-making process. He suggested that the churn may be one of the biggest sources of contamination of the cream after pastourization.

The various sources of butter contamination are the vat, pumps, pipelines, churn, wash water, salt, liner paper, among others. Macy and Combs (1927) found churns to be the source of molds in 65 per cent, salt in 33 per cent, starter in 40 per cent, water in 44 per cent and pipes and pumps in nearly 75 per cent of the creameries studied. Mold spores were carried by 90 per cent of the dry parchment and cloth circles examined.

Macy et al (1931) found that a great majority of the churns carried yeasts and molds and even new churns were not always free of molds. Wood, even up to the depth of 1 inch, may carry molds of one kind or other. They suggested that the molds are dislodged during the working process when the churns are subjected to a great strain and they then are incorporated in butter.

Olson and Hammer (1933) showed that highly contaminated churns

lowered the keeping quality of butter when the butter was unsalted and was held at 32 or 45°F.

Sorensen (1940) traced a few instances of rancid flavor development to breaks in vat linings, covers and in vat coils. He found that pumps and pipe lines seldom gave trouble unless more than one vat or churn had to be filled through the pipe at the same time. He found the churns to be the most consistent source of contamination in samples of butter that developed butyric or hydrolytic rancidity.

MATERIAL AND METHODS

Procedures for acquisition and treatment of samples. Of the 294 samples studied 93 were sent in by various Iowa creameries in the regular monthly scoring contests at Iowa State College and the other 201 samples were obtained through a large assembler of butter. The monthly contest samples were obtained in 20- to 60-pound tubs which were held at 33-35°F. The scoring was usually done by three judges 1 to 3 days after receipt. A 4-ounce sample was obtained from each tub for keeping quality test and a 2-ounce sample for microbial analysis. Triers were used to take out the samples from the tubs. Contamination of samples through the triers was avoided by wiping the triers thoroughly with tissue paper, dipping the triers in alcohol and flaming them before each use. Use of more than one trier allowed time to get the triers cooled before they were ready to be used again. Sterile, cylindrical, glass jars with metal screw tops were used to hold the samples and sterile wooden spatulas were used to help press the butter firmly into the jars.

For the other set of samples, sterile 4-ounce jars of butter were shipped in an insulated box. The samples were put in the jars aseptically, refrigerated and shipped. They always arrived in good condition. On arrival the butter was divided into three parts with the help of sterile wooden spatulas. One part was used for keeping quality test, a second for scoring while fresh and the third for microbial analysis.

All samples were stored at a refrigeration temperature of 3°F. They were held at about 50°F. for several hours before judging. Grading

was done according to U. S. numerical commercial grades for flavor by two or more experienced judges.

The samples for keeping quality test were incubated for 7 days at 21°C. in a thermostatically-controlled incubator. At the end of this period they were tempered and judged by two or three judges for flavor and odor. No points were deducted from any sample for oxidized flavor, as it was supposed that this method of handling is bound to give chances for oxidation, even if the samples would not show the defect otherwise.

The samples were refrigerated until they were plated. They were analysed for coliform organisms, gram-negative organisms, yeast and mold count and total count by the procedures described subsequently.

The samples for line run series were obtained from creameries operating under normal commercial conditions for that particular plant. During the process of manufacture, aseptic samples were taken in sterile jars at various points and stages. Samples of cream from the vat and the churn were taken from all parts of the vat or the churn with the help of sterile metal tubes, to get a representative sample. Other samples of liquid material were taken directly in the sample jar held under the stream of the product. Samples were taken at the following points and stages in so far as possible: (1) Raw cream from the vat (2) Pasteurized cream. (In case of vat pasteurized cream, samples were taken after cooling, right from the vat, while in the case of vacreated cream, this sample was taken hot at the top of the cooler, and a third sample was had at the bottom of the cooler). (3) In creamery A a temperature of 165°F. was used for 30 minutes in vat pasteurization, while in the

vacreator a temperature of 195°F. was employed for 1 second. (4) Cream from the holding vat just before pumping the cream to the churn. (5) a sample of the first cream entering the churn (cream through the pump and the pipes). (6) Cream from the churn after about 15 revolutions or churning for ten minutes in some cases. (7) Buttermilk from the buttermilk outlet, after enough had been discharged to minimize chances of local contamination. (8) Washed butter granules. (9) Salted finished butter.

Butter samples were taken in 4-ounce sterile containers by help of sterile wooden spatulas and an effort was made to get the samples from all parts of the churn. All samples were taken directly from the churn before any butter was removed.

In all, samples for 15 line run series were obtained, of which three were from churnings to which butter culture was added. Samples from all points and stages mentioned in the line run could not be obtained in all cases, with the result that some series are not complete.

In the study of the changes in the population of coliform organisms in butter stored at 38 and 48°F. and with varying concentrations of salt and degrees of working, butter was manufactured under controlled conditions. From the regular cream supplies of the college laboratory, samples of sweet cream were selected on the basis of taste and smell and put in two thoroughly cleaned, ten-gallon regular milk cans, 50 to 60 pounds in each. The cream tested between 30 and 32 per cent fat.

For pasteurization the cans were immersed in a water bath deep enough to have the water around the cans well above the level of the

cream inside. A third can with about 60 pounds of water in it was placed side by side in the bath and formed the control for checking temperatures. Live steam was injected into the water bath until the temperature of the water in the control can reached 130°F. Cream was held at that temperature for 30 minutes. At the end of this period the steam valve was closed completely and cold water was run in the bath until the control can showed a temperature of about 55°F. The cans were shaken occasionally all through the heating and the cooling periods. They then were removed to the refrigerator room at 35°F. and left there overnight.

Pure cultures of three strains of E. coli and two of A. aerogenes recently isolated in the Bacteriological Laboratories of the Department of Dairy Industry formed the flora for inoculation of the cream. The cultures were transferred in sterile litmus milk, every 24 hours for 2 or 3 transfers prior to their use in order to insure maximum activity when they were inoculated in the cream. The final transfer was made in sterile skim milk dispensed in 100 ml. quantities in 6-ounce medicinal ovals which was inoculated with 0.1 ml. of 24-hour culture.

The churn used was a semi-commercial Vane churn manufactured by the General Dairy Equipment Company, Minneapolis, Minnesota. The maximum capacity of the churn is supposed to be 650 pounds of cream, but it could very easily handle as little as 100 pounds of 30 per cent cream, the quantity which was used in making each of the present churnings. The churn was handled in such a way as to minimize contamination from that source. The coliform culture was sprinkled evenly over the cream in the churn at the rate of 0.5 ml. of culture for every pound of

cream. The cream then was churned, taking about 30 minutes before the butter was in granules of proper size. After draining the buttermilk, the granules were washed with filtered tap water and then cooled and hardened for 30 minutes with cold water at about 42°F. The butter then was worked partly to remove most of the free moisture. Four pounds of this butter were taken to the laboratory in each of the three stainless steel shot gun cans which were well steamed for 15 minutes in the Arnold steamer and then cooled in the refrigerator. In the laboratory the contents of each can was divided equally in two parts and each part was worked separately under aseptic conditions in a sterile enameled metal bowl. One half of each portion of butter was well worked to desirable degree of dryness, while the second portion was worked somewhat less than desirable. The first two samples were kept unsalted. The next four samples were worked as before but two were salted with 1.75 per cent of sterile salt and the other two with 2.5 per cent salt. Thus six different classes of butter were obtained as follows:

(1) Unsalted and well worked.

(2) Unsalted and poorly worked.

(3) 1.75 per cent salt and well worked.

(4) 1.75 per cent salt and poorly worked.

(5) 2.5 per cent salt and well worked.

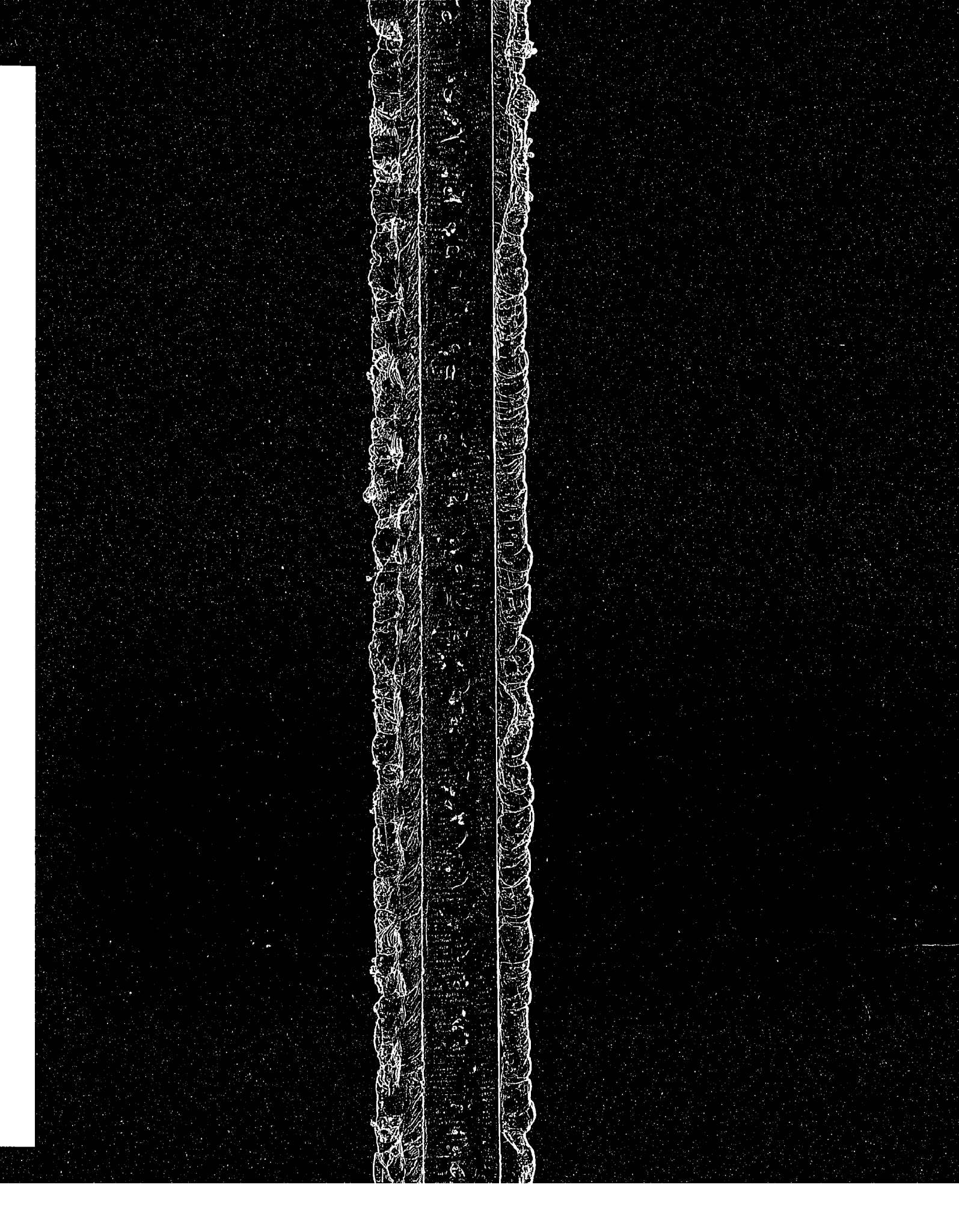
(6) 2.5 per cent salt and poorly worked.

The butter from each of the six lots was put in sixteen 2-ounce and two 4-ounce cylindrical screw capped, sterile glass jars. Half of the small jars were put at 48°F. and the rest at 38°F. The 4-ounce jars were used for salt and moisture analysis. Samples from each lot

were plated for coliform organisms at the following intervals: (1) within 24 hours, (2) 3 days, (3) 7 days, (4) 14 days, (5) 21 days, (6) 30 days, and (7) 90 days.

Two samples from each of the six lots were analyzed for per cent salt and moisture by the method given by Mortensen et al (1937).

General procedures for the microbial analyses. In as far as possible the bacterial populations were estimated by the agar plate method as described in Standard Methods for the Examination of Dairy Products, eighth edition (1941). Coliform organisms were determined by plating on violet red bile agar. One set of plates was incubated for 18 to 24 hours at 37°C. and another set for 30 to 36 hours at 30°C. Yeasts and molds were determined on acidified potato dextrose agar (pH 3.5) and the plates were incubated for 5 days at 21°C. "Total count" was determined on Tryptone-glucose-extract-milk agar, plates being incubated for 3 days at 30°C. or 5 days at 21°C. For gram-negative organisms nutrient agar was used, with added crystal violet dye to check the growth of most of the gram-positive bacteria. Two sets of plates were prepared and one incubated at 21°C. for 5 days and the other at 30°C. for 3 days. A stock solution of 0.1 per cent crystal violet was made in alcohol. This was added to the melted nutrient agar media just before dispensing in bottles and was thoroughly mixed. The medium then was sterilized as usual. For preliminary trials two strengths of crystal violet were used, the final dilutions being 1:65,000 and 1:150,000. The latter concentration only was used in interpreting the results except in the case of some of the first samples for which only the dye concentration of 1:65,000 had



been used. The higher concentration was shown to inhibit some of the gram-negative bacteria, even though the lower concentration permitted occasional colonies of coccoi to develop. A Quebec colony counter and a binocular microscope were used in making the plate counts.

The observations on the use of different temperatures and periods of incubation of plates along with the effect of the strength of crystal violet on the gram-negative count are given in table 1. In reporting the results of these trials in subsequent tables the coliform counts at 37°C., gram-negative counts at 30°C. with 1 part of crystal violet in 150,000 parts of agar (except a few early samples where only data on the 1:85,000 dye concentration were available) and the total plate counts at 21°C. were employed. The selection of the above enumeration procedures was based on the assumption that the procedure giving the higher count was the more suitable, provided it did not enumerate too many organisms not falling in the desired category. In order to decide whether or not a count was significantly higher than the corresponding one, a few arbitrary standards had to be fixed which formed the basis for comparisons. All counts below 10 per milliliter were discarded for the purposes of comparison, for in those cases the numbers of colonies on plates would be so small that the differences probably would not be significant. For those above 10 a mathematical mean of the two counts under comparison was taken and if the difference between the two counts was greater than 20 per cent of the mean, the two counts were presumed to be sufficiently different to warrant their use in calculation of differences.

The counts of the coliform organisms at 30 and 37°C., with respective

Table 1

Comparisons of counts of several organism types in commercial butter, using various indicated modifications of the enumeration procedures

Sample number	Coliform count per ml. 30°C. for 30 to 36 hours	Coliform count per ml. 37° for 18 to 24 hours	Gram negative count per ml. after 5 days at 21°C. with crystal violet concentration of: 1/85,000	Gram negative count per ml. after 5 days at 21°C. with crystal violet concentration of: 1/150,000	Gram negative count per ml. after 3 days at 30°C. with crystal violet concentration of: 1/85,000	Gram negative count per ml. after 3 days at 30°C. with crystal violet concentration of: 1/150,000	Total plate count per ml. after: 5 days at 21°C.	Total plate count per ml. after: 3 days at 30°C.
Samples losing no points during keeping quality test.								
1	< 2	< 2	< 2	-	< 2	-	38,000	21,500
2	< 2	< 2	< 2	-	4	-	5,500	6,000
3	< 2	< 2	6	-	6	-	7,800	9,700
4	< 2	< 2	6	-	2	-	184,000	175,000
5	2	< 2	14	-	26	-	7,300	11,300
6	< 2	< 2	70	-	54	-	16,200	13,500
7	2	< 2	110	-	106	-	6,600	6,800
8	< 2	< 2	40	-	48	-	19,100	23,100
9	< 2	< 2	74	-	80	-	9,400	9,000
10	< 2	< 2	< 2	-	2	-	8,300	7,300
11	< 2	< 2	< 2	-	< 2	-	3,400	2,900
12	< 2	< 2	10	-	12	-	12,700	13,700
13	< 2	< 2	< 2	-	< 2	-	8,400	6,200
14	< 2	< 2	2	-	4	-	7,100	7,200
15	< 2	< 2	2	-	6	-	12,000	11,700
16	< 2	< 2	4	-	10	-	28,000	26,500
17	< 2	< 2	< 2	-	< 2	-	20,700	17,100
18	< 2	< 2	4	-	8	-	6,200	8,800
19	< 2	< 2	< 2	-	4	-	8,300	9,300
20	< 2	< 2	12	-	18	-	5,500	3,800
21	< 2	< 2	4	-	4	-	23,100	13,600
22	2	2	2	-	2	-	6,200	5,100
23	< 2	< 2	< 2	-	< 2	-	>300,000	>300,000
24	< 2	< 2	< 2	-	< 2	-	5,300	5,300

19	< 2	< 2	< 2	-	4	-	8,300	9,300
20	< 2	< 2	12	-	18	-	5,500	5,800
21	< 2	< 2	4	-	4	-	23,100	13.600
22	2	2	2	-	2	-	6,200	5,100
23	< 2	< 2	< 2	-	< 2	-	>300,000	>300,000
24	< 2	< 2	< 2	-	< 2	-	5,300	5,300
25	< 2	< 2	42	-	74	-	11,500	14,400
26	< 2	< 2	2	-	2	-	151,000	191,000
27	< 2	< 2	4	-	4	-	14,700	8,200
28	< 2	< 2	2	-	2	-	5,200	1,760
29	< 2	< 2	< 2	-	4	-	27,400	26,900
30	< 2	< 2	< 2	-	< 2	-	9,300	8,300
31	< 2	< 2	< 2	-	< 2	-	15,600	10,500
32	2	< 2	8	-	8	-	48,000	47,000
33	< 2	< 2	32	-	30	-	269,000	229,000
34	< 2	< 2	2	-	< 2	-	52,000	52,000
35	< 2	< 2	12	-	8	-	7,200	8,400
36	< 2	< 2	2	-	4	-	5,500	3,500
37	< 2	< 2	< 2	-	40	-	59,000	48,000
38	< 2	< 2	32	-	26	-	17,600	15,100
39	< 2	< 2	< 2	-	2	-	129,000	86,000
40	< 2	< 2	< 2	-	< 2	-	49,000	28,900
41	< 2	< 2	44	-	76	-	330	670
42	10	8	52	-	88	-	49,000	57,000
43	< 2	< 2	98	-	138	-	30,000	31,000
44	< 2	< 2	6	-	14	-	32,000	24,900
45	48	54	> 3,000	-	> 3,000	-	31,000	31,000
46	< 2	< 2	4	-	4	-	67,000	54,000
47	< 2	2	< 2	-	4	-	4,300	2,350
48	2	2	< 2	-	8	-	2,110	1,620
49	< 2	< 2	22	-	20	-	51,000	51,000
50	< 2	< 2	< 2	-	6	-	> 300,000	> 300,000
51	< 2	< 2	< 2	-	4	-	161,000	228,000
52	< 2	< 2	< 2	-	2	-	3,900	6,500
53	< 2	< 2	< 2	2	< 2	2	19,000	16,000
54	< 2	< 2	2	2	4	2	30,000	29,000
55	< 2	< 2	2	2	2	2	> 300,000	>300,000
56	< 2	< 2	< 2	2	< 2	< 2	> 300,000	>300,000
57	< 2	< 2	400	600	10	15	116,000	60,000
58	< 2	< 2	< 2	2	< 2	< 2	6,100	330
59	< 2	< 2	< 2	2	< 2	< 2	54,000	53,000
60	2	< 2	2	12	< 2	2	18,000	19,000

54	< 2	< 2	2	2				
55	< 2	< 2	2	2	2	2	> 300,000	> 300,000
56	< 2	< 2	< 2	2	< 2	< 2	> 300,000	> 300,000
57	< 2	< 2	400	600	10	15	116,000	60,000
58	< 2	< 2	< 2	2	< 2	< 2	6,100	330
59	< 2	< 2	< 2	2	< 2	< 2	54,000	53,000
60	< 2	< 2	2	12	< 2	2	13,000	19,000
61	< 2	< 2	< 2	< 2	< 2	< 2	43,000	8,300
62	< 2	< 2	< 2	< 2	< 2	< 2	10,000	6,200
63	< 2	< 2	< 2	< 2	< 2	< 2	36,000	33,000
64	< 2	< 2	< 2	< 2	< 2	< 2	93,000	56,000
65	< 2	< 2	< 2	< 2	2	< 2	17,000	14,000
66	< 2	< 2	< 2	< 2	< 2	14	135,000	190,000
67	< 2	< 2	10	4	12	26	23,000	22,000
68	< 2	< 2	2	< 2	2	< 2	8,000	6,400
69	< 2	< 2	< 2	< 2	< 2	< 2	160,000	200,000
70	< 2	< 2	< 2	2	< 2	8	11,000	1,100
71	< 2	< 2	< 2	< 2	< 2	2	9,600	4,600
72	< 2	< 2	< 2	< 2	< 2	< 2	18,000	21,000
73	< 2	< 2	< 2	< 2	< 2	< 2	43,000	38,000
74	< 2	< 2	< 2	< 2	< 2	< 2	14,000	14,000
75	< 2	< 2	< 2	< 2	< 2	< 2	8,500	11,000
76	< 2	< 2	< 2	< 2	< 2	< 2	8,400	8,000
77	< 2	< 2	2	< 2	< 2	2	19,000	20,000
78	< 2	< 2	2	< 2	2	12	41,000	40,000
79	< 2	< 2	2	< 2	2	2	2,600	3,000
80	2	10	22	22	10	20	35,000	82,000
81	136	120	820	1350	430	550	> 300,000	> 300,000
82	< 2	< 2	< 2	< 2	< 2	< 2	15,000	15,000
83	< 2	< 2	< 2	< 2	< 2	< 2	4,700	4,000
84	< 2	< 2	< 2	< 2	< 2	< 2	10,000	15,000
85	< 2	< 2	< 2	< 2	< 2	< 2	36,000	34,000
86	< 2	< 2	< 2	< 2	< 2	< 2	16,000	12,000
87	< 2	< 2	< 2	< 2	< 2	< 2	10,000	9,400
88	< 2	< 2	< 2	< 2	< 2	2	290,000	290,000
89	< 2	< 2	< 2	< 2	< 2	< 2	13,000	13,000
90	8	20	10	22	10	22	13,000	14,000
91	< 2	< 2	< 2	< 2	< 2	< 2	5,500	5,600
92	< 2	< 2	8	6	2	70	100,000	110,000
93	< 2	< 2	< 2	< 2	< 2	< 2	170,000	140,000
94	< 2	< 2	38	54	46	64	31,000	35,000
95	< 2	< 2	4	8	10	6	34,000	30,000

89	< 2	< 2	< 2	< 2	< 2	< 2	13,000	13,000
90	8	20	10	22	10	22	13,000	14,000
91	< 2	< 2	< 2	< 2	< 2	< 2	5,500	5,600
92	< 2	< 2	8	6	2	70	100,000	110,000
93	< 2	< 2	< 2	< 2	< 2	< 2	170,000	140,000
94	< 2	< 2	38	54	46	64	31,000	35,000
95	< 2	< 2	4	8	10	6	34,000	30,000
96	2	< 2	2	2	4	4	25,000	25,000
97	< 2	< 2	< 2	< 2	2	< 2	12,000	71,000
98	< 2	< 2	< 2	< 2	< 2	< 2	300,000	300,000
99	< 2	< 2	< 2	< 2	< 2	< 2	11,000	11,000
100	4	< 2	< 2	< 2	< 2	< 2	7,800	5,100
101	< 2	< 2	< 2	< 2	< 2	< 2	5,400	5,200
102	< 2	< 2	4	< 2	< 2	< 2	14,000	12,000
103	< 2	< 2	10	12	16	12	5,900	3,700
104	< 2	< 2	4	< 2	< 2	4	19,000	19,000
105	< 2	< 2	< 2	< 2	< 2	< 2	51,000	50,000
106	2	2	2	< 2	2	2	31,000	23,000
107	< 2	< 2	6	4	2	200	54,000	43,000
108	< 2	< 2	< 2	< 2	< 2	< 2	7,100	12,000
109	2	< 2	2	< 2	< 2	2	450	550
110	2	2	< 2	< 2	< 2	2	3,100	5,900
111	< 2	< 2	2	< 2	< 2	2	12,500	18,000
112	< 2	< 2	10	8	10	10	69,000	51,000
113	< 2	< 2	2	12	12	24	34,000	46,000
114	< 2	< 2	< 2	28	< 2	24	5,300	6,500
115	< 2	< 2	< 2	< 2	< 2	< 2	> 300,000	> 300,000
116	< 2	< 2	< 2	< 2	< 2	< 2	42,000	28,000
117	< 2	< 2	< 2	< 2	2	< 2	> 300,000	> 300,000
118	66	84	230	210	280	250	210,000	160,000
119	< 2	< 2	< 2	< 2	< 2	< 2	14,000	12,000
120	< 2	< 2	< 2	< 2	< 2	< 2	190,000	210,000
121	< 2	< 2	< 2	< 2	< 2	< 2	93,000	87,000
122	< 2	< 2	< 2	< 2	< 2	< 2	139,000	170,000
123	< 2	< 2	< 2	< 2	< 2	< 2	280,000	300,000
124	2	6	73	71	96	76	125,000	120,000
125	6	6	< 2	< 2	2	8	160,000	160,000
126	4	< 2	62	210	48	300	> 300,000	> 300,000
127	< 2	< 2	2	< 2	< 2	4	18,000	13,000
128	< 2	< 2	2	< 2	< 2	14	15,000	21,000
129	< 2	< 2	2	< 2	20	46	15,000	17,000

124	2	6	78	71	36	76	125,000	120,000
125	6	6	< 2	< 2	2	8	160,000	160,000
126	4	< 2	62	210	48	300	> 300,000	> 300,000
127	< 2	< 2	2	< 2	< 2	4	18,000	13,000
128	< 2	< 2	2	< 2	< 2	14	18,000	21,000
129	< 2	< 2	2	< 2	20	46	15,000	17,000
130	2	2	14	20	16	32	76,000	52,000
131	< 2	< 2	14	36	14	36	140,000	150,000
132	< 2	< 2	< 2	< 2	< 2	< 2	120,000	110,000
133	< 2	< 2	< 2	< 2	< 2	< 2	300,000	-
134	< 2	< 2	4	< 2	2	< 2	> 300,000	> 300,000
135	4	4	120	110	< 2	12	97,000	90,000
136	< 2	< 2	< 2	< 2	< 2	2	27,000	20,000
137	< 2	< 2	< 2	< 2	< 2	< 2	27,000	25,000
138	< 2	< 2	< 2	< 2	2	< 2	14,000	9,000
139	< 2	< 2	< 2	< 2	2	< 2	6,000	10,000
140	< 2	< 2	< 2	< 2	2	4	9,600	7,800
141	2	< 2	2	< 2	< 2	4	130,000	140,000
142	2	4	2	8	4	4	39,000	40,000
143	< 2	< 2	< 2	2	< 2	2	74,000	53,000
144	< 2	< 2	< 2	< 2	< 2	< 2	120,000	96,000
145	< 2	< 2	< 2	< 2	< 2	2	30,000	29,000
146	< 2	2	< 2	< 2	< 2	< 2	31,000	26,000
147	< 2	2	6	6	2	6	120,000	120,000
148	< 2	< 2	< 2	< 2	< 2	< 2	> 300,000	> 300,000
149	> 600	> 600	> 600	> 600	> 600	> 600	120,000	110,000
150	< 2	< 2	< 2	< 2	< 2	< 2	14,000	15,000
151	2	< 2	< 2	< 2	< 2	2	46,000	37,000
152	< 2	< 2	< 2	< 2	< 2	< 2	650	960
153	18	26	< 2	< 2	24	20	14,000	19,000
154	< 2	< 2	< 2	< 2	< 2	< 2	120,000	140,000
155	< 2	< 2	6	4	< 2	2	26,000	18,000
156	2	< 2	6	6	2	2	60,000	-
157	< 2	< 2	< 2	< 2	< 2	< 2	1,100	770
158	< 2	< 2	2	< 2	< 2	< 2	7,800	4,400
159	< 2	< 2	2	< 2	< 2	< 2	99,000	70,000
160	< 2	< 2	< 2	< 2	< 2	< 2	13,000	11,000
161	< 2	< 2	< 2	< 2	< 2	< 2	54,000	38,000
162	< 2	< 2	6	10	8	12	70,000	24,000
163	< 2	< 2	< 2	< 2	< 2	< 2	190	90
164	18	22	< 2	6	20	38	100,000	93,000
165	150	120	120	120	120	150	25,000	19,000

158	< 2	< 2	2	< 2	< 2	< 2	7,800	4,400
159	< 2	< 2	2	< 2	< 2	< 2	99,000	70,000
160	< 2	< 2	< 2	< 2	< 2	< 2	13,000	11,000
161	< 2	< 2	< 2	< 2	< 2	< 2	54,000	38,000
162	< 2	< 2	6	10	8	12	70,000	24,000
163	< 2	< 2	< 2	< 2	< 2	< 2	190	90
164	18	22	< 2	6	20	38	100,000	93,000
165	150	120	120	120	120	150	25,000	19,000
166	< 2	< 2	8	4	< 2	< 2	> 300,000	> 300,000
167	24	46	< 2	< 2	26	44	51,000	40,000
168	< 2	< 2	< 2	< 2	< 2	< 2	210,000	180,000
169	14	16	16	28	34	36	20,000	11,000
170	44	64	22	50	54	60	46,000	38,000
171	< 2	< 2	< 2	< 2	< 2	< 2	8,000	8,400
172	< 2	< 2	12	4	4	2	50,000	30,000
173	< 2	< 2	2	< 2	< 2	2	1,300	620
174	< 2	< 2	< 2	< 2	< 2	< 2	37,000	25,000
175	2	2	< 2	< 2	2	4	1,300	900
176	2	< 2	< 2	< 2	< 2	2	5,600	3,000
177	< 2	< 2	< 2	< 2	< 2	< 2	1,900	1,900
178	< 2	< 2	6	< 2	< 2	6	23,000	11,000
179	< 2	< 2	< 2	2	2	4	62,000	50,000
180	36	50	< 2	< 2	42	74	41,000	47,000
181	< 2	< 2	2	4	1	20	7,200	-
182	170	120	< 2	< 2	150	160	30,000	41,000
183	12	16	12	20	4	26	14,000	14,000
184	< 2	< 2	< 2	< 2	< 2	< 2	120,000	120,000
185	2	4	16	34	30	38	14,000	15,000
186	< 2	< 2	30	34	38	38	18,000	13,000
187	16	20	< 2	< 2	16	38	28,000	30,000
188	< 2	< 2	38	46	36	68	12,000	14,000
189	< 2	< 2	2	4	< 2	8	23,000	21,000
190	< 2	< 2	2	4	2	< 2	22,000	30,000
191	< 2	< 2	< 2	< 2	< 2	< 2	45,000	56,000
192	< 2	< 2	< 2	< 2	< 2	< 2	26,000	23,000
193	< 2	< 2	< 2	< 2	< 2	2	18,000	24,000
194	< 2	< 2	< 2	2	2	< 2	25,000	35,000
195	< 2	< 2	< 2	2	2	< 2	7,000	13,000
196	12	8	18	12	20	24	300,000	300,000
197	10	12	46	52	74	110	27,000	24,000
198	< 2	< 2	< 2	2	< 2	4	25,000	20,000

193	< 2	< 2	< 2	< 2	< 2	2	18,000	24,000
194	< 2	< 2	< 2	< 2	2	< 2	25,000	35,000
195	< 2	< 2	< 2	< 2	2	< 2	7,000	13,000
196	12	8	18	12	20	24	300,000	300,000
197	10	12	46	52	74	110	27,000	24,000
198	< 2	< 2	< 2	2	< 2	4	25,000	20,000
199	< 2	< 2	< 2	2	< 2	< 2	9,600	12,000
200	< 2	< 2	22	18	14	24	59,000	-
	Samples losing 1 point during keeping quality test							
201	< 2	2	46	-	42	-	13,800	11,800
202	< 2	2	80	-	70	-	1,870	1,350
203	< 2	2	< 2	-	< 2	-	25,200	13,300
204	< 2	2	82	-	146	-	18,000	17,100
205	2	2	< 2	-	< 2	-	16,700	16,100
206	2	2	178	-	182	-	> 300,000	> 300,000
207	24	32	688	-	730	-	28,000	35,000
208	< 2	2	238	-	232	-	51,000	76,000
209	< 2	2	< 2	-	< 2	-	50,000	55,000
210	< 2	< 2	32	-	48	-	177,000	[illegible]
211	< 2	< 2	< 2	-	4	-	> 300,000	> 300,000
212	< 2	2	246	-	294	-	> 300,000	> 300,000
213	< 2	< 2	28	-	30	-	> 300,000	> 300,000
214	< 2	< 2	160	-	220	-	820	900
215	< 2	< 2	76	-	116	-	370	420
216	< 2	< 2	< 2	< 2	< 2	2	69,000	20,000
217	16	10	6	14	70	70	86,000	81,000
218	< 2	< 2	< 2	< 2	< 2	< 2	40,000	50,000
219	< 2	< 2	< 2	< 2	< 2	2	230,000	240,000
220	< 2	< 2	4	2	2	2	100,000	87,000
221	2	6	20	480	26	330	> 300,000	> 300,000
222	< 2	2	20	22	30	70	230,000	230,000
223	< 2	< 2	< 2	< 2	< 2	< 2	21,000	14,000
224	< 2	< 2	8	8	2	2	12,000	13,000
225	< 2	< 2	< 2	< 2	< 2	< 2	32,000	40,000
226	< 2	< 2	< 2	< 2	< 2	< 2	18,000	19,000
227	< 2	< 2	< 2	< 2	< 2	< 2	9,600	9,700
228	< 2	< 2	4	< 2	< 2	2	33,000	30,000
229	< 2	< 2	< 2	< 2	< 2	< 2	61,000	62,000
230	< 2	< 2	< 2	32	< 2	< 2	4,200	1,900
231	< 2	< 2	< 2	< 2	< 2	< 2	77,000	60,000
232	20	16	18	238	22	240	100,000	110,000

226	< 2	< 2	< 2	< 2	< 2	< 2	18,000	19,000
227	< 2	< 2	< 2	< 2	< 2	< 2	9,600	9,700
228	< 2	< 2	4	< 2	< 2	2	33,000	30,000
229	< 2	< 2	< 2	< 2	< 2	< 2	61,000	62,000
230	< 2	< 2	< 2	32	< 2	< 2	4,200	1,900
231	< 2	< 2	< 2	< 2	< 2	< 2	77,000	60,000
232	20	16	18	238	22	240	100,000	110,000
233	< 2	< 2	18	16	6	18	36,000	290,000
234	90	30	> 600	> 600	> 600	> 600	> 300,000	> 300,000
235	2	10	4	4	4	16	> 300,000	> 300,000
236	8	14	16	12	14	18	100,000	-
237	< 2	< 2	4	2	2	2	42,000	42,000
238	4	14	8	12	16	16	210,000	170,000
239	< 2	< 2	36	36	60	78	16,000	16,000
240	6	2	34	56	40	240	25,000	31,000
241	< 2	< 2	6	4	2	8	33,000	26,000
242	< 2	< 2	< 2	< 2	< 2	2	13,000	13,000
243	< 2	< 2	12	14	12	29	> 300,000	> 300,000
244	14	10	8	210	8	26	> 300,000	260,000
245	6	2	42	34	46	42	> 300,000	> 300,000
246	< 2	< 2	< 2	< 2	2	6	39,000	38,000

Samples losing 2 points during keeping quality test

247	< 2	2	< 2	-	2	-	53,000	34,000
248	< 2	< 2	46	-	34	-	9,700	9,300
249	4	4	8	-	4	-	7,700	7,100
250	< 2	< 2	2	-	2	-	2,420	1,810
251	> 3,000	> 3,000	> 3,000	-	> 3,000	-	> 300,000	> 300,000
252	< 2	< 2	330	-	310	-	67,000	26,400
253	< 2	< 2	620	540	72	100	> 300,000	> 300,000
254	< 2	< 2	< 2	< 2	< 2	4	200,000	220,000
255	< 2	< 2	90	100	92	118	20,000	17,000
256	12	8	2	8	2	14	> 300,000	> 300,000
257	20	20	2	3	4	26	35,000	15,000
258	< 2	< 2	4	< 2	4	< 2	3,200	4,200
259	< 2	< 2	14	98	10	96	59,000	32,000
260	< 2	< 2	< 2	2	< 2	2	11,000	11,000
261	< 2	< 2	< 2	< 2	< 2	< 2	36,000	38,000
262	< 2	< 2	2	10	2	2	98,000	110,000
263	14	16	< 2	< 2	18	16	8,000	8,300
264	2	< 2	< 2	< 2	4	4	3,600	6,100
265	2	8	2	2	14	8	92,000	100,000

260	< 2	< 2	< 2	2	< 2	2	11,000	11,000
261	< 2	< 2	< 2	< 2	< 2	< 2	36,000	38,000
262	< 2	< 2	2	10	2	2	93,000	110,000
263	14	16	< 2	< 2	18	16	8,000	8,300
264	2	< 2	< 2	< 2	4	4	3,600	6,100
265	2	8	2	2	14	8	92,000	100,000
266	> 600	> 600	> 600	> 600	> 600	> 600	210,000	210,000
267	< 2	< 2	< 2	< 2	< 2	< 2	240,000	240,000
268	66	64	52	44	48	38	130,000	150,000
269	52	48	> 600	> 600	> 600	> 600	130,000	150,000
270	590	600	> 600	> 600	> 600	> 600	300,000	300,000
271	120	120	72	100	160	256	120,000	100,000
272	20	12	2	10	20	34	160,000	160,000
273	60	80	90	110	130	140	19,000	19,000
274	> 600	> 600	> 600	> 600	> 600	> 600	89,000	91,000
275	0	2	170	150	140	150	39,000	26,000
276	18	20	2	2	34	40	210,000	-
			Samples losing 3 points during keeping quality test					
277	8	16	274	-	278	-	34,000	18,000
278	< 2	< 2	6	-	2	-	9,300	7,700
279	< 2	< 2	2	-	6	-	132,000	139,000
280	< 2	< 2	24	-	38	-	64,000	65,000
281	2	2	< 2	2	< 2	70	> 300,000	> 300,000
282	< 2	< 2	4	20	< 2	< 2	110,000	100,000
283	2	< 2	< 2	190	< 2	180	34,000	33,000
284	2	< 2	< 2	< 2	< 2	< 2	53,000	43,000
285	470	440	> 600	> 600	> 600	> 600	220,000	200,000
286	< 2	< 2	210	200	180	210	34,000	28,000
287	< 2	< 2	< 2	< 2	2	200	14,000	9,000
288	4	4	32	90	22	22	110,000	100,000
289	4	< 2	2	< 2	2	4	300,000	300,000
290	< 2	< 2	30	44	36	64	47,000	32,000
			Samples losing 4 points during keeping quality test					
291	< 2	< 2	< 2	-	2	-	21,100	19,300
292	< 2	< 2	4	8	2	2	47,000	51,000
293	4	< 2	4	10	10	12	26,000	24,000
294	> 600	> 600	> 600	> 600	> 600	> 600	> 300,000	> 300,000

incubation periods of 30 to 36 hours and 20 to 24 hours did not vary to any appreciable extent. On the basis of the calculations just described 40 samples showed usable levels of counts; of these, 14 samples showed a higher count in favor of 37°C. incubation temperature and 9 in favor of 30°C. The rest did not differ by more than 20 per cent of the mean of the two counts. Largely because this temperature is used most widely, 37°C. incubation was adopted for enumeration of coliform bacteria in the final comparisons of results.

In comparisons of total counts, out of the 188 samples studied at 21 and 30°C., 176 pairs did not differ by more than 20 per cent of the mean; 74 samples favored an incubation temperature of 21°C. as against 38 which showed a higher count at 30°C. Therefore, the former was taken for the final count.

For the gram-negative counts two factors had to be compared. First the temperature of incubation, 30 and 21°C. were compared using crystal violet strength of 1:35,000. Out of a total of usable counts on 97 samples, 39 were higher at 30°C. and 17 at 21°C. Using 1:150,000 dye concentration, out of a total of 82 usable counts, counts on 42 samples were found higher at 30°C. and on 10 samples at 21°C. Counts on the rest of the samples at both temperatures did not differ by more than 20 per cent of the mean of the counts on the two variables. For reporting the results, the counts at 30°C. were used.

The two strengths of crystal violet were then compared. With 30°C. as incubation temperature, out of a total of 59 usable counts, 29 were higher with 1:150,000 dye concentration and only 3 with 1:35,000. At

21°C. out of a total of 79 comparable counts, 50 favored 1:150,000 dye concentration and 3 showed higher counts with 1:85,000. The remaining samples in the two groups did not vary by more than 20 per cent of the mean of the two counts. In reporting these results counts with lower dye concentration of 1:150,000 were used.

RESULTS

Two hundred and ninety-four samples from various creameries of Iowa were examined for their coliform count, gram-negative count, yeast and mold count and total count. Their keeping quality at 21°C. for 7 days also was determined. A summary of the results obtained is presented in table 2, with the samples grouped according to loss in score in the keeping quality test. The data from this table have been summarized in a number of tables which show the interrelationships between the various values obtained in this study.

Relationship Between Various Groups of Organisms and Initial Score of Butter

Relationship between coliform count and initial score of commercial butter. Table 3 shows the relationship between the coliform count and the original score of the butter. The scores ranged from 87 to 93, with 90.2 per cent of all the samples scoring 90 or above. There is some tendency toward a relationship between the coliform count of the butter and its score upon receipt. However, there are samples with very low coliform counts which still have poor initial score. Although the opposite is not just as true, several samples with large numbers of coliform organisms scored as high as 91. A detailed examination of the three samples scoring 87 and giving a coliform count of less than 10 per ml. (table 2) shows that number 79 has a very low total count of 2,600 per ml. and that it has lost no points in the keeping quality test and showed

Table 2

Results of the microbiological analysis and keeping quality test on commercial butter

Sample number	Total plate count per ml. (5 days at 21°C.)	Yeast and mold count per ml. (5 days at 21°C.)	Gram-negative count per ml. (dye concentration 1:150,000 3 days at 30°C.)	Coliform count per ml. (18 to 24 hrs. at 37°C.)	Initial score of butter	Score after holding 7 days at 21°C.
			Samples losing no points during the keeping quality test			
1	38,000	24	< 2	< 2	90	90
2	5,500	4	4	< 2	90	90
3	7,800	26	6	< 2	91	91
4	184,000	16	2	< 2	89	89
5	7,300	4	26	< 2	91	91
6	16,200	14	54	< 2	91	91
7	6,600	192	106	< 2	90	90
8	19,100	102	40	< 2	91	91
9	9,400	192	80	< 2	92	92
10	8,300	8	2	< 2	91	91
11	3,400	78	< 2	< 2	90	90
12	12,700	24	12	< 2	92	92
13	8,400	4	< 2	< 2	90	90
14	7,100	176	4	< 2	92	92
15	12,000	4	6	< 2	92	92
16	28,000	4	10	< 2	90	90
17	20,700	4	< 2	< 2	91	91
18	6,200	150	8	< 2	91	91
19	8,300	28	4	< 2	92	92
20	5,500	4	18	< 2	92	92
[illegible]	[illegible]	[illegible]	4	< 2	92	92
22	6,200	26	2	2	92	92
23	> 300,000	4	< 2	< 2	91	91
24	5,300	64	< 2	< 2	93	93
25	11,500	404	74	< 2	92	92
26	151,000	120	[illegible]	[illegible]	[illegible]	[illegible]

20	5,500	4	18	[illegible]	[illegible]	[illegible]
22	6,200	26	2	2	92	92
23	> 300,000	4	< 2	< 2	91	91
24	5,300	64	< 2	< 2	93	93
25	11,500	404	74	< 2	92	92
26	151,000	100	2	< 2	91	91
27	14,700	72	4	< 2	92	92
28	5,200	16	2	< 2	92	92
29	27,400	14	4	< 2	90	90
30	9,300	6	< 2	< 2	92	92
31	15,600	36	< 2	< 2	90	90
32	43,000	44	8	< 2	91	91
33	269,000	90	30	< 2	88	88
34	52,000	118	< 2	< 2	90	90
35	7,200	12	8	< 2	90	90
36	5,500	16	4	< 2	89	89
37	5,900	48	40	< 2	91	91
38	17,600	278	26	< 2	92	92
39	129,000	96	2	< 2	90	90
40	49,000	34	< 2	< 2	90	90
41	330	< 2	76	< 2	90	90
42	49,000	210	88	8	92	92
43	30,000	818	138	< 2	90	90
44	32,000	980	14	< 2	91	91
45	31,000	162	3,000	54	90	90
46	67,000	60	4	< 2	90	90
47	4,300	32	4	< 2	90	90
48	2,110	46	8	< 2	92	92
49	51,000	6	20	< 2	93	93
50	>300,000	136	6	< 2	89	89
51	161,000	178	4	< 2	90	90
52	9,000	20	2	< 2	91	91
53	19,000	28	2	< 2	92	92
54	30,000	106	2	< 2	91	91
55	> 300,000	238	2	< 2	93	93
56	> 300,000	26	< 2	< 2	90	90
57	116,000	3,000	16	< 2	91	91
58	6,100	< 2	< 2	< 2	93	93
59	54,000	54	< 2	< 2	91	91
60	13,000	250	2	< 2	92	92
[illegible]	[illegible]	420	< 2	< 2	92	92

55	> 300,000	233	2	< 2	93	93
56	> 300,000	26	< 2	< 2	90	90
57	116,000	≯ 3,000	15	< 2	91	91
58	6,100	< 2	< 2	< 2	93	93
59	54,000	54	< 2	< 2	91	91
60	13,000	250	2	< 2	92	92
61	43,000	420	< 2	< 2	92	92
62	10,000	230	< 2	< 2	91	91
63	36,000	20	< 2	< 2	91	91
64	93,000	96	< 2	< 2	93	93
65	17,000	20	< 2	< 2	90	90
66	135,000	14	14	< 2	90	90
67	23,000	6	26	< 2	90	90
68	8,000	12	< 2	< 2	92	92
69	160,000	22	< 2	< 2	89	89
70	11,000	10	3	< 2	90	90
71	9,600	56	2	< 2	92	92
72	13,000	< 2	< 2	< 2	91	91
73	43,000	20	< 2	< 2	90	90
74	14,000	14	< 2	< 2	91	91
75	8,500	20	< 2	< 2	93	93
76	8,400	8	< 2	< 2	92	92
77	19,000	110	2	< 2	91	91
78	41,000	75	12	< 2	90	90
79	2,600	24	2	< 2	87	87
80	25,000	98	20	10	91	91
81	> 300,000	2	550	180	90	90
82	15,000	136	< 2	< 2	91	91
83	4,700	< 2	< 2	< 2	93	93
84	10,000	272	2	< 2	92	92
85	36,000	134	< 2	< 2	90	90
86	16,000	150	< 2	< 2	89	89
87	10,000	5	< 2	< 2	90	90
88	230,000	63	2	< 2	91	91
89	13,000	22	< 2	< 2	92	92
90	13,000	490	22	20	91	91
91	5,500	6	2	< 2	91	91
92	100,000	110	70	< 2	91	91
93	170,000	52	2	< 2	92	92
94	31,000	560	64	< 2	90	90
95	34,000	130	6	< 2	91	91
96	25,000	72	4	< 2	92	92

90	13,000	490	22	20	91	91
91	5,500	8	2	< 2	91	91
92	100,000	110	70	< 2	91	91
93	170,000	52	2	< 2	92	92
94	31,000	550	64	< 2	90	90
95	34,000	130	6	< 2	91	91
96	25,000	72	4	< 2	92	92
97	12,000	224	< 2	< 2	90	90
98	> 300,000	< 2	< 2	< 2	91	91
99	11,000	120	< 2	< 2	92	92
100	7,800	42	< 2	< 2	90	90
101	5,400	12	< 2	< 2	90	90
102	14,000	10	< 2	< 2	89	89
103	5,900	10	12	< 2	92	92
104	19,000	500	4	< 2	93	93
105	51,000	> 600	< 2	< 2	92	92
106	31,000	8	2	2	90	90
107	54,000	280	200	< 2	91	91
108	7,100	20	< 2	< 2	91	91
109	450	2	2	< 2	89	89*
110	3,100	16	2	2	93	93
111	12,500	70	2	< 2	91	91
112	69,000	210	10	< 2	92	92
113	34,000	24	24	< 2	91	91
114	5,800	20	24	< 2	91	91
115	> 300,000	40	< 2	< 2	90	90
116	42,000	30	< 2	< 2	91	91
117	> 300,000	170	< 2	< 2	91	91
118	210,000	> 600	260	84	89	89
119	14,000	2	< 2	< 2	91	91
120	190,000	18	< 2	< 2	90	90
121	98,000	14	< 2	< 2	90	90
122	139,000	26	< 2	< 2	91	91
123	280,000	> 600	< 2	< 2	88	88
124	125,000	170	76	6	90	90
125	160,000	160	8	6	92	91
126	> 300,000	34	300	< 2	90	90
127	13,000	74	4	< 2	91	91
128	19,000	28	14	< 2	91	91
129	15,000	84	46	< 2	92	92
130	76,000	8	32	2	91	91
131	140,000	14	36	< 2	90	90

124	125,000	170				
125	160,000	160	8	6	92	91
126	>300,000	34	300	< 2	90	90
127	18,000	74	4	< 2	91	91
128	19,000	28	14	< 2	91	91
129	15,000	84	46	< 2	92	92
130	76,000	8	32	2	91	91
131	140,000	14	36	< 2	90	90
132	120,000	60	< 2	< 2	90	90
133	300,000	4	< 2	< 2	90	90
134	>300,000	>600	< 2	< 2	90	90
135	97,000	>600	12	4	91	91
136	27,000	14	2	< 2	90	90
137	27,000	74	< 2	< 2	91	91
138	14,000	12	< 2	< 2	92	92
139	6,000	42	< 2	< 2	91	91
140	9,600	24	4	< 2	90	90
141	130,000	150	4	< 2	92	92
142	39,000	280	4	4	91	91
143	74,000	56	2	< 2	92	92
144	120,000	68	< 2	< 2	90	90
145	30,000	40	2	< 2	90	90
146	31,000	2	< 2	2	90	90
147	120,000	570	8	2	90	90
148	>300,000	430	< 2	< 2	91	91
149	120,000	28	>600	>600	87	87
150	14,000	2	< 2	< 2	90	90
151	46,000	32	2	< 2	89	89
152	650	< 2	< 2	< 2	89	89*
153	14,000	100	20	26	89	89
154	120,000	12	< 2	< 2	91	91
155	26,000	140	2	< 2	91	91
156	60,000	540	2	< 2	91	91
157	1,100	< 2	< 2	< 2	88	88*
158	7,300	8	< 2	< 2	89	89
159	99,000	28	< 2	< 2	91	91
160	18,000	18	< 2	< 2	91	91
161	54,000	8	2	< 2	92	92
162	70,000	14	12	< 2	87	87
163	190	2	< 2	< 2	89	89*
164	100,000	> 600	38	22	91	91
165	25,000	2	150	120	90	90

		[illegible]	< 2	< 2	91	91
161	54,000	8	2	< 2	92	92
162	70,000	14	12	< 2	87	87
163	190	2	< 2	< 2	89	89*
164	100,000	> 600	38	22	91	91
165	25,000	2	150	120	90	90
166	> 300,000	4	< 2	< 2	90	90
167	31,000	480	44	46	92	92
168	210,000	16	< 2	< 2	91	91
169	20,000	86	38	16	90	90
170	46,000	86	90	64	90	90
171	8,000	16	< 2	< 2	91	91
172	50,000	18	2	< 2	92	92
173	1,300	< 2	2	< 2	91	91
174	37,000	110	< 2	< 2	90	90
175	1,300	14	4	2	90	90
176	5,600	2	< 2	< 2	91	91
177	1,900	< 2	< 2	< 2	90	90
178	25,000	30	6	< 2	90	90
179	62,000	140	4	< 2	91	91
180	41,000	250	74	50	91	91
181	7,200	24	20	< 2	91	91
182	30,000	530	160	120	91	91
183	14,000	188	26	16	90	90
184	120,000	200	< 2	< 2	91	91
185	14,000	4	38	4	90	90
186	18,000	130	30	< 2	91	91
187	25,000	510	38	20	91	91
188	12,000	120	68	< 2	91	91
189	23,000	4	8	< 2	89	89
190	22,000	70	< 2	< 2	91	91
191	45,000	78	< 2	< 2	90	90
192	26,000	8	< 2	< 2	91	91
193	18,000	13	2	< 2	91	91
194	25,000	28	< 2	< 2	89	89
195	7,000	72	< 2	< 2	91	91
196	> 300,000	16	24	8	90	90
197	27,000	40	110	12	90	90
198	25,000	< 2	4	< 2	90	90
199	9,500	26	< 2	< 2	90	90
200	59,000	210	24	< 2	90	90

Sample lesion 1 point during [illegible]

195	7,000	72	<2	<2	91	90
196	>300,000	16	24	8	90	90
197	27,000	40	110	12	90	90
198	25,000	<2	4	<2	90	90
199	9,600	26	<2	<2	90	90
200	59,000	210	24	<2	90	90

Sample losing 1 point during keeping quality test

201	13,800	<2	42	<2	91	90
202	1,870	100	70	<2	92	91
203	25,200	12	<2	<2	92	91
204	18,000	24	146	2	93	92
205	16,700	10	<2	<2	92	91
206	>300,000	8	182	2	91	90
207	28,000	970	730	32	89	88
208	51,000	6	232	<2	92	91
209	50,000	78	<2	<2	91	90
210	177,000	740	48	<2	92	91
211	>300,000	26	4	<2	91	90
212	>300,000	400	294	2	90	89
213	>300,000	1,040	30	<2	90	89
214	820	2	220	<2	92	91*
215	370	<2	116	<2	91	90*
216	69,000	750	<2	<2	90	89
217	86,000	290	70	10	92	91
218	40,000	32	<2	<2	91	90
219	280,000	330	<2	<2	91	90
220	180,000	150	<2	<2	89	88
221	>300,000	220	330	6	87	86
222	230,000	74	70	2	91	90
223	21,000	120	<2	<2	92	91
224	12,000	6	2	2	91	90
225	32,000	150	<2	<2	92	91
226	13,000	14	<2	<2	93	92
227	9,600	174	<2	<2	92	91
228	33,000	10	2	<2	91	90
229	61,000	8	<2	<2	91	90
230	4,200	4	<2	<2	91	90
231	77,000	10	<2	<2	91	90
232	100,000	140	240	16	91	90
233	36,000	24	18	<2	90	89
234	>300,000	>600	>600	30	90	89
235	>500,000	590	16	10	90	89

228	33,000	10	2	< 2	91	90
229	61,000	8	< 2	< 2	91	90
230	4,200	4	< 2	< 2	91	90
231	77,000	10	< 2	< 2	91	90
232	100,000	140	240	16	91	90
233	36,000	24	18	< 2	90	89
234	>300,000	>600	>600	30	90	89
235	> 500,000	590	16	10	90	89
236	100,000	44	18	14	90	89
237	42,000	30	2	< 2	92	91
238	210,000	120	16	14	91	90
239	16,000	8	78	< 2	91	90
240	25,000	> 600	240	< 2	92	91
241	33,000	82	8	< 2	92	91
242	15,000	210	2	< 2	92	91
243	> 300,000	2,100	29	< 2	91	90
244	> 300,000	2,600	26	10	89	88
245	> 300,000	> 3,000	42	2	91	90
246	39,000	140	6	< 2	88	87
247	53,000	10	2	2	93	91
248	9,700	62	34	< 2	93	91
249	7,700	68	4	4	91	89
250	2,420	4	< 2	< 2	92	90
251	>300,000	80	> 3,000	> 3,000	89	87
252	67,000	36	310	< 2	92	90
253	>300,000	1,600	100	< 2	91	89
254	200,000	246	4	< 2	91	89
255	20,000	10	113	< 2	92	90
256	>300,000	104	14	8	91	89
257	35,000	540	26	20	90	88
258	3,200	10	< 2	< 2	92	90
259	59,000	22	96	< 2	92	90
260	11,000	30	2	< 2	92	90
261	36,000	4	< 2	< 2	92	90
262	93,000	420	2	< 2	93	91
263	8,000	82	16	16	91	89
264	3,600	26	4	< 2	91	89
265	92,000	2	8	8	92	90
266	210,000	180	> 600	> 600	90	88
267	240,000	> 600	< 2	< 2	91	89
268	180,000	> 600	38	64	90	88
269	180,000	> 600	> 600	48	92	90
270	>300,000	> 600	>600	> 600	90	88

258	3,200	10	< 2	< 2	92	90
259	59,000	22	96	< 2	92	90
260	11,000	30	2	< 2	92	90
261	36,000	4	< 2	< 2	92	90
262	98,000	420	2	< 2	93	91
263	8,000	82	16	16	91	89
264	3,600	26	4	< 2	91	89
265	92,000	2	8	8	92	90
266	210,000	180	> 600	> 600	90	88
267	240,000	> 600	< 2	< 2	91	89
268	180,000	> 600	38	64	90	88
269	180,000	> 600	> 600	48	92	90
270	> 300,000	> 600	> 600	> 600	90	88
271	120,000	30	256	120	91	89
272	160,000	180	34	12	90	88
273	19,000	68	140	80	92	90
274	89,000	92	> 600	600	89	87
275	39,000	78	150	2	92	90
276	210,000	1,100	40	20	90	88
277	54,000	94	278	16	92	89
278	9,300	124	2	< 2	92	89
279	132,000	212	6	< 2	92	89
280	64,000	30	38	< 2	92	89
281	> 300,000	48	70	2	91	88
282	110,000	150	< 2	< 2	92	89
283	34,000	> 600	180	< 2	91	88
284	38,000	16	< 2	< 2	92	89
285	220,000	> 600	> 600	440	91	88
286	34,000	> 600	210	< 2	93	90
287	14,000	< 2	200	< 2	93	90
288	110,000	> 600	22	4	92	89
289	> 300,000	> 600	4	< 2	92	89
290	47,000	22	64	< 2	90	87

Samples losing 4 points during keeping quality test

291	21,100	80	< 2	< 2	92	88
292	47,000	40	2	< 2	92	88
293	26,000	190	12	< 2	91	87
294	> 300,000	2,000	> 600	> 600	88	No grade V. unclean

Table 3

The relation of coliform count to initial score of commercial butter

Coliform count per ml.	Number of samples in each group based upon coliform count with an initial score of:							Totals
	93	92	91	90	89	88	87	
< 2	13	60	77	54	14	4	2	224
2-10	3	8	11	10	1	-	1	34
11-30	-	1	6	8	1	-	-	16
31-100	-	3	1	3	2	-	-	9
101-300	-	-	2	2	-	-	-	4
> 300	-	-	1	2	2	1	1	7
Totals	16	72	98	79	20	5	4	294

no evidence of bacterial deterioration either fresh or after holding. The low score was due to a pronounced cooked flavor. As will be discussed later, extremely low total count in commercial butter often results at the cost of the flavor of the sample. Number 221 had very high total, gram-negative and yeast and mold counts, which may be related to the low score. It also lost one point in the keeping quality test. No explanation for the low score of number 162 can be given on the basis of its microbial counts, for, although the total count is above the average (47,000 per ml.), counts of other groups are reasonably low. The fourth sample with a score of 87 (number 149) had a coliform count of more than 300 per ml. and other counts also are high, probably indicating previous microbial deterioration.

Five samples (numbers 33, 123, 157, 246 and 294, table 2) scored 89. Number 294 had a very high coliform count, while others had less than 2 per ml. Sample numbers 33 and 123 had very high total counts and yeast and mold counts which may be related to the cause of their low score. Sample number 157 had a very low total count of 1,100 per ml. But for a high yeast and mold count of 140 per ml. for sample 246, no explanation can be advanced for its low score.

Of the 20 samples which scored 89, 14 had a coliform count of less than 2 per ml. At least four of these samples with low coliform counts (numbers 4, 50, 69, 220) had abnormally high total counts ranging from 160,000 to more than 300,000 per ml. Three of them (numbers 109, 152, 163) had a very low total count ranging from 190 to 650 bacteria per ml., and their low score was due to improper or excessive heating of

the cream. Of the remaining seven, one had a high yeast and mold count (number 86) while total counts for the other six (numbers 36, 102, 151, 158, 189, 194) ranged from as low as 5,500 to 46,000 per ml. and no explanation on the basis of microbial population can be given for their low scores. It might be pointed out that none of these five lost any points on holding, and the low score could be due to the quality of raw cream. Of the twenty samples that scored 89, only five lost points on holding; those that lost most in score had the highest coliform counts.

Of the total butter samples, 79 or 26.8 per cent scored 90. Of these, 68 per cent had a coliform count of less than 2 per ml., while the remaining 32 per cent showed counts all the way from 2 to more than 300 per ml. However many of them had coliform count of less than 30 per ml. Only about 3.8 per cent of the samples in this group had a coliform count higher than 30 per ml. Only four samples from this group (numbers 31, 165, 266, 270) had coliform counts of more than 100 per ml. The two samples of this group which had a coliform count of more than 300 per ml. (numbers 266 and 270) lost two points each on holding.

Of all the samples, 98 or 33 per cent scored 91. Of these, 77 had coliform counts of less than 2 per ml. Only 4 of the whole lot had a count of more than 30 coliform organisms per ml. Still there were 3 samples (numbers 182, 271, 285) that had counts of more than 100, one having a count of more than 300 per ml. Of the four samples which had coliform counts above 30 per ml., the one with a count above 300 per ml. (number 285) lost 3 points on holding and one of the others (number 271) with a count of between 100 and 300 coliform organisms per ml. lost 2 points.

The fact that samples of butter with high coliform counts have less chance to score high is more apparent as one proceeds from a lower to a higher grade, but it becomes very clear when the top-grade samples scoring 92 or above are considered. All samples that scored 92 or above had less than 100 coliform organisms per ml. and those that scored 93 had less than 10 per ml. (strictly speaking less than 4).

The greater the number of coliform organisms, the less are the chances of a sample of butter having a high initial score. The percentage of samples with a coliform count higher than 100 per ml. in each score group is in a decreasing order as one passes from lower grade to a higher grade. The data also show that a low coliform count does not insure high scoring butter, for at least 20 samples out of 224 with coliform counts of less than 2 per ml. have scored less than 90.

Relationship between gram-negative count and initial score of commercial butter. Table 4 presents data which show little relationship between the gram-negative count of butter and its initial score. Samples having both low and high counts are distributed over the score scale from 87 to 93 or, conversely, samples in any score class show gram-negative counts ranging from less than 2 to over 300 per ml., except in the case of samples scoring 93. It is true that a large part of the samples having more than 300 gram-negative organisms per ml. show poor score and none of these has scored 93, but the total number of samples in this group is so small and the distribution so random that it would not be reasonable to draw specific conclusions. It can only be pointed out that with the increase in gram-negative count of butter the chances

Table 4

The relation of gram-negative count to initial score of commercial butter

Gram-negative count per ml.	Number of samples in each group based upon gram-negative count with an initial score of:							Totals
	93	92	91	90	89	88	87	
≤ 2	6	22	35	29	8	2	-	102
2-10	5	26	24	17	6	1	1	80
11-30	1	5	15	11	2	1	1	36
31-100	1	10	16	11	-	-	-	38
101-300	3	7	7	6	1	-	-	24
≥ 300	-	2	1	5	3	1	2	14
Totals	16	72	98	79	20	5	4	294

for getting a low score also increase somewhat, but only in a general way. Prediction of the score of a butter sample from its gram-negative count would be impossible.

Relationship between yeast and mold count and initial score of commercial butter. Table 5 gives the relation of yeast and mold count to initial score of commercial butter. Here again no relationship between the two criteria is apparent, as in any of the groups based on yeast and mold count, samples are found in all score groups and without any definite trend.

Relationship between total count and initial score of commercial butter. The relationship between the total count and the initial score of butter is indicated in table 6. Counts above 100,000 or below 3,000 tend to be associated in a degree greater than average with butter which has a low score. Of the 14 samples with counts below 3,000, 5 (numbers 79, 109, 152, 157 and 163) scored below 90, while the rest scored between 90 and 92. Burnt-protein or scorched-fat flavor was the cause of the low scoring samples in most instances. Similarly, of the 73 samples with a total count above 100,000 per ml., 12 scored below 90, two of them going as low as 87. There is only one sample in this group that scored 93. Among the 207 samples with counts between the above two extremes of 3,010 and 100,000, 12, or less than 6 per cent of the total number, scored below 90 and 15 or 8 per cent scored as high as 93. Of the 16 samples scoring 93, 15 belong to this group. It appears that samples of normal commercial butters having total counts between 3,000 and 100,000 have the best chances to score high.

Table 5

The relation of yeast and mold count to initial score of commercial butter

Yeast and mold count per ml.	Number of samples in each group based upon yeast and mold count with an initial score of:							Totals
	93	92	91	90	89	88	87	
< 2	3	-	5	3	1	1	-	13
2-10	2	14	16	14	5	-	-	51
11-30	4	16	21	19	4	-	3	67
31-100	3	15	17	13	4	1	-	58
101-300	1	13	23	12	3	1	1	59
>300	3	9	16	13	3	2	-	46
Totals	16	72	98	79	20	5	4	294

Table 6

The relation of total plate count to initial score of commercial butter

Total plate count per ml.	Number of samples in each group based upon total plate count with an initial score of:							Totals
	93	92	91	90	89	88	87	
≤ 1,000	-	1	1	1	3	-	-	6
1,010 - 3,000	-	3	1	2	-	1	1	8
3,010 -10,000	6	15	19	11	2	-	-	53
10,100 -30,000	4	22	27	18	6	-	-	77
30,100-100,000	5	22	25	21	2	1	1	77
101,000-300,000	-	8	14	14	4	2	1	43
≥ 300,000	1	1	11	12	3	1	1	30
Totals	16	72	98	79	20	5	4	294

Relationship Between Various Groups of Organisms and Loss in Score of Commercial Butter During the Keeping Quality Test

Relationship between coliform count and loss in score. Table 7 represents the data on the relationship between the coliform organisms and loss in score on holding. The score losses ranged from nothing to 4 points. The percentage of samples losing two or more points on holding increased as their coliform count increased. Of the 224 samples which had less than 2 coliform bacteria per ml., 75 per cent showed no loss in the 7 day keeping quality test, 13.5 per cent lost one point, 5.8 per cent lost two points, 4.4 per cent lost three points and 1.3 per cent lost four points. Of the samples giving a coliform count of 2 to 10 per ml., 20.6 per cent lost two or more points in score during the keeping quality test. A similar loss was found in 31 per cent of the samples with counts between 11 and 30, with 33 per cent when the count was between 31 and 100, with 25 per cent when the count was between 101 and 300 and 86 per cent when the counts were in excess of 300 per ml. The total number of samples in each of the last three groups was somewhat too small to permit much confidence to be placed in the absolute values of the averages.

Obviously butter samples scoring less than 90 when fresh, usually will not be stored. If in addition to rejecting all samples scoring below 90, those which score 90 or above but show a coliform count of over 300 per ml. were also rejected, three samples out of a total of 265 would have been discarded. All of these show a loss of two or more points on holding (numbers 266, 270, 285). If a coliform count of over

Table 7

The relation of coliform count to loss in score of commercial butter during the keeping quality test

Coliform count per ml.	Number of samples in each group based upon coliform count which showed a loss during the keeping quality test of:					Totals
	0 point	1 point	2 points	3 points	4 points	
< 2	167	31	13	10	3	224
2-10	17	10	5	2	-	34
11-30	7	4	4	1	-	16
31-100	5	1	3	-	-	9
101-300	3	-	1	-	-	4
> 300	1	-	4	1	1	7
Totals	200	46	30	14	4	294

100 per ml. had been set as the basis for rejection another four samples (numbers 81, 165, 132, 271) would have been discarded. Of these one lost two points, while the others did not lose any during the keeping quality test. If a coliform count of 30 per ml. as maximum count had been the basis for acceptance of samples for storage, seven more samples would have been rejected. Of this total of 14 samples that would have been rejected on this basis, six lost two points in storage and one lost three, while the rest did not lose any.

The relationship of coliform count to the keeping quality of butter is only a general one and it is not possible to predict the keeping quality of an individual sample of butter on the basis of its coliform count.

Relationship between gram-negative count and loss in score. Data on the relationship of the gram-negative count of butter to keeping quality are presented in table 8. The keeping quality of an individual sample cannot be predicted from the count of gram-negative organisms in the butter, but the chances of the samples keeping well are reduced as the number of these organisms increases. Of the 102 samples which had a gram-negative count of less than 2 per ml., 82.6 per cent did not drop in score during holding, 14.3 per cent lost one point, while 7.1 per cent lost two or more points. The percentage of samples losing two or more points on holding increased as the gram-negative counts increased. When the gram-negative count was between 2 and 10 per ml., 15 per cent of samples lost two or more points, and when it was between 11 and 30, this loss occurred with 14 per cent of the samples. When the count increased to between 31 and 100, the samples losing points increased sharply to 23.6 per cent. This sharp rise continued with the increase in count, and 33.3 per cent of

Table 8

The relation of gram-negative count to loss in score of commercial butter during the keeping quality test

Gram-negative count per ml.	Number of samples in each group based upon gram-negative count which showed a loss during the keeping quality test of:					Totals
	0 point	1 point	2 points	3 points	4 points	
< 2	81	14	4	2	1	102
2-10	62	7	7	3	1	80
11-30	24	7	3	1	1	36
31-100	22	7	6	3	-	38
101-300	8	8	4	4	-	24
> 300	3	3	6	1	1	14
Total	200	46	30	14	4	294

samples with counts between 100 and 300 per ml. lost two or more points, while 57 per cent of the samples which had a gram-negative count of over 300 per ml. showed a similar loss in score. Of the total samples in each group showing loss of zero, one, two, three and four points, the percentage of those that had higher gram-negative counts tended to increase with greater reduction in score during the keeping quality test. Despite the tendencies for some relationship between loss of score during the keeping quality test and the initial count of gram-negative bacteria, predictions of one value from the other would not be possible for individual samples.

Relationship between yeast and mold count and loss in score. Table 9 shows the data on the relationship of yeast and mold count to loss in score of butter during the keeping quality test. It is evident that the loss in score in general increases as the yeast and mold count of butter increases. However the discrepancies are many and it is not possible to predict the keeping quality of an individual sample of butter on the basis of its yeast and mold count.

Relationship between total count and loss in score. Table 10 shows the data on the relationship of total count to loss in score of butter when the latter is held at 21°C. for 7 days in the keeping quality test. The general relationship is evident from the table, although no definite predictions can be made about an individual sample on the basis of its total count, because the types and not the numbers of the organisms present are the important factor. Table 10 shows that samples having total counts of below 1,000 have not lost any points during holding, but such samples often score low initially because of excessive heating

Table 9

The relation of yeast and mold count to loss in score of commercial butter during the keeping quality test

Yeast and mold count per ml.	Number of samples in each group based upon yeast and mold count which showed a loss during the keeping quality test of:					Totals
	0 point	1 point	2 points	3 points	4 points	
< 2	10	2	0	1	-	13
2-10	35	10	6	-	-	51
11-30	54	6	4	3	-	67
31-100	40	6	8	2	2	58
101-300	41	10	4	3	1	59
> 300	20	12	8	5	1	46
Totals	200	46	30	14	4	294

Table 10

The relation of total plate count to loss in score of commercial butter during the keeping quality test

Total plate count per ml.	Number of samples in each group based upon total plate count which showed a loss during the keeping quality test of:					Totals
	0 point	1 point	2 points	3 points	4 points	
≤1,000	4	2	-	-	-	6
1,010-3,000	6	1	1	-	-	8
3,010-10,000	45	2	5	1	-	53
10,100-30,000	60	11	3	1	2	77
30,100-100,000	46	15	9	6	1	77
101,000-300,000	26	5	3	4	-	43
>300,000	13	10	4	2	1	30
Totals	200	46	30	14	4	294

of the cream during pasteurization. The lowest percentage of samples to lose 2 or more points in storage (7.8 per cent) was in the group having a count between 10,000 and 30,000; above or below this the percentage of samples showing losses increased. Of the samples with counts between 1,000 and 3,000, 12.33 per cent lost two or more points in holding and 11 per cent of those that had counts between 3,000 and 10,000 showed a similar loss. Among the samples that had counts ranging between 30,100 and 100,000, 20.3 per cent of them lost two or more points. The same loss in score occurred in 27.3 per cent of the samples with counts between 100,000 and 300,000 per ml. and with 23.2 per cent of those with counts greater than 300,000 per ml.

Relationship Between Numbers of Various Organisms Present in Commercial Butter

Relationship between coliform and gram-negative counts. Table 11 shows the relationship between counts of coliform and gram-negative bacteria. There is a direct relationship between the two groups in the sense that higher the coliform count, the higher is the count of the gram-negative organisms. However, many a sample with high gram-negative count has a low coliform count. The table shows that, although there were 224 samples that had counts of less than 2 coliform per ml., there were only 101 samples with counts of gram-negative organisms in the same range. With the remaining 123 samples of this group, the gram-negative count varied from 2 to more than 300 per ml. In all cases, as expected, the gram-negative count was higher than the coliform count.

Table 11

The relation of coliform count to gram-negative count of commercial butter

Coliform count per ml.	Number of samples in each group based upon coliform count with a gram-negative count per ml. of:						Totals
	< 2	2-10	11-30	31-100	101-300	> 300	
< 2	101	67	22	21	12	1	224
2-10	1	13	7	8	4	1	34
11-30	-	-	7	5	3	1	16
31-100	-	-	-	4	2	3	9
101-300	-	-	-	-	3	1	4
> 300	-	-	-	-	-	7	7
Totals	102	80	36	38	24	14	294

Relationship between coliform and yeast and mold counts. Table 12 presents data on the relationship between the coliform counts and the yeast and mold counts of butter. Apparently only a general relationship exists between the two groups of organisms. However, the yeast and mold count usually was higher than the coliform count, although there were a few samples that showed the reverse to be true. All samples with yeast and mold count of less than 2 per ml. had coliform counts in the same range, while the other 211 samples with coliform counts of less than 2 per ml. had yeast and mold counts ranging from 2 to more than 300 per ml.; 69 of these had yeast and mold counts in excess of 100 per ml.

Relationship between coliform and total counts. Data on the relationship between counts of these two groups are presented in table 13. The higher the coliform count the higher was the total count, although the changes did not observe any mathematical proportion. Many samples had low coliform counts and high total counts. Of the 224 samples that had less than 2 coliform organisms per ml., 100 had total counts above 30,000 per ml.

Although an increase in total count was in no way an index of the increase in coliform count, an increase in coliform count tended to be associated with an increase in the total count.

Relationship between yeast and mold count and gram-negative count. Table 14 shows that no relationship between the counts of these two groups of organisms is apparent.

Table 12

The relation of coliform count to yeast and mold count of commercial butter

Coliform count per ml.	Number of samples in each group based upon coliform count with a yeast and mold count per ml. of:						Totals
	≤ 2	2-10	11-30	31-100	101-300	> 300	
≤ 2	13	41	60	41	45	24	224
2-10	-	8	5	7	7	7	34
11-30	-	-	-	6	4	6	16
31-100	-	-	-	2	2	5	9
101-300	-	2	1	-	-	1	4
> 300	-	-	1	2	1	3	7
Totals	13	51	67	58	59	46	294

Table 13

The relation of coliform count to total plate count of commercial butter

Coliform count per ml.	Number of samples in each group based upon coliform count with a total plate count per ml. of:							Totals
	≤ 1,000	1,010 to 3,000	3,010 to 10,000	10,100 to 30,000	30,100 to 100,000	101,000 to 300,000	> 300,000	
≤ 2	6	6	48	64	56	28	16	224
2-10	-	2	4	3	11	5	9	34
11-30	-	-	1	6	5	3	1	16
31-100	-	-	-	2	4	3	-	9
101-300	-	-	-	2	-	1	1	4
> 300	-	-	-	-	1	3	3	7
Totals	6	8	53	77	77	45	30	294

Table 14

The relation of gram-negative count to yeast and mold count of commercial butter

Gram-negative count per ml.	Number of samples based upon gram-negative count with a yeast and mold count per ml. of:						Totals
	≤ 2	2-10	11-30	31-100	101-300	> 300	
≤ 2	7	25	27	19	16	8	102
2-10	2	12	23	17	21	5	80
11-30	-	5	9	6	6	10	36
31-100	2	3	5	8	10	10	38
101-300	2	5	2	5	3	7	24
> 300	-	1	1	3	3	6	14
Totals	13	51	67	58	59	46	294

Results on Line Run Samples

The results of the line run tests on samples are given in table 15. All samples of unpasteurized cream examined during the investigation had a high count of all of the types of organisms which were enumerated.

The counts of coliform organisms, gram-negative organisms and yeasts and molds in pasteurized cream were much below those for raw cream in most cases where comparative figures were available. However in many cases the counts on pasteurized cream were much higher than desirable. The reduction in the total count also was very marked. In cases where the pasteurized cream showed counts higher than expected, it is hard to determine whether these were due to inefficient or incomplete pasteurization or to post-pasteurization contamination, as no phosphatase tests were run on these samples.

Samples of the first cream entering the churn nearly always showed a higher count in all types of organisms than they had in the vat. The increases in the counts of the various types of organisms differ from creamery to creamery and even from churning to churning in the same creamery..

After a short agitation of the cream in the churn, there always was a considerable increase in counts of all types. Physical pickup from the churn wall and breaking up of the existing clumps in the cream undoubtedly are responsible for such increases. Here again the percentage change in the microbial counts of various types, from that of the cream as it entered the churn, differed from one churning to another. However, the percentage increase in yeast and mold count often was higher than the increase

Table 15

Microbiological results on line-run samples from creameries

Creamery and date	Sample	Coliform count per ml. 18-24 hours at 37°C.	Gram-negative count per ml. dye concentration 1:150,000 3 days at 30°C.	Yeast and mold count per ml. 5 days at 21°C.	Total count per ml. 5 days at 21°C.
A Aug. 12 1946	Pasteurized cream from vat	< 2	1,940	38	46,000
	First cream entering the churn	98	4,200	58	91,000
	Cream after 15 revolutions of the churn	1,710	5,400	90	113,000
	Buttermilk	2,100	6,600	360	226,000
	Washed butter granules	100	230	80	28,400
	Finished salted butter	24	140	230	39,000
A Aug. 17 1946	Raw cream	7,800,000	10,300,000	61,000	1,010,000,000
	Vacreated hot cream at top of the cooler	< 2	12	2	111,000
	Cream at the bottom of the cooler	< 2	4	< 2	105,000
	First cream entering the churn	1,090	1,300	12	142,000
	Cream after 15 revolutions of the churn	2,120	2,530	50	164,000
	Buttermilk	3,700	3,390	78	234,000
	Washed butter granules	172	246	10	1,110
	Finished salted butter	< 2	32	14	670

(Continued next page)

Table 15 (con'd)

Creamery and date	Sample	Coliform count per ml. 18-24 hours at 37°C.	Gram-negative count per ml. dye concentration 1:150,000 3 days at 30°C.	Yeast and mold count per ml. 5 days at 21°C.	Total count per ml. 5 days at 21°C.
A	Raw cream	15,300,000	19,800,000	115,000	378,000,000
	Vacreated hot cream at top				
Aug.	of the cooler	2	223	24	9,700
24	Cream at the bottom of the				
1946	cooler	8	22	4	8,100
	Cream from the vat	1,170	2,790	84	8,500
	First cream entering the				
	churn	1,240	2,800	124	9,900
	Cream after 15 revolutions				
	of the churn	1,460	4,800	146	11,100
	Buttermilk	1,650	7,100	166	27,000
	Washed butter granules	78	86	56	2,100
	Finished salted butter	< 2	50	68	650
A	Pasteurized cream from vat	< 2	< 2	< 2	45,000
	First cream entering the churn	< 2	10	< 2	53,000
Oct.	Cream after 15 revolutions				
12	of the churn	80	110	92	99,000
1946	Buttermilk	590	790	440	200,000
	Washed butter granules	30	40	68	25,000
	Finished salted butter	172	196	220	34,000

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Table 15 (con'd)

Creamery and date	Sample	Coliform count per ml. 18-24 hours at 37°C.	Gram-negative count per ml. dye concentration 1:150,000 3 days at 30°C.	Yeast and mold count per ml. 5 days at 21°C.	Total count per ml. 5 days at 21°C.
A	Raw cream	11,700,000	15,300,000	35,000	710,000,000
	Vat pasteurized and cooled cream	< 2	< 2	< 2	105,000
Oct. 12 1946	First cream entering the churn	< 2	18	< 2	111,000
	Cream after 15 revolutions of the churn	68	100	94	171,000
	Buttermilk	108	201	194	225,000
	Washed butter granules	84	98	138	13,900
	Finished salted butter	40	26	406	29,800
A	Raw cream	16,600,000	18,800,000	3,900	790,000,000
	Vacreated hot cream at top of the cooler	< 2	< 2	< 2	129,000
Nov. 2 1946	Cream at the bottom of the cooler	< 2	< 2	< 2	127,000
	Cream after addition of culture	< 2	< 2	2	200,000
	First cream entering the churn	8	12	30	290,000
	Cream after 15 revolutions of the churn	146	202	870	900,000
	Buttermilk	1,870	2,130	6,100	1,700,000
	Washed butter granules	158	180	940	46,000
	Finished salted butter	134	283	4,860	383,000
A	Raw cream	10,900,000	81,000,000	71,000	1,090,000,000
	Vacreated hot cream at top of the cooler	40	100	< 2	217,000

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Table 15 (con'd)

Creamery and date	Sample	Coliform count per ml. 18-24 hours at 37°C.	Gram-negative count per ml. dye concentration 1:150,000 3 days at 30°C.	Yeast and mold count per ml. 5 days at 21°C.	Total count per ml. 5 days at 21°C.
Nov. 18 1946	Cream entering vat after being cooled	70	320	< 2	290,000
	Cream after addition of culture	120	578	8	350,000
	First cream entering the churn	214	11,000	91	390,000
	Cream after 15 revolutions of the churn	410	27,000	112	430,000
	Buttermilk	830	82,000	182	540,000
	Washed butter granules	114	390	20	28,000
	Finished salted butter	6	58	86	39,000
A	Raw cream	6,700,000	11,300,000	53,000	810,000,000
Nov. 25 1946	Vacreated hot cream at top of the cooler	< 2	< 2	< 2	198,000
	Cream entering vat after being cooled	< 2	< 2	< 2	206,000
	Cream after addition of culture	72	173	10	295,000
	First cream entering the churn	109	383	17	380,000
	Cream after 15 revolutions of the churn	300	3,900	18	460,000
	Buttermilk	410	4,300	70	800,000
	Washed butter granules	96	430	58	27,500
	Finished salted butter	4	84	106	43,000

(continued next page)

Table 15 (con'd)

Creamery and date	Sample	Coliform count per ml. 18-24 hours at 37°C.	Gram-negative count per ml. dye concentration 1:150,000 3 days at 30°C.	Yeast and mold count per ml. 5 days at 21°C.	Total count per ml. 5 days at 21°C.
B	Cold water entering churn	0 (in 5 ml.)	230	2	830
	Rinse water from churn	6	3,100	2	2,500
March	Pasteurized cream from vat	520	> 600	4	550,000
20	First cream entering the churn	>600	> 3,000	4	470,000
1948	Cream after 10 minutes churning	21,000	270,000	68	1,370,000
	Buttermilk	>30,000	350,000	190	2,060,000
	Washed butter granules	40	1,440	8	11,300
	Finished salted butter	< 2	< 2	32	28,000
C	Pasteurized cream from vat	100	2,080	20	63,000
	First cream entering the churn	510	8,700	18	102,000
April	Cream after 10 minutes of				
28	churning	580	9,100	26	194,000
1948	Buttermilk	1,760	23,600	60	340,000
	Washed butter granules	< 2	< 2	14	1,930
	Finished salted butter	< 2	20	6	3,400
D	Pasteurized cream vat no. 2	> 30,000	> 30,000	400	> 3,000,000
	First cream entering churn				
April	no. 1 (first run)	> 30,000	> 30,000	420	> 3,000,000
30	Cream after 10 minutes of				
1948	churning	> 30,000	> 30,000	448	> 3,000,000
	Buttermilk	> 30,000	> 300,000	600	> 3,000,000
	Washed butter granules	6,500	> 30,000	94	298,000
	Finished salted butter	376	410	74	61,000

(continued next page)

Table 15 (con'd)

Creamery and date	Sample	Coliform count per ml. 18-24 hours at 37°C.	Gram-negative count per ml. dye concentration 1:150,000 3 days at 30°C.	Yeast and mold count per ml. 5 days at 21°C.	Total count per ml. 5 days at 21°C.
D	Pasteurized cream from vat no. 3	>30,000	>30,000	149	>3,000,000
April 30 1948	First cream entering churn no. 1 (second run)	>30,000	>30,000	166	>3,000,000
	Cream after 10 minutes of churning	>30,000	>30,000	280	>3,000,000
	Buttermilk	>30,000	>300,000	570	>3,000,000
	Washed butter granules	25,900	>30,000	56	>300,000
	Finished salted butter	100	164	22	167,000
D	Pasteurized cream from vat no. 1	-	-	-	-
April 30 1948	Buttermilk, churn no. 2 (first run)	10,500	53,000	200	1,000,000
	Washed butter granules	218	1,500	22	44,000
	Finished salted butter	2	12	14	23,000
D	Pasteurized cream from vat no. 3	>30,000	>30,000	140	>3,000,000
April 30 1948	Cream entering churn no. 2 (second run)	>30,000	>30,000	146	>3,000,000
	Cream after 10 minutes of churning	>30,000	>30,000	270	>3,000,000
	Buttermilk	>30,000	>300,000	300	>3,000,000
	Washed butter granules	12,000	>30,000	46	>300,000
	Finished salted butter	4	14	24	198,000

(continued next page)

Table 15 (con'd)

Creamery and date	Sample	Coliform count per ml. 18-24 hours at 37°C.	Gram-negative count per ml. dye concentration 1:150,000 3 days at 30°C.	Yeast and mold count per ml. 5 days at 21°C.	Total count per ml. 5 days at 21°C.
D April 30 1943	Pasteurized cream from vat no. 3	> 30,000	> 30,000	140	> 3,000,000
	First cream entering churn no. 3	> 30,000	> 30,000	156	> 3,000,000
	Cream after 10 minutes of churning	> 30,000	> 30,000	280	> 3,000,000
	Buttermilk	> 30,000	> 300,000	360	> 3,000,000
	Washed butter granules	7,100	> 30,000	52	> 300,000
	Finished salted butter	28	38	38	218,000

in other groups, although this was not true in all cases. The counts of all the four groups of organisms in buttermilk usually were second only to those of raw cream. The counts per milliliter of buttermilk were considerably higher in all cases than those of the cream put in the churn. The buttermilk carried away a large portion of the cream organisms and consequently the washed butter granules showed a comparatively lower count than did the cream. However, there was no precise relationship between the counts of washed granules and those of buttermilk or cream.

An important change as far as the counts and the ratio of the various microbial types are concerned took place during the working of the butter. The coliform and gram-negative counts as a rule decreased, in many cases very considerably. Of the 15 samples only one showed an increase in coliform count at this point. This was the only sample showing an increase in all the four types of organisms (table 15, creamery A - Oct. 12). The gram-negative count increased in only three samples at this stage. The yeast and mold counts increased during working in all cases except the five samples from creamery D, all of which were taken on the same day, and the one sample from creamery C. In the case of creamery D the yeast and mold count was very low compared to the counts of other groups. The total count increased during working of some samples and decreased with others. In all of the samples from creamery D and two from other sources, the total counts decreased. The samples investigated were salted and therefore, they show not only the effect of working, but also that of salt. The picture certainly would be different if the butter had been unsalted.

The only samples that showed a decrease in the counts of all the four types studied were from creamery F. The salt content of these samples varied between 2 and 2.5 per cent.

Behavior of Coliform Organisms in Butter During Storage at 38 and 48°F. with Varying Salt Concentrations and Degrees of Working

The data on this portion of the study are presented in table 16. With the small scale of manufacture the texture of butter in this series was inferior to that of commercially made butter. Some difficulty was also experienced in controlling salt and moisture concentrations, and the various samples studied do show considerable variation. Five churnings were made. Of the cultures used for inoculation, two were from the Aerobacter group and three from the Escherichia group.

Effect of salt. The general effect of salt on both organism groups was the same. The greatest decreases in count were immediately after the addition of salt. It may be recalled that in the line run samples, the coliform count and the gram-negative count frequently showed a sharp drop from washed butter granules to finished salted butter, a drop which was attributed to the incorporation of salt. The greatest reduction in number, as estimated by plate count within 24 hours after the butter was finished, was about 99 per cent. This was with a typical strain of E. coli in a well-worked sample containing 2.46 per cent salt. The least decrease (93.7 per cent) was with a culture of A. aerogenes in a poorly worked sample having 1.08 per cent salt. At both the temperatures studied salt maintains its inhibitory effect throughout the storage period. This

Table 16

Changes in the population of coliform organisms in butter made and held under controlled conditions

Degree of working	Holding temperature °F.	Salt %	Moisture %	Counts per ml. after holding periods of: less than 24 hrs.	3 days	7 days	14 days	21 days	30 days	60 days
				Escherichia coli (Strain 1)						
Well worked	38	0	17.81	180,000	125,000	103,000	76,000	31,000	6,500	100
Well worked	48	0	17.81	-	130,000	110,000	105,000	44,000	14,900	40
Poorly worked	38	0	16.95	134,000	93,000	55,000	27,000	4,300	4,000	< 100
Poorly worked	48	0	16.95	-	128,000	85,000	67,000	30,000	30,000	< 100
Well worked	38	1.39	16.56	1,000	470	160	140	100	2	< 2
Well worked	48	1.39	16.56	-	930	200	130	100	42	< 2
Poorly worked	38	1.29	17.07	4,900	960	340	140	46	2	< 2
Poorly worked	48	1.29	17.07	-	1,430	630	620	144	20	< 2
Well worked	38	2.48	15.92	400	80	72	10	8	2	< 2
Well worked	48	2.48	15.92	-	244	40	22	4	2	< 2
Poorly worked	38	2.29	17.13	700	260	130	32	10	10	2
Poorly worked	48	2.29	17.13	-	370	130	40	6	2	< 2
				Escherichia coli (Strain 2)						
Well worked	38	0	16.85	190,000	129,000	100,000	72,000	41,000	10,100	108
Well worked	48	0	16.85	-	141,000	121,000	83,000	50,000	16,000	52
Poorly worked	38	0	18.42	141,000	89,000	57,000	31,000	8,000	6,500	94
Poorly worked	48	0	18.42	-	132,000	93,000	71,000	39,000	13,000	68
Well worked	38	1.11	15.90	5,300	1,200	350	230	190	10	< 2
Well worked	48	1.11	15.90	-	3,100	870	610	340	62	2
Poorly worked	38	1.14	17.01	8,300	3,200	1,300	510	270	16	< 2
Poorly worked	48	1.14	17.01	-	4,200	2,500	1,300	310	82	4

(Continued next page)

Table 16 (con'd)

Degree of working	Holding temperature °F.	Salt %	Moisture %	Counts per ml. after holding periods of: less than 24 hrs.	3 days	7 days	14 days	21 days	30 days	60 days
Well worked	38	2.52	14.32	1,100	390	82	60	12	< 2	< 2
Well worked	48	2.52	14.32	-	350	60	22	4	< 2	< 2
Poorly worked	38	2.46	15.92	2,900	290	340	80	10	< 2	< 2
Poorly worked	48	2.46	15.92	-	320	200	98	210	10	2
					Escherichia coli (Strain 3)					
Well worked	38	0	15.80	106,000	78,000	41,000	33,000	26,000	19,400	800
Well worked	48	0	15.80	-	77,000	1,600,000	3,000,000	2,000,000	1,180,000	1,170
Poorly worked	38	0	15.12	81,000	66,000	55,000	36,000	25,000	5,900	1,200
Poorly worked	48	0	15.12	-	69,000	1,400,000	1,800,000	1,440,000	610,000	7,100
Well worked	38	1.01	14.70	3,800	2,100	1,530	1,030	430	410	76
Well worked	48	1.01	14.70	-	4,100	3,900	18,400	> 30,000	14,000	850
Poorly worked	38	1.01	15.56	5,100	7,000	2,000	1,350	910	390	2
Poorly worked	48	1.01	15.56	-	5,200	4,100	23,000	31,000	18,100	590
Well worked	38	2.12	13.95	3,100	2,600	1,780	820	410	300	10
Well worked	48	2.12	13.95	-	3,000	2,100	11,200	16,800	6,100	170
Poorly worked	38	2.10	15.14	3,700	3,000	2,100	1,000	770	215	2
Poorly worked	48	2.10	15.14	-	3,500	2,900	6,100	19,800	10,000	8,800
					Aerobacter aerogenes (Strain 1)					
Well worked	38	0	14.6	115,000	85,000	88,000		43,000	26,000	21,000
Well worked	48	0	14.6	115,000	160,000	> 300,000		14,800,000	137,000,000	30,000,000
Poorly worked	38	0	14.23	95,000	63,000	55,000		34,000	14,400	19,200
Poorly worked	48	0	14.23	-	127,000	> 300,000		1,850,000	6,500,000	5,800,000
Well worked	38	1.02	13.15	4,000	4,200	1,800		1,730	1,000	520
Well worked	48	1.02	13.15	-	113,000	> 30,000		156,000	340,000	300,000

(continued next page)

Table 16 (con'd)

Degree of working	Holding temperature °C.	Salt %	Moisture %	Counts per ml. after holding periods of: less than 24 hrs.	3 days	7 days	14 days	21 days	30 days	60 days
Poorly worked	38	1.03	14.32	7,300	4,900	3,400		1,300	300	430
Poorly worked	43	1.03	14.32	-	19,000	45,000		160,000	140,000	600,000
Well worked	38	1.98	12.36	3,400	3,800	2,100		1,500	500	500
Well worked	43	1.98	12.36	-	9,000	20,000		48,000	48,000	65,000
Poorly worked	38	1.87	13.16	4,500	5,100	2,200		1,690	1,000	700
Poorly worked	43	1.87	13.16	-	13,300	>30,000		300,000	205,000	212,000
					Aerobacter aerogenes (Strain 2)					
					(2 days)	(4 days)	(7 days)	(14 days)	(21 days)	(30 days)
Well worked	38	0	15.10	126,000	84,000	81,000	59,000	41,000	3,000,000	4,400,000
Well worked	43	0	15.10	-	171,000	3,000,000	30,000,000	137,000,000	212,000,000	55,000,000
Poorly worked	38	0	14.73	90,000	82,000	79,000	52,000	29,500	125,000	470,000
Poorly worked	43	0	14.73	-	151,000	3,000,000	10,200,000	96,000,000	300,000,000	159,000,000
Well worked	38	1.77	13.16	3,600	4,400	3,600	2,900	1,650	1,700	7200
Well worked	43	1.77	13.16	-	6,900	4,100	30,000	15,600	16,900	12,300
Poorly worked	38	1.57	14.20	4,900	6,000	2,920	3,100	2,300	2,020	920
Poorly worked	43	1.57	14.20	-	11,200	6,500	30,000	20,300	20,100	8,200
Well worked	38	2.25	12.92	3,000	3,100	1,220	470	1,80	1,300	320
Well worked	43	2.25	12.92	-	5,100	4,300	26,000	17,000	22,400	9,100
Poorly worked	38	2.62	12.59	5,000	3,600	900	250	990	1,890	240
Poorly worked	43	2.62	12.59	-	3,000	1,630	5,100	3,100	16,000	2,500

effect increased with increase in the concentration of salt, although not in proportion to the quantity of salt added. The rate of destruction of bacteria by salt is greater at the time of addition than it is in the latter part of the storage period. The effect of salt as storage advances will be discussed later with the effect of time on the bacteria in butter. It should suffice to mention here that counts in salted samples always were lower than in the unsalted samples and, barring a few exceptions where the differences were within experimental error, all samples with higher salt concentrations showed lower counts than those which had lower salt concentrations.

Effect of working. The general effect of working was demonstrated by the lower counts in the well-worked as compared to the poorly-worked salted samples. Thorough working brings about a better distribution of salt and moisture throughout the butter mass and usually is expected to result in lower counts in the salted samples.

The results with the unsalted samples in these series were a little different than expected. Counts in all cases of poorly-worked samples were lower than for the well worked ones. The poorly-worked samples had a tendency to show a little irregularity in counts in some series.

Effect of temperature. Temperature exerts considerable influence on the growth of microorganisms in butter. This effect was marked even in a short period of 48-72 hours and was maintained throughout the storage life of butter. As a rule lower temperature was associated with lower counts. Holding at 38°F. was less favorable than 48°F. for all of the five cultures used in these studies. Within any pair of samples, other things being the same, the count at 48°F. was higher than at 38°F.

Effect of time of storage. As indicated before, the general trend with two of the E. coli organisms (Table 16 - strains 1 and 2) was that their number gradually decreased throughout the storage period. This was true in all cases whether the butter was salted or unsalted, well-worked or poorly-worked, held at 38 or 48°F. and with low or high concentrations of salt. The rate of reduction in number, however, was greater with salted samples at 38°F. than at 48°F. At the end of 1 month the samples containing strain 1 with both low and high salt concentrations had less than 2 or only very few organisms while with strain 2 this was true only at the higher salt concentration. At the end of 60 days both strains practically died out in salted samples and their numbers in unsalted samples also were very low. The third sample with E. coli in it showed a different behavior as the time in storage advanced. At 38°F. it showed a reduction all the way, whether salted or unsalted. At 48°F. in the unsalted samples a decrease in count was observed during the first 72 hours, and then the count increased for 2 weeks, after which a drop followed during the remainder of the storage period. However, in the presence of salt, the counts showed a tendency to remain somewhat stationary for 7 days, and then to increase for 3 weeks and finally to be followed by a conspicuous drop.

The two strains of A. aerogenes behaved a little differently from each other. Strain 1 (table 16) almost always showed a decrease in count all through the storage at 38°F. and this was true of both salted and unsalted samples. The only exception was the unsalted poorly-worked sample which showed a slight increase at the end of 60 days. Even at the end of the 60-day period the organisms in these samples had not died out completely.

At 48°F. the unsalted samples showed a constant increase in count for 30 days, after which the counts decreased. However, the decrease was not very definite in the poorly-worked samples. The well-worked, mildly-salted sample showed a constant increase in count for 30 days, after which there was a slight decrease in count. The poorly-worked sample in this group showed the increase only for 3 weeks, and then a slight drop was followed by an increase at the end of the 60 day period. With the increase in the concentration of salt, in the case of well-worked samples, the growth continued all through the storage and a high point was reached in 60 days. With poor working this point was reached earlier and was followed by a drop after which the counts were more or less stationary.

Storage studies with A. aerogenes strain 2 were obtained for a period of 30 days only. This strain showed a continued drop for 2 weeks at 38°F. in the unsalted sample, after which even at that low a temperature an increase occurred. With about 1.6 per cent of salt added to it, this trend did not change very much, except that at the end of 2 weeks, instead of showing an increase in the count similar to the unsalted samples, the count tended to remain stationary for some time and then to decrease. Perhaps this was due to the light inhibitory effect of salt present in the sample. When the concentration of salt was increased the drop in count was a little sharper but only for a week and then the counts increased for the next two weeks, to be followed by a decrease. This probably was due to the fact that the higher concentration of salt destroyed more organisms but those remaining at the end of a 2 week period were the selected ones that possessed or had developed the ability to resist high concentrations of salt. These organisms then might be responsible for the subsequent

increase in count.

At 48°F. the unsalted well-worked samples showed an increase all through the storage. The poorly worked samples reached the peak at the end of 3 weeks and then showed a decrease. The salted samples showed a very irregular change in population at this temperature, but the maximum counts reached were greater in the lightly salted samples than in those containing more salt.

DISCUSSION

A general lack of close relationship between the counts and initial butter quality was observed. Butter flavor and aroma, and consequently the score, depend on such a subtle complex of physical, biological, biochemical and chemical reactions that it is not surprising at all that microbial counts fail to show a definite relationship to the butter score or fail to provide a basis for the prediction of the keeping quality of the samples. The individual taste and liking of various judges also may be a factor in the relationship under some circumstances, because of the differing degrees to which even trained judges may be impressed by different levels of various defects.

Therefore selection of one microbial type from the several which may influence butter quality is difficult. To say that the one type chosen is the key to the grading situation is not possible. The results of investigations such as the present one should be interpreted in the light of these limitations. However, a new test or a new application of an old test may yield results which may be of value in the grading of butter, particularly from the standpoint of commercial keeping quality.

The majority of the samples studied gave counts of less than two coliform organisms per ml. Therefore it can be concluded that production of commercial butter having low coliform counts is possible without undue difficulty, particularly when the butter is salted. This agrees with the observation of Crossley (1946) who studied 126 samples of butter and found that 43 per cent gave negative tests for coliform organisms, although

quantitative data were not presented.

Coliform organisms are comparatively salt-sensitive and therefore their number in commercial butter is effectively reduced, apparently without causing a proportional quantitative decrease in at least some other groups. This explains why a low coliform count does not necessarily mean a low count of other organism types or freedom from microbial deterioration during keeping quality tests. However, a high coliform count does indicate the possibility of contamination with other groups of microorganisms, making the butter more vulnerable to microbial deterioration. The relationship of coliform bacteria to the initial score therefore is expected to be only a general one in which the low count samples may have scores ranging all the way from the lowest to the highest. Coliform organisms are only one type and may or may not be associated with defect-producing bacteria. This has been demonstrated for creamery water supplies. In the present investigations there is a tendency for the samples with high coliform counts to score low, but the discrepancies are many. Rice (1938) did not find any significant difference in score of samples highly contaminated with coliform bacteria or with fewer bacteria of this group. However, it is reasonable to believe that a high coliform count is often only due to carelessness or faulty equipment and there is a tendency for the operator who is lax enough to have high coliform counts to be lax enough to fall somewhat below desirable levels of other plant operations.

No one of these counts employed served as an index of the keeping quality of an individual sample of butter. It is only in a general way that the plate count of the various types of organisms show a relationship

to keeping quality. Many samples with high counts fail to develop defects and defects appear in some samples with low counts.

In holding tests the various organisms in butter get a chance to carry on their activities at a greater speed, so that the changes they might produce in a long-time storage may be observed in a week or 10 days and the samples be accepted or rejected for storage. Here again the relationship of coliform count and loss in score on holding would be expected to be only a general one. Samples with a low coliform count may undergo no loss or may lose several score points for the reasons given before, i.e., the salt may have destroyed all the coliform organisms without bringing a proportional reduction in the number of other organisms which may be responsible for butter deterioration. Besides there is no necessary association between coliform bacteria and those producing defects, as one can be present without the other. However, it will be expected that the chances of deterioration will be lessened with a low coliform count. The present investigations indicate this and thus are at variance with the results of Parfitt (1936) who found no relationship between the keeping quality of butter and the presence or absence of the Escherichia-Aerobacter group. It will also be expected that when coliform counts are higher the total count also will be higher, but whether or not the additional organisms are spoilage organisms depends on the type of contamination. The rate of multiplication also may be an important factor. Coliform organisms may multiply faster and somewhere during the holding of butter they may have increased in number without a corresponding increase in other groups. The two factors may be responsible for some samples showing a

high coliform count and still not showing any loss during storage. However, as a general rule, the majority of the samples with high coliform counts will be expected to show a greater tendency to lose points during storage. This analysis is confirmed in the present investigations.

A fairly large portion of the samples have counts of less than two gram-negative organisms per ml., as estimated by the methods used in these investigations. The gram-negative group includes the coliform organisms, along with others. It tends to influence initial butter scores in the way coliform organisms do, but the relationship is less definite. The effect on the initial score of butter will depend, to some degree, not on the degree of initial contamination but on the opportunity provided to these organisms to multiply. In the case of a very high gram-negative count under average conditions, at least some multiplication may occur and an effect on butter deterioration may be expected. The data tend to point toward that.

The observations as to the effect of the number of this group on the loss in score in holding should be expected to be similar to those with coliform organisms. The results reported tend to confirm this relationship.

Looking through the literature one comes across more work on the influence of yeasts and molds on initial score and keeping quality of butter than in the case of any one other group. Some workers did not observe any relationship, while others found yeast and mold counts to be an index of keeping quality and of fresh score of butter. In the present work, no relationship at all could be established between the yeast and mold count and the score of fresh butter. This is quite logical because

yeasts and molds usually do not cause much deterioration of butter and because most of the butter samples pick up yeasts and molds from the churn during the working but they do not get a chance to develop and cause deterioration because of the presence of salt in butter and the usual low temperature at which commercial butter is stored. However, in the present work they have shown a little tendency to bear a general relationship with loss on holding at 21°C. Thus, the chances for butter samples to lose points on holding increase as the number of yeasts and molds increase, not because they alone are a significant factor in deterioration but because they are associated with post-pasteurization contamination which may introduce extraneous organisms capable of causing pronounced defects.

The data reveal that samples with very low total counts have no better chances to score high than do those that have very high counts. The total count of butter samples is the most variable of counts studied. This is because of various factors, such as condition of the raw cream, the time and temperature of pasteurization, the degree of sterilization of the various pieces of equipment, the use of a butter culture, with or without ripening, the quantity of salt if used and the storage conditions. When butter cultures are used the total count can have little significance because the numbers of other bacteria ordinarily will be so small proportionately that they would not be reflected in the count. However, very few of the samples studied showed any evidence of the use of butter culture.

There is no treatment given to butter or cream after pasteurization, except the washing of the granules, that may destroy or reduce the microbial flora of the cream, until the butter is salted. Salt certainly

reduces the number, but there is now a tendency to use a smaller quantity of salt than was used some years ago. Therefore, a high grade butter with a rather high total count may not be rare. On the other hand, all samples of cream have a rather high total count when they are brought to the creamery. Even with a very high efficiency of pasteurization, a considerable number of bacteria may be left in cream. An extra exposure of cream to a higher temperature or time gives the cream a cooked or burnt-protein flavor or scorched-fat flavor, either of which results in poor butter. Thus ordinarily it does not seem to be possible to reduce the number of organisms below a certain level without injuring the flavor of butter.

The best chances of scoring high, according to these data, seem to be in the range with counts from 3,000 to 30,000 per ml. As the counts go above or below these levels, the average score of samples in the group tends to go down. This is in accordance with the findings of Loftus-Hills et al (1939) who found that their choicest samples had 12,000 bacteria per ml., their next best lots which they called first had 26,000 bacteria and the next to that which they called seconds had 5,200.

As far as total counts are concerned, they offer a possible means for checking the pastourization, sterilization of equipment and general sanitation within the creamery. It is evident however that the relationship to quality need not be quantitative. There are many examples in microbiology where the alterations in an organic material are not a matter of numbers of microorganisms but rather of their types and specific capabilities. This is so true of butter. Russel and Hastings (1920) stated that all microorganisms in butter tend to influence its keeping quality unfavorably to a greater or lesser degree, and the lactic acid bacteria are

the least injurious of all. Therefore it would be expected to find at least a general relationship between the total count of fresh butter and the loss in holding test. The data show some tendency that way.

High coliform counts are associated with high gram-negative counts and high total counts. This is to be expected for coliforms are themselves gram-negative. A high gram-negative count on the other hand will not necessarily be accompanied by high coliform count. The same is true for total count. Coliform organisms occur in pasteurized cream as post-pasteurization contamination, and there is reason to believe that when coliform bacteria find their way into butter, others, too, may have gained access. At the same time the sample may show a high total count with a low coli count or even in its complete absence. The possibility of the use of butter culture has been already discussed which supports this phase of butter microflora.

Commercial butter often has a higher yeast and mold count than a coliform count. This suggests that either there is a greater contamination of yeasts and molds in the finished butter as compared to that of coliform organisms or the conditions in butter are more favorable for the survival of yeasts and molds than of coliform organisms. Undoubtedly both these factors are operative.

During the manufacture of butter, the cream and butter have opportunities to pick up microorganisms at various points during the processing operations from the pieces of equipment and the materials they contact. The type of organisms and their number will depend on type and condition of equipment and the general sanitation of the plant.

If pasteurization is carried out properly and proper sanitation practices followed, the cream as ready to be pumped to the churns should be free of coliform and apparently all other non-sporulating gram-negative bacteria, just as it should be free of yeasts and molds. Since coliform and other non-sporulating gram-negative bacteria are destroyed by the pasteurization times and temperatures commonly used in creameries, presence of these organisms in the product at any time during the manufacturing operation may be considered indicative of contamination. Although all microbial groups in the present study showed a tendency to increase during processing operations, in most cases the coliform and also gram-negative bacteria were a better index of the sterilization efficiency of the vat, pump, pipes and the filter, while yeasts and molds were a good index of churn sanitation. The general distribution on poorly cleaned equipment, the ease with which small numbers may be enumerated and the comparatively short time needed for enumeration by the plate count technics make the coliform bacteria particularly suited as an index of contamination. The data indicate that coliform and gram-negative counts on line run samples are good indices of plant sanitation.

The line run tests indicate very clearly that one reason for the lack of relationship between coliform counts, and also gram-negative counts to a certain extent, and either initial score or keeping quality is the inability of most of these bacteria to tolerate the salt concentrations commonly employed in commercial butter. When counts of coliform bacteria can be in the thousands per milliliter in the buttermilk and be as low as less than 2 per milliliter on the finished salted butter, a count

on such butter hardly can be considered a good index of sanitation during manufacturing. Yeasts and molds generally show a significantly greater tolerance than do coliform or gram-negative bacteria and thus seem better suited as a sanitary index, despite their tendency to miss or minimize certain contamination preceding the churning operation.

The results on line run samples indicate definitely that contamination in the plants studied was at a level much higher than desirable. In many runs the less sensitive index employing total count was adequate to detect contamination because of the very poor sanitary conditions which prevailed.

Under normal conditions butter is subjected during most of its commercial life to temperatures which may permit some bacterial growth. Storage in the frozen condition will prevent microbial development but most butter is not subject to such conditions all the time. A number of factors affect the microbial population of butter during the time it is merchandised.

The data on the effect of strain of coliform organisms, amount of salt added, degree of working and temperature of holding indicate definitely that the coliform population of a sample of butter is not static. The manner in which the population changes varies with each of the factors studied, and prediction of the trends of coliform population in a given sample of butter would be extremely difficult, even if the history of the sample were known with a considerable degree of detail.

Some strains of coliform bacteria will multiply at a storage temperature of 38°F. while others will not. To say that a given number of coliform bacteria in a sample of butter represented so much initial contamination

would be extremely hazardous. It is true that the coliform population in general decreased as the butter was held, particularly at a temperature of 58°F., but at higher temperatures A. aerogenes multiplied in butter containing as much as 2 per cent salt. Hammer and Yale (1932) also have shown that Acrobacter species showed more growth in butter at 7°C. than did the Escherichia group. The present studies were made with populations appreciably greater than those which would be encountered in normal commercial samples, in order to have sufficient numbers of bacteria for accurate enumeration by the plate count procedure. However, the populations were not so great but that the behavior of the usual number of coliform bacteria in butter may be presumed to follow much the same pattern of change.

It has been demonstrated in numerous instances that salt inhibits the growth of microorganisms. However, both salted and unsalted samples of butter have shown deteriorations, developing sometimes within a few days after churning. More often these losses have been associated with high counts or the presence of certain specific organisms. The effect of salt on bacterial changes in butter has been exhaustively reviewed by Hammer and Long (1941).

The amount of salt that will prove effective against the organisms in butter depends on the strains present in it. The Aerogenes group in general showed greater resistance than the Escherichia group. The effect of salt, however, will also depend on the degree of initial contamination and the ability of the particular organism to adapt itself to salt. Winslow et al (1932) reported that in broth large numbers of organisms tend to neutralize the inhibitory effect of salt and stated that there

always is a mass effect caused by large numbers of living or dead cells which tends to neutralize any inhibitory action. Clemmons (1926) inoculated various concentrations of *E. coli*, *Micrococcus albus* and *Bacillus mesentericus* in bouillons containing from 1 to 12 per cent salt. The larger inoculations of the organisms showed greater salt tolerance than the smaller inoculations. With *M. albus* an inoculation of 50 cells failed to grow in 4 per cent salt bouillon, whereas an inoculation of 50,000 cells grew in 11 per cent salt bouillon.

That the salt tolerance of certain organisms can be increased by continued cultivation on salt agar was noted by Giltner and Brown (1915). Garrard and Lochhead (1939), while working with pickle brine, reached a similar conclusion.

The observations in these investigations show that the bactericidal effect of salt is well marked with all strains and is about the same. However, the abilities of various strains to adapt themselves to salt at the temperatures employed differ widely.

The effect of the degree of working and the distribution of salt in butter is an important factor in the control of bacterial changes in butter. The literature on the subject has been reviewed by Long and Hammer (1937) and Hammer and Long (1941).

Thorough working helps finer distribution of moisture droplets. As the number of bacteria in butter is limited, the higher the number of moisture droplets, the larger is the proportion of sterile ones and the less food material is available to bacteria. In other words, more and better working deprives microorganisms in butter of most of the bacterial food and thus retards their growth. Therefore it would be expected that

the well-worked butter will show lower counts than the underworked, and the data on salted butter in these investigations support this interpretation. On the other hand, the unsalted poorly-worked samples show a lower count than the well-worked samples. The explanation for this phenomenon is not apparent from the data available.

These data indicate that finding few or no coliform bacteria in butter, as frequently was the case in the survey of commercial samples, may only mean that the bacteria originally present in the butter as a result of contamination have died as the result of salting the butter and possibly because of low holding temperatures. This correlates with the results obtained on the line run samples, where butter with low coliform count frequently was obtained from cream which had been contaminated grossly during the operations following pasteurization.

The finding of considerable numbers of coliform bacteria in a sample of butter probably would indicate gross contamination in most instances, but there are circumstances when considerable proliferation of these organisms may occur, even in salted butter.

In unsalted butter, considerable proliferation of coliform bacteria might occur at temperatures in the range of 48°F., if strains corresponding to several of those tested were present, and might even occur at 38°F. if some strains of A. aerogenes were present. At 38°F. the count of E. coli dropped rather consistently during the storage period employed, indicating that the type of coliform organism would be very important in determining the population changes which would occur, even in unsalted butter.

The gram-negative organisms were not studied in the detail used with

the coliforms, particularly as to behavior in controlled laboratory samples, but the data on line run samples are such as to indicate that the behavior of most strains of gram-negative bacteria may be expected to fall within the range established for the coliform group. None of the data indicate the probability that the gram-negative count would be any more suitable and reliable index of contamination or keeping quality than would the coliform count.

The primary field of usefulness of the coliform count seems to be in the detection of sources of processing contamination when used on line-run samples. For this purpose the procedure apparently is capable of detecting contamination gaining access to the product before the cream enters the churn more satisfactorily than does the yeast and mold count. The comparatively short time required for results and the sensitivity of the test for small numbers of coliform bacteria among large numbers of other bacteria are additional advantages of the procedure. The inability of the coliform test to detect contamination with spoilage organisms from wash water also is shared by the yeast and mold count. As a test to be used on commercial butter samples of miscellaneous histories, as an index of contamination or keeping quality, the coliform test seems to have little applicability because of the many opportunities for erroneous interpretation due to the unpredictable multiplication and death behavior of this group of organisms in butter.

SUMMARY

Two hundred and ninety-four samples of commercial butter were analyzed for coliform organisms, gram-negative organisms, yeasts and molds and total count. Initial butter scores and scores after 7 days at 21°C. were obtained.

The coliform counts were about the same whether the plates were incubated at 37°C. for 18 to 24 hours or at 30°C. for 30 to 36 hours. An incubation temperature of 30°C. for 3 days showed a tendency to give higher gram-negative counts than did 21°C. for 5 days. A crystal violet concentration of 1:150,000 gave higher gram-negative counts than one of 1:85,000. The total counts were higher with a greater number of samples when plates were incubated at 21°C. for 5 days as compared to 30°C. for 3 days.

A low coliform count did not insure high scoring butter, but samples with a high count showed a tendency to score low upon receipt. The same tendency, but to a lesser degree, was shown in the case of the gram-negative count. The yeast and mold counts did not bear any relationship to the score of butter on receipt. Both very low and very high total counts tended to be associated with low scoring butter. The best chances for high scoring buttor were with a total count between 3,000 and 100,000 per ml.

Low coliform counts did not insure good keeping qualities, although high counts, particularly counts over 300 per ml., diminished the chances of the score being maintained during the keeping quality test. Gram-negative counts showed the same tendency, although the trend was less

definite. Samples with high yeast and mold counts showed greater deterioration in keeping quality than those with lower counts. The total plate counts failed to show any definite relationship to the loss in score during the keeping quality test.

The gram-negative counts increased with an increase in coliform count, but many samples showed a high gram-negative count and a low coliform count. No definite relationship existed between the coliform count and the yeast and mold count. High coliform counts were accompanied by high total counts, although certain lots of butter with high total counts showed low coliform counts. Higher gram-negative counts showed a tendency to be associated with higher yeast and mold counts, although there were many discrepancies.

Samples from 15 line run series were analyzed for all the four types of organisms. Pasteurization efficiency apparently was high in most cases, but there were evidences of post-pasteurization contamination of some of the samples. The pump and the pipe line in most cases were sterilized inadequately, and they added to the counts of all types. Cream after a short churning always had a higher count of all types than it had before the churning started. Buttermilk carried away a large portion of the microorganisms and showed a very high count of all types. Washed granules had comparatively lower counts, although the amount of decrease varied from churning to churning. During the process of working, yeast and mold counts frequently increased due to the pick-up of large numbers of organisms from the churn wall and probable breaking of clumps; but usually the bacterial counts showed a decrease over that of washed unworked granules,

apparently because of the action of salt. The yeast and mold count showed an increase due mostly to the physical pickup of the organisms and also probably due to breaking up of the mold mycelia into numbers of fragments. Apparently salt was not as effective against the yeasts and molds and the bacteria which make up the total count as it was against the coliform and the gram-negative bacteria.

Five semi-commercial churnings were made with highly-pasteurized cream inoculated with pure cultures of three strains of E. coli and two strains of A. aerogenes. Butter granules from the churn were worked into the laboratory and two degrees of working and three concentrations of salt - including no salt - were employed. Salt exerted a very marked germicidal effect on all five cultures of coliform organisms, but as storage proceeded the effect of salt was more marked on two strains of E. coli (Strain 1 and 2) than on the third strain of this group and the two strains of A. aerogenes.

Mild salting was more effective in reducing the count than no salt and high salting was more effective than mild salting. In the case of the first two strains of E. coli, all salted samples became more or less sterile in 60 days.

Low temperature was more effective against the E. coli than against A. aerogenes. One strain of the former (strain 3) withstood low temperatures better than did the other two. During storage the counts of two strains of the E. coli (strain 1 and 2) decreased both at 38°F. and 48°F. and in either the presence or the absence of salt. The third strain of E. coli and the first of A. aerogenes showed a constant decrease in count at 38°F.

while at 48°F. there was a decrease both in the beginning and at the end with a short growth period in between. The second strain of A. aerogenes showed this behavior both at 38 and 48°F.

Poorly worked salted samples showed higher counts than well worked ones; with unsalted samples the trend was opposite.

CONCLUSIONS

Coliform count cannot be used as an index of initial score of butter or of keeping quality of an individual sample. There is, however, a general tendency for samples with high counts to score low and show a poorer keeping quality. Similar statements apply to counts of gram-negative bacteria.

Both counts on line run samples and controlled laboratory tests show that salt may destroy a large percentage of the coliform and gram-negative bacteria originally present in a sample of butter. Temperature and time of holding and degree of working also influence growth and survival of coliform bacteria in butter.

The field of satisfactory applicability of the coliform count in butter testing seems to be confined to determining sources of post-pasteurization contamination in line-run samples.

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