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Detection and significance of enterococci in dairy products

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DETECTION AND SIGNIFICANCE OF ENTEROCOCCI IN DAIRY PRODUCTS

by

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INTRODUCTION

Many tests have been devised to judge bacterial quality of milk or milk products. Each has its limitations and measures only a circumscribed segment of product quality.

Much emphasis has been placed on the total bacterial count as an index of quality. Milk with low total count is regarded as "quality milk" without further question. The total count, admittedly, is a valuable test, but does not provide complete information for evaluating a milk supply. Total counts respond to massive contamination but do not properly reflect numerically small but physiologically significant infection. Milk cooling may conceal unsanitary conditions when quality is measured by this test, particularly with the better cooling facilities, including bulk tanks, now used on many dairy farms. Reduction tests and the direct microscopic count are of even less value. Dairy bacteriologists have, therefore, turned toward enumeration of specific groups of organisms by the use of selective media as means of revealing improper milk handling methods.

The presence of coliform organisms in large numbers in fresh milk is almost invariably regarded as evidence of carelessness at some stage of its handling. However, this test has been criticized by many workers on grounds that it is lacking in specificity and has little or no correlation with other tests and the keeping quality of milk. A plate or tube method gives no indication of the probable source of the organisms, whether they be of fecal or nonfecal origin, Escherichia, Aerobacter or intermediate types. Since routine procedure in the dairy or public health laboratories does not usually include positive identification of colonies found growing

on the various selective media, it has been suggested that these tests are of limited value. The use of the presumptive coliform test has been dropped from regulations controlling raw milk grading in England; in France, it is now required to count only the indole-producing coliforms in milk. Unfortunately, there is evidence that the true fecal types may lose the power of indole formation. In the United States few public health authorities assay raw milk samples either qualitatively or quantitatively for the presence of coliforms, but a limit is set for the numbers permissible in certified milk. However, with the increased use of farm bulk tanks and pipeline milkers, more laboratories are now turning to the coliform count on raw milk to help ascertain the quality of sanitation used in milk production.

Because coliforms do not generally survive proper pasteurization, a positive coliform test on pasteurized milk products virtually always indicates recontamination. However, since the coliform organisms are a diverse group their reaction to the adverse microenvironment encountered in certain dairy products, such as butter, may be completely unpredictable.

Coliforms are widely distributed in nature and are found in water, grain, soil and plants. They may be present in virgin soil and water away from sources of pollution. They are also capable of multiplying in soil and water. Coliforms organisms do not always constitute the major flora of the intestines. They are usually outnumbered in the feces of man and animals by certain species of streptococci. Recently, bacteriologists have turned their attention toward the enumeration of enterococci in water and various other products. Enterococci are invariably found in feces, sewage and contaminated water; they are not found in potable water, most

virgin soil and sites out of contact with man and animal. Presumably they do not multiply outside the alimentary canal except in rich nutrient menstrua. Evidence indicates the greater ability of enterococci over the coliform group to survive in antagonistic environments. Enterococci may survive for long periods of time in frozen foods. Consequently many bacteriologists are now proposing the use of enterococci as a more dependable index of sanitation.

Little work has been reported on the presence or significance of enterococci in dairy products. Comparative studies with other indicator organisms are almost lacking. This investigation was undertaken to collect much needed information on, and assess the sanitary significance of enterococci in raw grade A and manufacturing grade milk, butter and other dairy products.

REVIEW OF LITERATURE

Enterococci

Taxonomy

Recently, interest in the classification of enterococci has been revived. Because of the distribution and import of this group, this concern has not been confined to any particular subdivision of microbiology. As an unfortunate result, the terms "Streptococcus faecalis," "enterococcus," "enterococci," "fecal streptococci," "Group D streptococci," and even "streptococci" are being used too loosely and too interchangeably to describe the streptococcal organisms inhabiting the intestinal tract of man and animals, water, soil, feces, foods, and dairy products. A critical examination of the physiological and serological classification of these organisms may help to clarify some of the ambiguities present in their nomenclature.

Thiercelin (165) first used the term "enterococcus" to describe, on a morphological basis, a gram-positive diplococcus of intestinal origin. Andrews and Horder (6) called a group of non-hemolytic streptococci characteristic of the human intestine, Streptococcus faecalis. Dible (40) thought that the two names were synonymous and suggested that they be applied to a group of heat resistant diplococci commonly found in human feces. Orla-Jensen (119) identified and named two of these heat resistant diplococci, Streptococcus faecium and Streptococcus glycerinaceus without relating them either to Streptococcus faecalis or to the "enterococcus." Sherman (148) used the term "enterococcus" to designate a group of

streptococci comprising hemolytic, non-hemolytic and gelatin-liquefying types. He thought that the Streptococcus faecium and Streptococcus glycerinaceus of Orla-Jensen (119) were both synonymous with Streptococcus faecalis of Andrews and Horder (6). Sherman (148, 147) regarded the "enterococcus group" as comprising Streptococcus faecalis and its varieties, liquefaciens and zymogenes, and the Streptococcus durans of Sherman and Wing (153).

According to Bergey's Manual of Determinative Bacteriology (16) the enterococci (organisms belonging to the "enterococcus" group of streptococci) comprise S. faecalis, S. faecalis var. liquefaciens, S. faecalis var. zymogenes, and S. durans. These are the fecal streptococci which share the Sherman criteria (148), i.e., growth at 10 and 45 C, at pH 9.6, in 6.5% NaCl broth, in 0.1% methylene blue milk, survival at 60 C for 30 min., and production of ammonia in peptone broth. S. faecium, Orla-Jensen (119), has not been recognized as either a separate species or variety in this manual. There is considerable evidence, however, in recent literature to suggest that this species (or variety) deserves individual recognition.

Skaudhauge (156) differentiated S. faecalis from S. faecium by showing that the former grows in a medium containing a 0.04% concentration of potassium tellurite, whereas the latter is inhibited. Barnes (10) observed that the two species share the Sherman criteria (148) but have different reduction and fermentation reactions, in addition to the different tellurite tolerance. Barnes (10), and Barnes and Ingram (11) reported that S. faecalis usually produces reduction, acid and coagulation in

litmus milk in 24 hr, but S. faecium shows less reduction and sometimes only acid production. London and Appleman (91) found that in vigorously aerated glucose medium cultures, S. faecalis produces acetic acid and acetylmethylcarbinol in a ratio of 1:1, but the acetic acid-acetylmethylcarbinol ratio for S. faecium is 35:1. Kereluk (76) studied 307 isolates of enterococci from frozen meat pies. He separated S. faecium strains from S. faecalis and its varieties by use of the identification scheme of Barnes (10). He concluded that S. faecium is a separate and easily distinguishable species of enterococcus deserving independent status.

The use of serological methods has substantiated the biochemical and cultural differentiation of enterococci from other streptococci. All four members of the traditional enterococcus group and S. faecium possess Group D antigen.

Shattock (145) first suggested that S. bovis also contains D antigen, is serologically identical to other enterococci and should be included in this group. Using preparations of type-specific antisera, Sharpe and Shattock (143) designated 24 serological types within the Group D streptococci. Sharpe's (141) serological type of S. faecium became number 25; 14 new types described by Sharpe and Fewins (142) were added to the previous list, thus totaling 39 serological types in Group D. Shattock (144) thought that there were more serological types within Group D, and that the three broad divisions proposed by her within this group on physiological grounds (viz., S. faecalis and its varieties; S. faecium and S. durans; and S. bovis and S. equinus) could be substantiated by serological studies. Jones and Shattock (71), Medrek and Barnes (102), Papavassiliou (122) and

several other workers have reported on the serology of Group D streptococci. Findings generally support the conclusions drawn by Shattock (144). Elliott (42) found the cell-wall carbohydrate in Group D to be type-specific and suggested that the D antigen is probably a polymer of glucosyl glycerophosphate. Shattock (144) stated that in Group D streptococci the group antigen is not an integral part of the cell wall, but is in the cell contents which remain when the cell wall has been removed. Slade and Shockman (157) found the protoplast membrane fraction of S. faecalis positive for the D antigen. They also noted that repeated washing of the membrane fraction results in gradual removal of the antigen.

Medrek and Barnes (102) reported that some Group D streptococci, when grown under certain conditions, did not yield HCl extracts which would react serologically with Group D antisera. Sharpe (141) noted that a common type antigen was present in both a strain of S. lactis and a strain of S. faecium which would allow them to react with both Group D and Group N antisera. Gunsalus et al. (51) found that three strains of S. lactis R had Group D serological reactions.

Smith and Shattock (162) isolated strains of S. equinus from horse feces and found they belonged to Group D. The D antigen was produced by all the strains examined, but was not always extractable with HCl, although broken cells always gave D antigen. Unlike S. bovis, which has numerous serological types based on their capsular antigens--a characteristic in which it is distinct from other species within Group D (Medrek and Barnes, 102), the type antigens of S. equinus have not yet been studied. Bergey's Manual of Determinative Bacteriology (16) mentions that no

group-specific antigen was demonstrated for this species, although Sherman (147) noticed that some of his strains of S. equinus reacted weakly with Group D antisera.

Apart from the serological patterns, there are well-defined physiological differences between S. bovis, S. equinus, S. faecium and the four original members of the enterococcus group which still find wider use with the majority of bacteriologists. S. equinus characteristically does not ferment lactose and gives no reaction in litmus milk, whereas S. bovis ferments lactose and produces acid. Both grow at 45 C but do not share any of the other criteria of Sherman (148) for enterococci. S. bovis hydrolyzes starch, is insensitive to lysozyme systems (Hartsell and Caldwell, 56), and is the only streptococcal species capable of using ammonium salts as a sole source of nitrogen for growth (Wolin et al., 177). Hartsell and Caldwell (56), however, favored use of lytic techniques. According to their findings S. faecalis and its varieties were lysed by a combination of lysozyme and trypsin, but not lysozyme alone; S. faecium and S. durans were lysed by lysozyme alone; and S. bovis was completely resistant to lysis. On the basis of their data they questioned even the varietal designation for S. faecalis varieties liquefaciens and zymogenes, and suggested that they be called S. faecalis with the ability to liquefy gelatin and to hemolyze blood agar (regarded as variable characteristics). It may be noted, however, that these two varieties were accorded independent designations before publication of the present edition of Bergey's Manual of Determinative Bacteriology (16).

Colobert and Blondeau (27) recently reported that S. faecalis, as

identified according to Sherman's scheme (148), proved to be constituted of a great number of biotypes. According to them S. faecalis proprium and S. faecium represent only two of these biotypes to which may be added S. durans and a hitherto undescribed type which they called S. innominatus. It was further suggested by Defayolle and Colobert (39) that S. faecalis should be considered a collective strain (species), in which evolutive buds are, in fact, functional biotypes, which might be identified under names of varieties: proprium, faecium, durans, liquefaciens, innominatus, etc. Sherman et al. (149) found the fermentation tests in S. faecalis of diverse nature and regarded them of minor descriptive value. No justification was seen in differentiation of the species on the basis of fermentation tests. Shattock (146) reviewed the classification of S. faecalis and associated streptococci. Bartley and Slanetz (13) suggested that all streptococci commonly inhabiting the intestinal tract of man and animals be included within the enterococci. Kenner et al. (74) named S. bovis, S. mitis, S. salivarius and S. equinus, along with the enterococci, to be included in "fecal streptococci." These latter authors felt that undue emphasis was given to enterococci.

There are bound to be wide variations in the distribution, source and survival of all species of fecal streptococci. To consider them all of the same sanitary significance would hardly be justified. The term "enterococcus" is now being abused in the literature to such an extent that a good term may be doomed to elimination.

Detection

Many procedures and media for detection of the enterococci have been described. Hartmann (55) used sodium azide in a medium for the selective growth of mastitic streptococci. This compound, while permitting the growth of streptococci, suppressed the growth of other organisms. McKenzie (100) used thallium acetate, and Fleming (47) used potassium tellurite for a similar purpose. Both sodium azide and thallous acetate have since been used in many media for the detection and enumeration of enterococci and other fecal streptococci. The tetrazolium reduction activity of enterococci and other bacteria was studied by Laxminarayana and Iya (86) who found enterococci to be the most active of the organisms studied. This property of enterococci has been utilized for obtaining counts on agar plate media by observing the color reactions of colonies. Both triphenyl-tetrazolium chloride and diphenyl-tetrazolium chloride are used in different selective media for enterococci.

Hajna and Perry (52) described "SF" medium which they claimed to be highly selective for fecal streptococci from water, sewage, milk and for growth of fecal streptococci when transferred from other primary media. Mallmann and Seligmann (99) compared the Sodium Azide broth of Mallmann (97), the SF broth of Hajna and Perry (52), and the Azide Dextrose broth of Rothe as described by them (99). They found the Azide Dextrose broth to be the best medium for the quantitative determination of fecal streptococci. Litsky et al. (90), using Glucose Azide broth as a presumptive medium, designed a new confirmatory medium, Ethyl Violet Azide broth, which they reported as selective for enterococci. Zaborowski et al. (179)

compared several liquid media and considered Azide Dextrose broth (99) satisfactory for detection and enumeration of enterococci in frozen foods. Using Glucose Azide broth, Childs and Allen (26) determined the most probable numbers of S. faecalis by 'direct,' 'subculture,' and 'resuscitation' methods, and found the last method the most specific for the organisms concerned. Chesbro and Evans (25) used a carbonate-buffered medium adjusted to pH 10.0 and recommended it as a superior enrichment broth for the detection of enterococci in fecal samples.

Several workers have used the membrane filter technique to enumerate enterococci in water: Slanetz et al. (159); Slanetz and Bartley (158); Morelis and Colobert (108); Kenner et al. (74); and others. Slanetz and Bartley (158) and Kenner et al. (74) advocated broadening of the enterococcus group to include all fecal streptococci, for which, they claimed, their media were selective and satisfactory. These workers preferred the use of the membrane filter and agar plate method over the multiple tube procedure. Morelis and Colobert (108) used a much higher azide concentration, semi-anaerobic conditions and shorter incubation.

Barnes (9) proposed two methods for the isolation and enumeration of Group D streptococci. The first consists of a presumptive count in Lab-Lemco-Peptone Glucose broth containing thallous acetate, followed by confirmation by streaking on Tetrazolium Glucose agar. The other method uses direct plating with this medium containing agar. Differentiation between the colonies of S. faecalis and its varieties, and those of the other Group D streptococci can be made on plates of this medium. The selectivity of this medium is, however, questionable. Barnes (8) stated

that by increasing the sodium azide content of many media in order to increase selectivity, S. bovis is often eliminated. The thallous acetate media could be safer in this respect.

Mallmann and Kereluk (98), and Kjellander (77) developed selective plating media for detection and enumeration of enterococci in water, while Ross and Thatcher (136) reported another medium for similar use in foods. White and Sherman (172) devised a medium, Penicillin Azide agar, for the enumeration of enterococci in raw milk. They reported that their medium, although completely selective, partially inhibited the growth of S. durans. A plating medium for the isolation and enumeration of enterococci in dairy products was developed by Reinbold et al. (134).

Few comparative studies have been made to determine the suitability of different media. Saraswat et al. (139) studied ten media to select a plating medium for the isolation and enumeration of enterococci in dairy products. They selected Citrate Azide agar (134) after increasing the sodium azide content of the medium to 0.4 g/liter. High recovery, selectivity but not undue inhibition of enterococci and ease in obtaining and interpreting results were the criteria used in selecting the medium by these workers who thought that the organisms sharing the Sherman criteria (148), including S. faecium, formed the enterococcus group. S. bovis and S. equinus did not grow on this medium.

Distribution

Water, plants, insects, etc. Enterococci have been reported to be widely distributed in nature. Buttiaux (21) studied the incidence of

different species and varieties of enterococci in untreated water supplies and reported their frequency in the following order: S. faecium, 82; S. faecalis, 16; S. faecalis var. liquefaciens, 8; S. faecalis var. zymogenes, 1; S. durans, 1; and S. bovis, 1. These organisms were present in almost 10% of the water samples examined. The samples were not known to be contaminated with fecal matter.

Recently, interest in the occurrence of enterococci in plants and comparable materials, hitherto not considered sources of these organisms, has been shown by some workers, chiefly Mundt and his co-workers. Mundt (111) observed that enterococci were invariably present or absent in certain plant species. They occurred in small numbers in enclosed tassels and silks of corn, and in greater numbers after the floral parts had emerged. Interposition of a mechanical barrier reduced the incidence of recovery from flowers. He concluded that enterococci may be regarded as temporary residents on plants, capable of limited reproduction, and may be disseminated among plants by insects and wind. Mundt and Johnson (113) isolated Group D streptococci from plants and studied their physiological characteristics. They concluded that, although there was some evidence for the existence of an independent plant flora, no common property had been uncovered until then to confirm such existence. In a recent publication, however, Mundt et al. (112) suggested that S. faecalis var. liquefaciens is a potential epiphyte and that it reproduces on growing plants. They indicated that this organism is capable of adaptation to an environment substantially different from that of the intestinal tract.

In another study, Eaves and Mundt (41) reported that the

non-hemolytic and non-proteolytic S. faecalis, S. durans, and S. bovis were only infrequently present in insects, while the occurrence of S. faecium and the proteolytic variants of S. faecalis was quite high. Since flowers are visited briefly but repeatedly by insects, a mechanical transfer from insect to plant to insect is suggested. The latter types were distributed fairly equally on insect legs, wings and mouth parts, whereas all the types were present in the gastro-intestinal tract.

Hugh et al. (65) isolated enterococci from the oral cavity of 4% of 297 normal adults, and more frequently from the buccal cavity of patients with ulcerated and malignant conditions of the digestive tract. The presence of enterococci in the oral cavity was not correlated with the state of oral hygiene. S. faecalis was the most frequently encountered enterococcus species isolated from the oral cavity.

Feces In a survey of feces from human beings of different ages and from swine, cows, and sheep, Buttiaux (22) observed that streptococci were always present in the feces of man and swine, but were not necessarily present in the feces of cows and sheep. S. faecalis and its varieties were more frequently found in man than in animals, but did exist in animals. S. faecium was always present in sheep; it was found quite frequently in cows, swine and also in man. Medrek and Barnes (101) reported that S. bovis was the predominant species in cattle and sheep, while other Group D streptococci--S. faecalis, S. faecium and S. durans, were rarely found in cattle, although they formed a significant proportion of the population in sheep. Kenner et al. (75) determined enterococcus densities in moist feces and reported that the median density in millions per g was 0.16 for the cow, 2.29 for human, 2.10 for fowl,

9.42 for sheep and 8.40 for the pig. According to these authors, the enterococcus group amounted to 77% of the total fecal streptococci isolated from human pollution and to only 10% of the fecal streptococci found in pig feces. Cooper and Ramadan (29) isolated fecal streptococci from the excreta of man, cattle and sheep and divided them into groups, some of which were characteristic of the sources. According to these authors characterization of a strain as typical S. faecalis would indicate a human source, while a starch-positive S. bovis would definitely point to animal origin. S. faecalis var. zymogenes, the most common hemolytic streptococcus in the human intestine, was isolated by Smith (163) from the feces of the horse and the cow. The fact that this organism appears to be a normal inhabitant of the bovine intestine would be of interest in connection with its occurrence in milk.

In a study of feces from 100 pigs, Mieth (104) isolated 438 strains of enterococci and examined them biochemically and serologically. In view of the relatively high incidence of S. faecalis (6 pigs) and of its variety liquefaciens (22 pigs), and of the probability that the enterococcal flora is food dependent and not specific for the host, he concluded that it is not possible to consider these organisms as indicators of human contamination. In another study, involving 105 humans of different ages, Mieth (105) found S. bovis in the feces of infants fed cow's milk; the feces of adults yielded S. faecalis and var. liquefaciens and S. faecium. S. durans and S. faecalis var. zymogenes appeared infrequently in the feces of healthy people. In another investigation of 58 heifers and cows, Mieth (106) found that 124 of 166 streptococcal strains from feces were

S. bovis and 28 were S. faecium; the rest were atypical enterococci and other species of streptococci and diplococci.

Colobert and Blondeau (28) reported variable distribution of S. faecalis (implying the enterococcus group) in human and swine stools. None of these were found exclusively in man or swine, particularly S. faecalis proprium, often considered characteristic of the human intestinal flora. S. faecalis proprium amounted to 10% of the enterococcal flora found in swine. Therefore, according to these authors, it was not possible to recognize the origin of contamination by means of characterization of the strains examined. The precision given by factorial analysis did not modify their conclusion. Barnes et al. (12) in a survey of the numbers and types of Group D streptococci occurring in three bacon factories, found S. faecium, a normally occurring organism in the gut of the pig and often isolated from canned hams, outnumbered by S. faecalis which is rare in the pig thereby suggesting human contamination.

Foods Ross and Thatcher (136) found enterococci present up to a maximum of 140,000 organisms/g in 60 samples of food products from Canada and the United States. A reduction in counts up to 67.5% occurred on cooking; only 10 out of 60 samples showed survival with individual specimens retaining up to 1,900 organisms/g. Larkin et al. (84), using hot water as in blanching, successfully decontaminated beans inoculated with S. faecalis. A temperature of 88 C for 1 min. was sufficient to obtain a 100% kill. Kereluk (76) listed the organisms of the enterococcus group isolated from various frozen meat pies in decreasing order of greatest recovery as S. faecalis, S. faecalis var. liquefaciens, and S. durans.

Dairy products Abd-El-Malek and Gibson (1) identified streptococci isolated from raw and pasteurized milk of varying purity, as enterococci. White and Sherman (172) found that milk with high bacterial counts contained large numbers but significantly small percentages of enterococci. Since large variations in both numbers and percentages occurred, these workers thought it inadvisable to use the enterococcus count of milk as a criterion of quality. Sasaki et al. (140) isolated 348 strains of bacteria from 172 samples of raw milk collected from 19 plants throughout Japan; of these, 14.9% were S. faecalis, suggesting that the enterococci form a significant portion of the microbial flora of milk in that country. More S. faecalis were found in summer and autumn than in winter and spring.

Iyengar et al. (68) tested for heat tolerance and found that S. faecalis and var. liquefaciens were completely destroyed after 30 min. heat treatment at 63 C in skim milk, when their concentration was less than 50,000/ml. With higher concentrations of cells in the milk, partial or full resistance was shown. Sherman et al. (149) reported that S. faecalis survived heating for 30 min. at 65 C in skim milk. Abd-El-Malek and Gibson (1), studying enterococcal strains isolated from milk, reported that the formation of acetoin from citrate and glucose was common, but not a universal property of the enterococcus group. Williams (174) found that the addition of autolysates from milk cultures of B. subtilis to milk cultures of S. faecalis resulted in the formation of gas in 2 days at 37.5 C.

Czulak and Hammond (34) used an active culture of S. durans in the

'short-time' method of cheese making in preference to S. thermophilus because of the greater sensitivity of the latter organism to sodium chloride. Kosikowsky and Dahlberg (80) found that S. faecalis was adaptable to the conditions in ripening Cheddar cheese. When added as starter, these organisms increased rapidly during the cheese making process, and persisted as the dominant flora throughout the ripening period, declining in numbers only by about 50%. Dahlberg and Kosikowsky (37) also reported that the use of S. faecalis as starter hastened the ripening of Cheddar cheese. Well-ripened cheese of medium flavor was produced in 4 1/2 months at 50 F when the mixed starter was used. Anderson (4) suggested that the use of S. faecalis starters was essential for the manufacture of high grade Emmental cheese from pasteurized milk. Walter et al. (170) obtained a United States patent on a process for manufacturing Cheddar cheese using S. durans as a starter. Kosikowsky (79) reported that cheese of the Mozzarrella type made from properly pasteurized milk with his DK (S. faecalis) starter ripened as well as the raw milk cheese control. The flavor and yield of the pasteurized milk cheeses were good. Pizza pies made with them were of excellent quality. Concentrations of from 0.2 to 0.5% DK starter were thought adequate for commercial operations. However, the commercial application of this starter has not been made as yet.

Three strains resembling S. faecalis were isolated by Feagan (45) from farm milks in Australia, which, when used as starter produced a malty aroma in cheese curd. Raadsveld (128) reported that a water-soluble bitter constituent of bitter Gouda cheese was a polypeptide analogous to a bitter substance found in milk cultures of S. faecalis. Substances of

similar structure could be isolated from normal cheese as well. Pette(126) attributed the amounts of H_2S in excess of those normally present in Gouda cheese to the growth of streptococci resembling S. faecalis in most characteristics. Tittsler et al. (169) noted that S. faecalis var. liquefaciens greatly increased proteolysis and produced objectionable flavors, while S. faecalis had no such effect on cheese quality.

Higginbottom (61) found no relationship between the keeping quality and plate count of reconstituted roller-dried milks. S. faecalis, along with S. thermophilus, was predominant in spray-dried whey. A plate count bacterial standard not to exceed 5,000/g was suggested for high quality roller-dried milk products. Crossley and Johnson (33) noted from the bacterial flora of 671 milk powder samples that S. durans was a predominant organism. A variable decline in bacterial numbers occurred during storage. Jarchovská and Müller (69) stated that contamination of dried milk with enterococci to the extent of 250 to 2,500 organisms/g was traced to cracks in the agitator, in the evaporator and faulty seals. Replacement of the seals and repair of the agitator resulted in a considerable improvement in the bacteriological quality of dried milk.

Sherman et al. (150) identified a strain of organism, previously reported to be implicated in outbreaks of food poisoning, as S. faecalis. Among 34 strains of enterococci isolated by Evans and Chinn (43) from human pathological cases and other sources, one strain from milk powder was designated as S. durans; the other, from pasteurized milk, was designated as S. faecalis var. zymogenes. Dangler and Steffen (38) isolated a significantly high number of enterococci, i.e. more than 1 million/g

from goats' milk cheese directly implicated in food poisoning outbreaks. Similarly 64 to 145 million enterococci were isolated from three 'check-up' samples of goats' milk cheese and Mexican cheese by these workers.

Osler et al. (120), from experiments on enterococcal food poisoning in man, reported that six out of 26 human volunteers developed symptoms of acute gastric or intestinal disturbance when samples of food on which cultures of S. faecalis had grown for 5 hr. at 37 C were ingested. Four strains of S. faecalis were used in these experiments, three of which were isolated from human feces and the fourth from a can of evaporated milk implicated in an outbreak of gastro-enteritis. Two of the fecal strains produced no symptoms of food poisoning; in the case of the other two strains, 6 of the 17 persons who ingested them became ill. No ill effects were produced with 20 hr. old cultures of the same strains. Buchbinder et al. (18) isolated S. faecalis and S. faecalis var. liquefaciens from foods, including evaporated milk, which were believed to be responsible for four outbreaks of food poisoning.

Dack et al. (36) observed no ill effect in 25 subjects fed with doses of 40×10^9 to 317×10^9 S. faecalis organisms, in 10 persons given 100 to 500 g of cheese made with a strain of S. faecalis as starter, or in six people given 100 to 300 mg of tyramine, the metabolic product of this species supposed to cause food poisoning. Nevertheless, doses of 182×10^9 organisms of S. faecalis var. liquefaciens produced diarrhea in three out of four subjects.

Bellamy and Gunsalus (15) thought that for the production of tyrosine decarboxylase by S. faecalis more specific growth conditions and require-

ments were needed than for active growth. Shattock (145) considered the production of tyrosine decarboxylase a characteristic of S. faecalis and its varieties, but regarded the probability of formation of tyramine quite untenable unless these organisms were present in considerably large numbers.

Auld and Parker (7) isolated S. faecalis from clinically affected quarters of cows which, when inoculated with a mastitic serum and treated with penicillin, responded well to treatment. They also found this enterococcus species in up to three quarters of the normal udders of some cows.

Other Indicator Organisms

Coliforms

Taxonomy The term "coliform was suggested by Breed and Norton (17) to include those aerobic, facultatively anaerobic, gram-negative, non-spore forming bacteria which ferment lactose with gas production. Numerous coliform types are known. Parr (125) suggested a classification consisting of five groups: Escherichia coli, intermediate, Aerobacter aerogenes, Aerobacter cloacae, and Klebsiella species. Malcolm (94, 96) classified coliform strains isolated from milk as E. coli (one type), A. aerogenes (one type), A. cloacae (one type) and intermediate (11 types). The British Ministry of Health (107) suggested that seven types are adequate for water analysis requirements, comprising E. coli (two types), A. aerogenes (two types), intermediate (two types) and A. cloacae (one type). Wilson et al. (175) investigated the suitability of the coliform

test for grading milk and proposed the addition of eight 'irregular' types to the seven types employed in water analysis.

The Report of Coli-Aerogenes (1956) Sub-Committee of the Society for Applied Bacteriology (135) included E. coli (three types), Citrobacter freundii (two types), Klebsiella aerogenes (two types), K. cloacae (one type) and Erwinia carotovora (one type). In Bergey's Manual of Determinative Bacteriology (16) the genus Citrobacter is not recognized and its species freundii is regarded as Escherichia freundii, while the species aerogenes and cloacae listed under Klebsiella in the above-mentioned report, still are included in the genus Aerobacter. The genus Klebsiella of Bergey's Manual of Determinative Bacteriology (16) does not include the latter two species. Cowan (31) has reviewed the taxonomy of the coliform bacteria with particular reference to the above-mentioned report. There is wide disagreement over the classification of Enterobacteriaceae between workers of different nationalities to whom bacteria of the coliform group, particularly the intermediate types, have different meanings. All workers, however, agree that the coli type constitutes the normal coliform flora of human and bovine feces, and the aerogenes and cloacae types appear to be common in soil and vegetation, but infrequent in feces if ordinary methods of isolation are used.

Standard Methods for the Examination of Dairy Products (3) includes a few unmentioned species of other lactose-fermenting genera in addition to the Escherichia and Aerobacter species. Furthermore, this reference mentions that the application of the coliform test is intended neither to detect fecal pollution specifically nor to identify E. coli in dairy

products, but rather 'to measure the general care' used to minimize bacterial contamination of dairy products.

Detection Both agar plate and multiple tube methods are used for coliform determination. Because of the increased reproducibility and probability of prompt confirmation of any doubtful colonies in appropriate media, the solid media procedures are preferred. Standard Methods for the Examination of Dairy Products (3) recommends Brilliant Green Lactose Bile broth and Lactose broth of the liquid, and Violet Red Bile agar, Deoxycholate Lactose agar, Endo agar and Eosin Methylene Blue agar of the solid media for the determination of coliform organisms.

Many workers, however, have evaluated the different techniques employed for detecting and enumerating coliform bacteria in milk and dairy products. Kalshoven (72) preferred the Violet Red Bile agar plate method, although with this medium Morris and Cerny (110) experienced difficulties with heavily contaminated milk. Murray (115) recommended a 30 C plate incubation temperature over 37 C. Simonart and Lambert (154), and Olsen (117) suggested the use of penicillin in place of the basic dyes used at present in the selective media for the coliform determination, as the inhibiting agent against other organisms.

Distribution

Water, soil, feces, etc. In a survey of the coliform bacteria in feces and waters, Henriksen (57) reported from Norway that strains giving a negative Voges-Proskauer reaction and a positive indole or 44 C reaction or both, should be considered E. coli regardless of the results

of other tests. Only 29% of the water strains and 1.9% of the fecal strains gave reaction patterns which were uncommon or unobserved in the fecal strains. He maintained that the main value of methods for detecting E. coli in water may be to distinguish between recent and remote pollution. Thomas et al. (167) found that the coliform content of surface soil from a polluted site, using Violet Red Bile agar plates, often exceeded 10^4/g at 30 and 10^3/g at 37 C. E. coli was found to be present in small proportion only. In unpolluted soil, high coliform counts were much less frequent and E. coli type I was relatively rare. In a study of various geographical areas, Geldreich et al. (49) noticed that fecal coliforms were usually absent or were present in relatively small numbers only in undisturbed soils, with most counts being less than 1.8/g. There was a marked increase in numbers in soils of the polluted group, with densities between 3,300 and 49,000/g. Intermediate types represented 76% of the 2,348 strains isolated from undisturbed soils as compared to only 17% of 665 polluted soil strains.

In another investigation involving 4,512 strains of coliform organisms from human, 2,339 from livestock, and 1,896 strains from poultry feces, Geldreich et al. (48) found that the EC broth and Boric acid-Lactose broth procedures had a 96.3 and 95.3% positive correlation, respectively, with the coliform types from fecal sources. These findings suggested that the EC or BALB-positive coliform strains in water or wastes indicate relatively recent fecal pollution.

Foods In an evaluation of the EC (44.5 C) confirmation test for the estimation of E. coli type I as an index of sanitary quality of

frozen sea foods, Raj and Liston (129) found that 48 out of 163 samples gave positive EC tests; but only 16 (or 33%) of the positive samples actually contained fecal E. coli.

Dack (35) reported that some special types of coliforms present in sufficient numbers in foods may cause illness. The relatively few coliforms found occurring naturally in frozen orange juice concentrate did not pose a public health problem.

Dairy products Sherman and Wing (152) observed that in the case of high grade raw milk containing less than 10,000 organisms/cc, the coliform test may have a place as a supplementary index of quality. If it was used for such milk, the authors contended, a standard coliform count of less than 100/cc would not seem to be unreasonable. For certified milk, they thought, a standard of less than ten coliforms/cc did not appear unduly stringent, in view of the fact that 48% of the milk samples they examined, containing less than 10,000 organisms/cc, had a coliform count of less than ten/cc.

The American Association of Medical Milk Commissioners, Inc. (3) recognize a standard for the coliform count of not more than ten/ml for certified raw milk. Coliform density standards for raw milk to be pasteurized do not appear to be widely used in the United States. Only one state, New Hampshire, requires that the density of coliform organisms shall not exceed 100/ml in raw milk to be pasteurized (3). The Milk Ordinance and Code (127) prescribes the coliform standards for both grade A and B pasteurized milk and milk products at not more than ten/ml, and at not more than 200,000 and 1,000,000 total bacterial plate counts/ml

for grade A and B raw milks respectively.

Fay (44) observed that the standard of ten coliforms/ml for certified milk was rigid and impractical for market grades of milk produced under somewhat less exacting conditions. He also suggested that the use of the coliform count on raw milk in farm bulk tanks may prove valuable in the future, not only as an index to contamination from poor practices surrounding the milk operation, but to poor cleaning and sanitizing of the farm bulk tank itself.

Finkelstein (46) reported that, in raw milk, coliform bacteria were present to the extent of less than 100/cc on an average where care was used, and averaged 588/cc where varying indifferent methods were used for production on the farm. Proper pasteurization at 145 F destroyed practically all coliforms in milk.

Hiscox and Briggs (62) reviewed the inadequacy of the coliform test for milk, and observed (63) that in France only an indole-positive coliform count on milk is made. Smit (161), from the Netherlands, regarded the presence of coliform bacteria in fresh milk as almost unavoidable. He also noted that a considerable number of the coliforms found in milk were true E. coli which had lost the power of indole formation. Smillie (160) noted that a much higher proportion of test failures than formerly experienced was due to heavier coliform contamination in milk of low bacterial count.

Kampe (73) found that in certified milk, A. aerogenes dominated the coliform flora all through the year, while in ordinary milk, E. coli constituted a greater part of the coliform density throughout the year.

Murray (116) reported that 52.4% of 1,114 samples of bottled raw milk gave positive presumptive results in MacConkey's broth. Of the 262 cultures obtained from positive samples, none of the strains of E. coli was an enteropathogenic serotype. Anderson and Storgards (5) reported that of 190 strains of coliforms isolated from raw and pasteurized milk, none belonged to E. coli type I. Thom (166) traced coliform contamination to milking equipment on 17 occasions, to the farm tank on eight occasions, and to cows only on four occasions. He also noted that when gram-negative rods were predominant in milk, rapid multiplication occurred at 4 C.

Gopalkrishna and Laxminarayana (50) found that in the case of farm-produced milk in India, a majority of the samples tested gave coliform counts below 1,000/ml with corresponding total counts below 100,000/ml. Irregular types were predominant in milk followed by E. coli and A. aerogenes. Morris and Edwards (109) noted a long lag phase of coliform growth in raw milk and their destruction by a bactericidal substance present in raw milk.

Standard Methods for the Examination of Dairy Products (3) states that butter made with good sanitary methods shall not have a coliform count of more than ten/ml. Madsen (92) tested butter, buttermilk and wash water for the presence of coliforms and found that the presence of these organisms in butter was correlated with inadequate pasteurization or insanitary factory conditions. Thomson (168) found that 19.1% of 719 samples of export salted butter from New Zealand gave confirmed coliform tests using MacConkey's broth as the presumptive medium.

Hammer and Yale (53) put coliform organisms into butter during

churning and noted that in 10 days at 7 C, Escherichia species did not grow in salted butter. In unsalted butter, however, some of them did. Aerobacter species sometimes grew in the salted butter and regularly grew in the unsalted. In 10 days at 18 C, both Escherichia and Aerobacter species grew in salted as well as in unsalted butter. The Aerobacter species grew more rapidly, however, and reached higher numbers than the Escherichia species. These authors further noted that 2.0 to 2.9% of the organisms of the Escherichia-Aerobacter group initially present per ml of cream were retained per ml of fresh unsalted butter. When water known to contain coliform organisms was used to wash experimental butter, Corley and Hammer (30) found that the coliforms were regularly present in unsalted butter and were sometimes found in the salted butter. Commercial butter from plants using water that commonly contained coliform organisms regularly contained these organisms when not salted and sometimes contained them when salted.

Singh and Nelson (155) found that out of 294 samples of commercial butter, many had coliform counts of less than two/ml. They also noted that the field of applicability of the coliform count for butter appeared to be for use on the line-run samples to detect sources of contamination. Too many uncontrolled factors affect the coliform count of commercial butter samples to permit satisfactory use of the test for control purposes.

In a survey of 170 churnings of washed and non-washed butters, White and Smith (171) found that 90% of the washed and 85% of the non-washed samples showed no coliforms when 2.5 ml of a 1:10 dilution of butter were plated. Only 5% of the washed and 10% of the non-washed butters had initial coliform counts exceeding ten/ml. Crossley (32) reported the

incidence of coliforms in pasteurized cream, the storage vat, the churn before starting, washed butter granules and salted butter at 5.0, 68.2, 73.3, 83.3 and 61.1% respectively. In 310 isolates from line-run butter, 46.4% were coli, 8.4% were intermediate, 43.9% were aerogenes-cloacae, and 1.3% were irregular types.

Yale (178) determined the coliform counts of 35 lots of experimentally made pasteurized milk cheese, using Violet Red Bile agar. The rate at which the coliform organisms died off, varied greatly with different lots of cheese, so that the coliform count of cheese a few days, or a few weeks old, was not an accurate index of the initial coliform content. Sadek and Eissa (138) in a study of 100 cheese samples, with a salt content of 2.1 to 10.2% and an acidity of 0.12 to 1.5%, found that the incidence of coliform contamination varied from less than two to less than 1,000/g. Acidity higher than 1.2% had an inhibitory effect on the growth of coliforms. Rasic (133) reported that during the manufacture of white cheese, there was active multiplication of coliform bacteria. A rapid decline in their numbers followed during the ripening of the 16 samples of cheese taken. They practically disappeared after a month's time. The period of the dying off of these bacteria corresponded to the lowest pH levels in the cheese, when the actual brine concentration was over 6%.

Crossley and Johnson (33) noticed that contamination of spray-dried milk and whey powders could take place between pasteurization and drying. Coliform species, particularly E. coli, disappeared rapidly on storage. The authors concluded that the coliform test is only of limited value when applied to stored powders, especially when the conditions and

duration of storage are unknown.

Herschdoerfer and Ward (58) observed that indole production by coliform bacteria does not occur when the pH of the medium drops to 6.0 or below. They also stated that, in England, they do not attach any significance to coliforms or to E. coli type I as an indicator of the standard of hygiene in ice cream making. Rao and Dudani (132), in a survey of 92 ice cream samples taken from manufacturing plants, found that 50% had a standard plate count below 250,000/ml and 21% had a coliform count below ten/ml. A tentative plate count standard of 250,000/ml and not more than ten coliform/ml for ice cream in the Delhi area in India was suggested.

Yeasts and Molds

In making a yeast and mold count it is necessary to inhibit bacterial growth by acidifying the medium. Standard Methods for the Examination of Dairy Products (3) recommends the use of Potato Dextrose agar with reduction of the reaction to pH 3.5 ± 0.1 with sterile 10% tartaric or lactic acid. Standard Methods for the Examination of Dairy Products (3) also states that in good quality butter the yeast and mold count should not exceed 20/ml. Higher counts in freshly churned butter samples indicate one or more of the following: ineffective cleaning and sterilizing procedures, inefficient pasteurization, or carelessness in cleaning and handling equipment. A high yeast and mold count, as with the coliform count, does not accurately measure either the quality of raw materials or the keeping quality of butter.

Based on the analysis of over 2,000 samples of salted butter made from sour cream, Parfitt (124) proposed a standard of less than 50, 50-100, 101-500 and more than 500 yeasts and molds/ml for butter produced under good, fair, poor and very poor conditions respectively.

Comparative Studies on Indicator Organisms

Water, soil, feces, and plants

Litsky et al. (89) found a positive correlation of +0.84 between the numbers of E. coli and enterococci in water samples taken from the Connecticut River during a 2-year period. Based upon the median value of all samples collected in this study, the density of enterococci was approximately 7.6 times that of E. coli. Winter and Sandholzer (176) noted that in polluted waters, coliform bacteria persisted for a greater distance from the source of pollution than did the enterococci. Kjellander (77) reported that fecal streptococci did not survive in natural waters for any length of time while the coliform bacteria not only survived, but even multiplied in waters. The fecal streptococci were regularly found in polluted waters in larger numbers than E. coli. This suggested that fecal streptococci are more sensitive indicators of fecal pollution than are the E. coli. It was further stated that S. faecalis and its varieties, S. faecium and S. durans were more resistant to chlorine compounds than were S. bovis and the atypical strains which were sensitive to chloramine. The coliforms occupied a position between the two streptococcal groups mentioned. Burman (19) agreed with the findings of other workers regarding the relatively greater ability of fecal streptococci than E. coli to survive in various natural and antagonistic environments, but challenged the tolerance of these organisms in a chlorinated water supply. According to Malaney et al. (93) the median population densities in lightly polluted farm ponds were 33/ml for coliforms and 3.6/ml for enterococci. Horrock

(64) reported finding fecal streptococci in great abundance in sewage and in waters which were known to be sewage polluted, but which contained no trace of E. coli. In a study involving 215 water samples, Leclerc and Catsaras (87) found S. faecium four times oftener than S. faecalis among the isolated species of fecal streptococci. In non-drinkable waters, fecal streptococci were found in 80% and E. coli in 70% of the cases.

Cataldi and Montagna (24) reported little difference in the fecal flora of breast and bottle fed infants. Enterococci were found in all 45 samples of feces examined while coliforms were found in only 42. Ostrolenk and Hunter (121) examined specimens of feces representing ten animals; enterococci were present in one-tenth to one-millionth of a gram of feces in 49 samples, while coliforms were present in from one-hundredth to one-10 millionth of a gram of feces. Smith (164) found that the bacterial flora of the feces of all animals examined was closely similar in the early life irrespective of the feces; there also was a common pattern of the composition of the fecal flora in adults of the same species; there were low numbers of E. coli in the feces of rabbit, horse, and cattle. Zubrzycke and Spaulding (180) reported on the basis of two series of stool cultures that members of the genus Bacteroides together with enterococci, coliforms, diphtheroides and lactobacilli constituted more than 99% of the total human fecal flora. Buttiaux and Mossel (23) observed that, from the point of view of hygiene, all fecal contamination was equally dangerous, whether originating from domestic or wild animals or from man.

In a study involving 369 samples of undisturbed soil, Medrek and Litsky (103) found that 73.4% contained coliform bacteria. E. coli were

present in 1.4% and enterococci in 2.2% of the samples examined. Mundt et al. (114) isolated enterococci from 62% of samples of plants and soils taken from agricultural and inhabited areas, and from 22% of similar samples taken from unpopulated areas reasonably devoid of human and large animal life. In 46 instances coliform bacteria were associated with the enterococci. Few of the coliforms were of the genus Escherichia. In 13 instances enterococci occurred without coliforms, in 21 instances coliforms were isolated without enterococci, and in 20 instances neither type of organisms was obtained. Most enterococcus isolates appeared to be similar to S. faecium and few were similar to S. faecalis. These authors also isolated enterococci from the atmosphere of a freezing-processing plant.

Foods

Allen and Fabian (2) found little difference between the viability of E. coli and S. faecalis in the less acid foods. However, the latter organism remained viable longer than E. coli in the more acid foods, especially orange juice and mayonnaise. From the bacteriological examination of unbottled soft drinks, Ramadan and Abd-Elnaby (131) found 98.9% of the isolated enterococci to be of animal origin. They thought enterococci were more reliable indicators of pollution than the coliform group. Larkin et al. (81) examined 64 samples of commercially frozen fruits, fruit juice concentrates, and vegetables. Fecal streptococci were found more consistently, and usually in greater numbers, than coliform bacteria. These authors in another study (83) observed that S. faecalis and S. faecalis var. liquefaciens apparently did not decrease in numbers in

inoculated orange concentrate stored at -10 F for 147 days, while the numbers of E. coli fluctuated considerably.

From a study of 456 commercial, freshly frozen chicken pies, Hartman (54) reported that enterococcus counts were more closely related to total counts than were the coliform counts, while the coliform counts were more closely related to enterococcus counts than to total counts. Wilkerson et al. (173) found that freezing inoculated turkeys at -30 C and storing at -2 to -10 C reduced percentages of coliforms more than those of enterococci. Burton (20) suggested that the enterococci might prove superior to the coliform organisms as an indication of fecal contamination in frozen foods, as fecal streptococci were most likely to survive the storage temperature, although the coliforms seemed to be the best test organisms before freezing and storing.

Raj et al. (130) pointed out that the consistently high recoveries of enterococci from frozen sea foods and the low and erratic recoveries of coliforms from the same samples were indirect evidence of the value of enterococci as better indicators of contamination in sea foods. Larkin et al. (85) also reported that fecal streptococci were present more frequently and in larger numbers than the coliform bacteria in frozen fish products. These authors in another study (82) with E. coli, S. faecalis and S. faecalis var. liquefaciens inoculated onto green beans, compared the viability of these organisms stored at 0 F for more than 200 days. The numbers of enterococci remained constant, while the numbers of E. coli decreased significantly during storage.

Dairy products

After studying 192 samples of raw milk and 19 samples of pasteurized milk, White and Sherman (172) reported that enterococci constituted 0.4 and 0.1% respectively, of the total bacterial population. Higginbottom (60) found less than ten coliforms/ml in 14 raw milk samples before the change and in ten samples after the change from surface cooling to refrigerated farm bulk tank for milk storage, while the numbers of enterococci were reduced from 30 to ten/ml in the change-over. Olsen (118), from the results of the study of a large number of samples taken consecutively from many farms, showed that, even though a greater amount of coliform infection was found in the higher than the lower plate count samples, a surprisingly large percentage of samples with high plate counts were, nevertheless, to all intent and purposes, free from coliform infection. He further stated that the coliform determination of raw milk, in contrast to heat-treated milk, can only be regarded as a supplement to the determination of total bacterial count, and that so far as raw milk was concerned, the coliform determination could not be compared for accuracy with the latter.

Johns (70) kept two heifers in unusually unclean conditions to determine the value of the coliform test in assessing the cleanliness of cow's udder and teats. By milking with bacteriologically clean machines, he found that the total bacterial count increased roughly ten-fold, but the coliform count remained surprisingly low, only two counts exceeding ten/ml with a maximum of 83/ml, suggesting that the coliform test cannot be relied upon to reflect unclean udders and teats. Lethem (88) stated that the emphasis of control should change from buildings and equipment, to

methods of handling which might be difficult to control.

Hunter (66) noted that the number of coliforms was greatly influenced by temperature and was closely correlated with the total count of milk. Of 21,569 mixed samples of cow's milk taken under fair conditions of cleanliness, Malcolm (95) found 48.3% free from coliform bacteria in 0.1 cc amounts. The positive samples had an average total count of 160,000 and the coliform-negative samples averaged 25,000 bacteria/cc. Bartram and Black (14) noticed excellent correlation between coliform and total counts of milk; 93.5% of the samples having total counts below 10,000/cc were coliform-negative, while the average total count of the positive and negative samples was 80,000 and 6,700/cc, respectively. Hiscox and Briggs (62), in a review, questioned the value of the coliform test for milk on the grounds of its failure to detect the nature and source of contamination and the limitations of the techniques used.

Higginbottom (59) observed that the growth of E. coli type I could be reduced to one-half of that occurring in pure culture by the growth of associated bacteria. The reduction in gas formation was observed in the presence of S. faecalis and its variety liquefaciens, and S. lactis. Iya and Frazier (67) also noticed that S. lactis suppressed, slightly, the growth of A. aerogenes in a mixed culture grown at 20 C. The effect, however, decreased when the temperature was increased.

Parfitt (123) found, from the analysis of over 1,000 samples of butter from 60 different plants, that only 16.9% had a positive coliform test in 0.1 ml quantities. No relationship was found between the yeast and mold count of the butter and the presence of coliform organisms or between the

keeping quality and the presence or absence of the coliform group in butter. Of 1,058 samples of Australian butter taken from 49 plants, Roughley and McLeod (137) reported 76.9% had ten coliform bacteria or less/ml and 64.5% of the samples had 20 yeasts and molds or less/ml.

Kjellander and Nygren (78) examined 287 samples of spray-dried milk and found that only 1% had more than 30 coliforms/10 g while 54% of the samples contained more than 30 fecal streptococci/10 g of dry milk.

EXPERIMENTAL METHODS

Collection, Handling and Treatment of Samples

Unless otherwise noted, all milk, butter and cheese samples were collected, cooled, transported and plated according to the procedure described in Standard Methods for the Examination of Dairy Products (3).

A total of 119 samples of bulk-cooled grade A raw milk from 16 producers delivering milk to the Iowa State University Dairy were obtained. Other grade A raw milk samples were obtained from 211 producers supplying milk to four Central Iowa dairy plants. Most of this milk came from farm bulk tanks. Some samples were taken from cans as they were delivered at the plants. The temperature of the samples varied from 37 to 43 F when collected.

To determine the effect of storage on the growth of different groups of bacteria, 90 of these samples were held at 7 C for 7 days. After plating, 49 of the raw milk samples were laboratory pasteurized (62.8 C for 30 min) to study the survival of the various groups of bacteria. The procedure described in Standard Methods for the Examination of Dairy Products (3) was used.

The 120 samples of manufacturing grade raw milk, representing a like number of individual producers, were collected from cans as they were delivered at four Central Iowa dairy plants.

The 375 samples of commercial butter used in this study were, in most instances, obtained from various Iowa butter contests and exhibits. Some, however, were taken directly from creameries within the state.

Line-run samples were collected from 20 different churnings at eight Iowa creameries. Samples were taken at the following points or procedures along the line: raw cream, pasteurized cream, cream from the holding vat, cream from the churn at the start of churning, cream from the churn after 2 min. of churning, buttermilk, unwashed butter granules, washed butter granules, salted, finished butter and salted, finished butter after holding at 7 C for 7 days.

The 72 samples of Cheddar cheese were obtained from two Iowa cheese contests and three Iowa cheese plants. Six samples were cured at 38 F for 5 months and were examined at monthly intervals.

Experimental Butter

Strains of coliform bacteria were isolated from butter and were then tested for salt tolerance in nutrient broth. Several selected strains were identified; a single strain each of E. coli and A. aerogenes that could not grow in more than 4.0% salt and a single strain each of E. coli and A. aerogenes that could grow readily in 10.0% salt were chosen. These strains were designated as salt-sensitive (SS) and salt-resistant (SR). Similarly, one typical strain of S. faecalis and one of S. durans, both capable of growing readily in 8.0% salt, were selected from a large number of enterococcus cultures isolated from dairy products.

Glass churns, plastic filter screens, filter cloths, distilled water used for washing and stainless steel beakers used for working and holding the butter were sterilized in an autoclave at 121 C for 1 hr. Whipping cream was heated in sterile pyrex flasks at about 93 C in a steam chest

for 30 min. It was then quickly cooled in ice water and was stored over night at 3 C.

One ml each of a 24-hr culture of enterococcus and coliform were added per kg of cream in the churn. Nine churnings of cream were inoculated as follows:

1. S. faecalis + E. coli (SS)
2. S. faecalis + E. coli (SR)
3. S. faecalis + A. aerogenes (SS)
4. S. faecalis + A. aerogenes (SR)
5. S. durans + E. coli (SS)
6. S. durans + E. coli (SR)
7. S. durans + A. aerogenes (SS)
8. S. durans + A. aerogenes (SR)
9. S. faecalis + A. aerogenes (SR) + butter culture.

The cream was churned observing precautions to avoid contamination. The butter granules were washed in sterile distilled water and the butter was worked in a sterile stainless steel beaker with a mechanical mixer. The mixing screw was flamed in alcohol and was cooled before use. The butter from each churning was divided into two lots, one of which was kept without salt; 2% salt was added to the second lot. Half of the unsalted and salted butter from each churning was worked properly; the remaining half was worked poorly. The absence of free moisture droplets as shown by indicator paper and appearance served as criteria to determine proper working. Appearance of free moisture indicated insufficient working. It took 6 min to work the butter properly. The improperly worked

butter was mixed for only 1.5 min. In the last experiment, butter culture was added in the amount of 2.0% to the salted butter and 3.0% to the unsalted butter.

Appropriate controls were used to assure freedom from accidental contamination by unwanted enterococci and coliforms. All butter samples were immediately placed at 3 C and were kept at room temperature only as long as was necessary for handling.

Ten 2-oz samples from each lot of butter were placed in screw-capped jars. The samples were held and examined as indicated:

1. After 4 hr at 3 C.
2. After 24 hr at 3 C.
3. After 3 days at 3 C.
4. After 1 week at 3 C.

   (After 1 week at 3 C, one sample was placed in the freezer at -20 C to be examined after 8 weeks of storage).
5. After 2 weeks at 3 C.
6. After 3 weeks at 3 C.

   (The remaining samples were then transferred to a 10 C cabinet).
7. After 3 weeks at 3 C and 1 week at 10 C.
8. After 3 weeks at 3 C and 2 weeks at 10 C.
9. After 3 weeks at 3 C and 3 weeks at 10 C.
10. After 1 week at 3 C and 7 weeks at -20 C.

The experimental butter made in the preceding manner contained nine different combinations of organisms, two different salt concentrations,

and two different working treatments, amounting to 36 different lots. Thus 360 samples of experimental butter (10 different sampling periods for each lot) were plated for enterococci, coliform and yeast and mold content. The chemical composition for each lot was determined by a routine Kohman analysis.

Enumeration Procedures

Total count

Standard plate counts were made on all raw samples of milk using a 32 C plate incubation temperature. Total plate counts on cheese samples were obtained by incubation of the plates at 21 C for 5 days.

Enterococcus count

Enterococcus counts were made on all samples of milk, butter, and cheese studied in this work. The Citrate Azide agar of Reinbold et al. (134), modified by increasing the sodium azide concentration to 0.4 g/ liter, was used as described by Saraswat et al. (139).

Coliform count

Coliform counts were made on all samples of milk, butter, and cheese. Violet Red Bile agar and a plate incubation temperature of 35 C were used. The medium was prepared as described in Standard Methods for the Examination of Dairy Products (3) except that sterilization at 121 C for 12 min was employed. Individual bottles were kept at 3 C and were used within the week following preparation.

Yeast and mold count

Yeast and mold counts were made on all samples of butter used for this thesis. Acidified Potato Dextrose agar plates were incubated at room temperature inside the drawers of the working table to avoid contamination.

RESULTS

Relation of Enterococcus and Coliform Counts to the Standard Plate Count of Milk

Grade A raw milk

The Standard Plate, enterococcus and coliform counts of 330 grade A and 120 manufacturing grade raw milk samples were used in this study. Ninety samples of grade A raw milk were held at 7 C for 7 days to study the effect of storage on the bacterial populations. An additional 48 samples were laboratory-pasteurized to determine the effect upon survival of the various bacterial groups. Each count presented in the following 10 tables is an arithmetic average of duplicate plate counts.

The Standard Plate, enterococcus and coliform counts of grade A raw milk are presented in Table 1. The results show wide variations between counts. Higher total counts are usually accompanied by higher counts of both enterococci and coliform bacteria. But many samples with high total counts have surprisingly low enterococcus and coliform counts. Similarly, samples with higher enterococcus counts tend to have higher coliform counts. Many samples with high enterococcus counts also have relatively low coliform counts and vice versa. The averages of all samples examined were: Standard Plate Count, 100,000/ml; enterococcus count, 200/ml; and coliform count, 130/ml. The percentages of the average enterococcus and coliform counts of the total count were 0.20 and 0.13, respectively.

Table 1. Standard Plate, enterococcus and coliform counts of grade A raw milk

Sample No.	Standard Plate Count/ml	Entero-coccus Count/ml	Coliform	Sample No.	Standard Plate Count/ml	Entero-coccus Count/ml	Coliform
1.	660,000	50	3,500	36.	65,000	140	8
2.	150,000	350	10	37.	14,000	6	3
3.	38,000	35	43	38.	53,000	5	23
4.	150,000	88	54	39.	1,700,000	4,000	20
5.	29,000	4	2	40.	21,000	3	1
6.	8,000	7	3	41.	55,000	9	11
7.	87,000	180	49	42.	140,000	10	160
8.	150,000	33	12	43.	81,000	520	320
9.	1,800,000	630	33	44.	62,000	150	20
10.	7,000	15	10	45.	480,000	190	38
11.	36,000	35	12	46.	4,200,000	90	80
12.	6,000	14	67	47.	430,000	32	7
13.	18,000	4	15	48.	940,000	26	92
14.	1,000,000	3,000	2,300	49.	150,000	140	87
15.	470,000	17	22	50.	150,000	50	14
16.	170,000	1,100	1	51.	12,000	460	20
17.	27,000	120	13	52.	9,000	8	13
18.	1,800,000	780	390	53.	22,000	330	530
19.	18,000	14	36	54.	51,000	12	4
20.	82,000	48	22	55.	26,000	5	34
21.	59,000	42	11	56.	32,000	54	350
22.	340,000	990	12	57.	40,000	45	8
23.	9,000	100	8	58.	4,000	1	4
24.	180,000	140	7	59.	5,000	7	4
25.	12,000	11	37	60.	12,000	1	2
26.	34,000	2,400	170	61.	11,000	25	6
27.	4,000	62	15	62.	300,000	2,400	1,700
28.	110,000	3,600	450	63.	20,000	1	11
29.	5,000	1	5	64.	77,000	36	31
30.	47,000	98	2	65.	11,000	50	19
31.	10,000	18	48	66.	18,000	13	15
32.	130,000	17	8	67.	13,000	780	18
33.	34,000	63	13	68.	4,000	42	9
34.	39,000	1	3	69.	35,000	20	66
35.	170,000	60	140	70.	21,000	50	280

Table 1 (Continued)

Sample No.	Standard Plate Count/ml	Entero-coccus Count/ml	Coliform	Sample No.	Standard Plate Count/ml	Entero-coccus Count/ml	Coliform
71.	4,000	31	52	106.	47,000	1	14
72.	8,000	8	9	107.	22,000	28	5
73	4,000	1	4	108.	61,000	53	67
74.	10,000	1	11	109.	18,000	9	10
75.	11,000	19	12	110.	16,000	2	4
76.	13,000	16	35	111.	76,000	8	21
77.	38,000	10	14	112.	42,000	280	80
78.	23,000	290	44	113.	28,000	440	13
79.	34,000	17	300	114.	36,000	230	3
80.	4,000	50	12	115.	38,000	450	6
81.	6,000	160	11	116.	15,000	75	5
82.	1,000	63	12	117.	22,000	2	33
83.	45,000	960	510	118.	150,000	56	29
84.	16,000	80	6	119.	10,000	61	37
85.	18,000	10	140	120.	12,000	41	1
86.	58,000	85	450	121.	6,000	1	9
87.	3,000	40	78	122.	6,000	1	3
88.	140,000	240	31	123.	9,000	26	2
89.	1,000	1	2	124.	18,000	44	29
90.	9,000	43	10	125.	20,000	55	33
91.	250,000	110	190	126.	13,000	3	3
92.	35,000	16	9	127.	25,000	150	24
93.	14,000	43	16	128.	34,000	14	6
94.	24,000	110	4	129.	5,000	10	32
95.	32,000	640	14	130.	70,000	18	400
96.	12,000	56	5	131.	18,000	100	60
97.	17,000	18	58	132.	64,000	12	110
98.	59,000	67	210	133.	97,000	210	340
99.	8,000	72	82	134.	18,000	76	170
100.	4,000	44	5	135.	4,000	2	5
101.	5,000	1	1	136.	3,000	10	2
102.	75,000	3	1	137.	6,000	3	13
103.	12,000	39	14	138.	4,000	14	6
104.	74,000	230	630	139.	260,000	180	420
105.	2,000	1	1	140.	84,000	120	43

Table 1 (Continued)

Sample No.	Standard Plate Count/ml	Entero-coccus Count/ml	Coliform	Sample No.	Standard Plate Count/ml	Entero-coccus Count/ml	Coliform
141.	3,000	120	8	176.	16,000	13	1
142.	21,000	110	410	177.	4,000	2	3
143.	4,000	19	8	178.	8,000	25	2
144.	46,000	3,400	1,600	179.	300,000	660	93
145.	20,000	130	4	180.	65,000	71	15
146.	60,000	22	110	181.	20,000	150	83
147.	68,000	180	550	182.	280,000	230	41
148.	13,000	61	83	183.	7,000	17	8
149.	3,000	26	29	184.	17,000	40	390
150.	3,000	6	3	185.	27,000	92	35
151.	7,000	2	7	186.	11,000	48	24
152.	6,000	12	1	187.	3,000	6	42
153.	150,000	200	1,200	188.	21,000	6	43
154.	53,000	110	48	189.	1,000	3	2
155.	5,000	130	13	190.	9,000	1	16
156.	26,000	2,800	630	191.	4,000	17	6
157.	6,000	20	15	192.	18,000	75	760
158.	49,000	44	3,200	193.	140,000	59	17
159.	16,000	220	120	194.	52,000	100	4
160.	43,000	16	310	195.	500,000	1,100	46
161.	9,000	9	71	196.	7,000	14	25
162.	9,000	2	55	197.	52,000	68	480
163.	12,000	14	4	198.	360,000	40	420
164.	10,000	1	5	199.	7,000	140	62
165.	10,000	11	1	200.	40,000	92	48
166.	270,000	290	1,700	201.	62,000	2,200	93
167.	51,000	75	96	202.	1,000	9	6
168.	27,000	120	5	203.	9,000	6	4
169.	94,000	1,800	44	204.	11,000	5	1
170.	7,000	8	8	205.	5,000	12	44
171.	55,000	25	130	206.	13,000	25	8
172.	20,000	100	11	207.	98,000	42	500
173.	47,000	10	120	208.	11,000	12	27
174.	22,000	7	94	209.	5,000	7	130
175.	11,000	3	15	210.	3,000	8	10

Table 1 (Continued)

Sample No.	Standard Plate	Entero-coccus	Coliform	Sample No.	Standard Plate	Entero-coccus	Coliform
	Count/ml				Count/ml		
211.	6,000	4	60	246.	19,000	52	450
212.	2,000	1	15	247.	9,000	2	1
213.	15,000	250	82	248.	7,000	16	8
214.	2,000	9	18	249.	42,000	40	210
215.	13,000	13	23	250.	34,000	12	120
216.	2,000	3	14	251.	27,000	67	35
217.	4,000	8	8	252	10,000	3	3
218.	6,000	14	36	253.	35,000	11	4
219.	110,000	9	7	254.	220,000	250	46
220.	3,000	15	20	255.	5,000	1	2
221.	1,000	3	14	256.	44,000	570	110
222.	1,000	3	5	257.	10,000	3	27
223.	3,000	2	10	258.	9,000	2	21
224.	2,000	2	17	259.	13,000	230	400
225.	64,000	34	260	260.	99,000	100	380
226.	22,000	19	120	261.	53,000	270	48
227.	3,000	4	17	262.	100,000	130	160
228.	2,000	4	9	263.	100,000	750	130
229.	2,000	1	1	264.	95,000	11	49
230.	5,000	22	3	265.	66,000	110	320
231.	3,000	4	3	266.	100,000	65	91
232.	2,000	11	13	267.	17,000	96	24
233.	38,000	9	88	268.	6,000	39	4
234.	310,000	27	350	269.	15,000	15	48
235.	8,000	4	26	270.	8,000	9	5
236.	2,000	1	1	271.	26,000	40	7
237.	21,000	9	330	272.	12,000	65	3
238.	38,000	110	1	273.	23,000	25	23
239.	60,000	45	86	274.	67,000	160	13
240.	62,000	4	210	275.	4,000	2	1
241.	400,000	2,600	1	276.	11,000	1	2
242.	93,000	2,200	8	277.	26,000	12	39
243.	22,000	17	70	278.	13,000	130	3
244.	22,000	26	1	279.	96,000	150	16
245.	31,000	49	9	280.	20,000	180	20

Table 1 (Continued)

Sample No.	Standard Plate Count/ml	Entero-coccus Count/ml	Coliform	Sample No.	Standard Plate Count/ml	Entero-coccus Count/ml	Coliform
281.	140,000	3,200	28	306.	13,000	380	55
282.	27,000	8	1	307.	18,000	59	1,200
283.	20,000	60	1	308.	10,000	7	3
284.	10,000	1	1	309.	24,000	1,100	2,100
285.	13,000	730	20	310.	14,000	3	31
286.	57,000	16	13	311.	7,000	150	24
287.	6,000	2	3	312.	13,000	6	18
288.	57,000	5	8	313.	9,000	65	5
289.	4,000	48	4	314.	11,000	19	970
290.	3,000,000	11	10	315.	360,000	25	23
291.	28,000	3	28	316.	4,000	19	3
292.	4,000	20	1	317.	10,000	26	5
293.	1,500,000	1	2	318.	10,000	2	41
294.	25,000	20	2	319.	37,000	12	3
295.	22,000	250	60	320.	51,000	40	2
296.	23,000	130	10	321.	71,000	62	4
297.	39,000	10	170	322.	9,000	110	32
298.	100,000	920	4	323.	14,000	11	1
299.	7,000	1	1	324.	9,000	18	8
300.	11,000	110	71	325.	73,000	1	2
301.	22,000	10	21	326.	100,000	3	1
302.	43,000	8	30	327.	750,000	450	170
303.	760,000	170	450	328.	31,000	1,800	47
304.	130,000	7	630	329.	20,000	950	17
305.	24,000	4	1	330.	18,000	300	7
				Average	100,000	200	130
				% Total Count	100	0.20	0.13

The frequency distribution of samples according to bacterial content is presented in Table 2.

Table 2. Frequency distribution of the bacterial content of 330 samples of grade A raw milk

Standard Plate Count/ml	Per cent of samples in range					
	Enterococcus count/ml			Coliform count/ml		
	≤ 1-10	11-100	>100	≤ 1-10	11-100	>100
≤ 1-30,000	24.8	25.8	9.7	28.2	27.0	5.2
30-200,000	5.5	14.8	11.2	8.2	12.7	10.6
>200,000	0.3	2.7	5.2	1.2	3.6	3.3
Total	30.6	43.3	26.1	37.6	43.3	19.1

The data in Table 2 show that although only 8.2% of the samples had total counts of more than 200,000/ml, 69.4% had more than ten enterococci and 62.4% samples had more than ten coliforms/ml.

The data relating to the variations in the levels of the Standard Plate, enterococcus, and the coliform counts are presented in Table 3.

Table 3. Variations in the Standard Plate Counts of 330 samples of grade A raw milk corresponding to variations in the enterococcus and coliform counts

	Enterococcus count/ml			Coliform count/ml		
	≤ 1-10	11-100	>100	≤ 1-10	11-100	>100
No. of samples	101	143	86	124	143	63
Average SPC/ml	35,000	110,000	180,000	66,000	110,000	150,000
Ratio of SPC between groups	1	3.1	5.1	1	1.7	2.3

The average total count of the samples with high average enterococcus counts was considerably larger than the average total count of the samples with low enterococcus count. The average total count of samples with ten enterococci or less/ml was nearly one-half of that of the samples with ten coliforms or less/ml. The average total count of the samples with high coliform count was also larger than the average total count of samples with low coliform count. But the ratios were not as wide as with the enterococcus count. The average total count of samples with high enterococcus count was larger than the average of samples with high coliform count.

The analysis of variance of the Standard Plate, enterococcus, and coliform counts is presented in Table 4.

Table 4. Analysis of Variance of the Standard Plate, enterococcus and coliform counts of samples of grade A raw milk

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F Value[1]
Standard Plate Count	329	41,072,715		
Enterococcus and coliform counts	2	1,780,201		
Coliform count	1	214,783	214,783	1.79
Enterococcus count	1	1,235,029	1,235,029	10.28
Deviations	327	39,292,514	120,161	

[1]F Value = variance ratio.

The F values for the enterococcus and the coliform counts were 10.28 and 1.79. This suggests that the relationship between the enterococcus count and the total count is highly significant. On the other hand, there is no significant relationship between the coliform count and the total count of grade A raw milk.

The effect of storage on the bacterial counts is shown in Table 5. Under the conditions of storage described, the enterococci grew slowly while the coliform bacteria multiplied at a more rapid rate. The rate of increase of the total count was intermediate.

Table 5. Effect of storage at 7 C for 7 days on the Standard Plate, enterococcus and coliform counts of grade A raw milk

Sample No.	Initial			After 7 days at 7 C		
	Standard Plate	Entero-coccus	Coliform	Standard Plate	Entero-coccus	Coliform
	count/ml					
1.	5,000	1	5	170,000,000	100	3,800,000
2.	47,000	98	2	420,000,000	6,000	2,700,000
3.	10,000	18	48	310,000,000	6,000	4,500,000
4.	130,000	17	8	370,000,000	100	4,000,000
5.	34,000	63	13	430,000,000	3,000	2,800,000
6.	39,000	< 1	3	240,000,000	< 1	300,000
7.	170,000	60	140	900,000,000	1,000	2,800,000
8.	65,000	140	8	410,000,000	1,000	1,100,000
9.	14,000	6	3	370,000,000	1,000	20,000
10.	53,000	5	23	210,000,000	100	3,900,000
11.	1,700,000	4,000	20	65,000,000	130,000	7,000,000
12.	21,000	3	< 1	380,000,000	100	1,000
13.	55,000	9	11	130,000,000	1,000	4,000,000
14.	140,000	10	160	370,000,000	100	38,000,000
15.	81,000	520	320	430,000,000	1,000	16,000,000

Table 5 (Continued)

Sample No.	Initial			After 7 days at 7 C		
	Standard Plate	Entero-coccus	Coliform	Standard Plate	Entero-coccus	Coliform
	count/ml					
16.	62,000	150	20	190,000,000	18,000	500,000
17.	480,000	190	38	800,000,000	29,000	2,200,000
18.	4,200,000	89	80	800,000,000	28,000	3,700,000
19.	430,000	32	7	1,100,000,000	21,000	21,000
20.	940,000	26	92	1,300,000,000	100	2,000,000
21.	150,000	140	87	1,300,000,000	1,000	22,000,000
22.	150,000	50	14	210,000,000	2,000	1,800,000
23.	12,000	460	20	36,000,000	5,000	39,000
24.	9,000	8	13	260,000,000	330	160,000
25.	22,000	330	530	180,000,000	1,400	280,000
26.	51,000	12	4	9,000,000	50	30,000
27.	26,000	5	34	150,000,000	110	630,000
28.	32,000	54	350	250,000,000	500	4,000,000
29.	40,000	45	8	140,000,000	400	6,000,000
30.	4,000	1	4	33,000,000	300	600,000
31.	5,000	7	4	60,000,000	100	4,000,000
32.	12,000	1	2	60,000,000	50	550,000
33.	11,000	25	6	9,000,000	180	97,000
34.	300,000	2,400	170	110,000,000	13,000	9,000,000
35.	20,000	1	11	32,000,000	40	200,000
36.	77,000	36	31	60,000,000	350	1,300,000
37.	11,000	50	19	37,000,000	520	12,000
38.	18,000	13	15	200,000,000	270	11,000
39.	13,000	780	18	190,000,000	9,000	25,000
40.	4,000	42	9	26,000,000	550	300,000
41.	35,000	20	66	120,000,000	3,300	3,700,000
42.	21,000	50	280	110,000,000	1,100	2,500,000
43.	4,000	31	52	60,000,000	310	85,000
44.	8,000	8	9	33,000,000	200	160,000
45.	4,000	< 1	4	56,000,000	< 1	3,000
46.	10,000	< 1	11	82,000,000	< 1	3,000
47.	11,000	19	12	74,000,000	360	500,000
48.	13,000	16	35	200,000,000	410	3,800,000
49.	38,000	10	14	77,000,000	140	260,000
50.	23,000	290	44	44,000,000	700	130,000

Table 5 (Continued)

Sample No.	Initial Standard Plate	Initial Entero-coccus	Initial Coliform	After 7 days at 7 C Standard Plate	After 7 days at 7 C Entero-coccus	After 7 days at 7 C Coliform
			count/ml			
51.	34,000	17	300	200,000,000	40	3,500,000
52.	4,000	50	12	190,000,000	1,500	1,500,000
53.	6,000	160	11	77,000,000	430	6,000
54.	1,000	63	12	200,000,000	2,500	3,000
55.	45,000	960	510	510,000,000	130,000	70,000
56.	16,000	80	6	110,000,000	1,400	1,000
57.	18,000	10	140	190,000,000	100	5,100,000
58.	58,000	85	450	300,000,000	1,900	4,500,000
59.	3,000	40	78	88,000,000	160	160,000
60.	250,000	110	190	350,000,000	4,700	8,500,000
61.	35,000	16	9	58,000,000	800	4,100,000
62.	14,000	43	16	69,000,000	1,500	95,000
63.	24,000	110	4	260,000,000	74,000	11,000
64.	32,000	640	14	190,000,000	81,000	2,000
65.	12,000	56	5	110,000,000	4,700	1,000
66.	17,000	18	58	100,000,000	6,300	5,000,000
67.	59,000	67	210	7,000,000	23,000	6,000,000
68.	8,000	72	82	120,000,000	3,400	13,000
69.	4,000	44	5	150,000,000	5,400	1,000
70.	5,000	<1	<1	7,000,000	30	1,000
71.	75,000	3	<1	61,000,000	40	1,000
72.	12,000	39	14	19,000,000	2,900	230,000
73.	74,000	230	630	980,000,000	7,500	16,000,000
74.	47,000	<1	14	7,000,000	<1	1,000
75.	28,000	440	13	160,000,000	8,700	53,000
76.	36,000	230	3	110,000,000	6,000	8,000
77.	38,000	450	6	260,000,000	13,000	10,000
78.	15,000	75	5	35,000,000	900	2,000
79.	22,000	2	33	130,000,000	80	680,000
80.	150,000	56	29	470,000,000	3,900	80,000
81.	10,000	61	37	120,000,000	1,500	370,000
82.	12,000	41	1	110,000,000	3,700	2,000
83.	6,000	<1	9	8,000,000	10	1,000
84.	6,000	1	3	27,000,000	20	2,000
85.	9,000	26	2	83,000,000	160	3,000

Table 5 (Continued)

Sample No.	Initial			After 7 days at 7 C		
	Standard Plate	Entero-coccus	Coliform	Standard Plate	Entero-coccus	Coliform
	count/ml					
86.	18,000	44	29	330,000,000	1,500	240,000
87.	20,000	55	33	90,000,000	1,700	12,000
88.	13,000	3	3	27,000,000	60	5,000
89.	25,000	150	24	240,000,000	1,200	11,000
90.	34,000	14	6	370,000,000	1,400	240,000
Average	120,000	170	70	300,000,000	7,600	2,400,000
% increase over initial count				250,000	4,500	3,400,000

The effect of pasteurization on survival of different types of bacterial groups has been presented in Table 6.

Table 6. Effect of laboratory pasteurization on the Standard Plate, enterococcus and coliform counts of grade A raw milk

Sample No.	Raw			Pasteurized		
	Standard Plate	Entero-coccus	Coliform	Standard Plate	Entero-coccus	Coliform
	count/ml					
1.	6,000	160	11	580	<1	<1
2.	1,000	63	12	530	<1	<1
3.	45,000	960	510	1,600	<1	<1
4.	16,000	80	6	110	<1	<1
5.	18,000	10	140	810	<1	<1
6.	58,000	85	450	560	<1	<1
7.	3,000	40	78	130	<1	<1
8.	140,000	240	31	130	<1	<1
9.	1,000	<1	2	60	<1	<1
10	9,000	43	10	390	<1	<1

Table 6 (Continued)

Sample No.	Raw			Pasteurized		
	Standard Plate	Entero-coccus	Coliform	Standard Plate	Entero-coccus	Coliform
	count/ml					
11.	250,000	110	190	150	<1	<1
12.	35,000	16	9	120	<1	<1
13.	14,000	43	16	500	<1	<1
14.	24,000	110	4	250	<1	<1
15.	32,000	640	14	800	<1	<1
16.	12,000	56	5	350	<1	<1
17.	17,000	18	58	2,000	<1	<1
18.	59,000	67	210	3,000	<1	<1
19.	8,000	72	82	100	<1	<1
20.	4,000	44	5	100	<1	<1
21.	5,000	<1	<1	150	<1	<1
22.	75,000	3	<1	250	<1	<1
23.	12,000	39	14	850	<1	<1
24.	74,000	220	630	850	<1	<1
25.	2,000	<1	<1	200	<1	<1
26.	47,000	<1	14	200	<1	<1
27.	22,000	28	5	150	<1	<1
28.	61,000	53	67	750	<1	<1
29.	18,000	9	10	150	<1	<1
30.	16,000	2	4	2,000	<1	<1
31.	76,000	8	21	250	<1	<1
32.	42,000	280	80	350	<1	<1
33.	28,000	440	13	430	<1	<1
34.	36,000	230	3	120	<1	<1
35.	38,000	450	6	380	<1	<1
36.	15,000	75	5	260	<1	<1
37.	22,000	2	33	770	<1	<1
38.	150,000	56	29	700	<1	<1
39.	10,000	61	37	20	<1	<1
40.	12,000	41	1	30	<1	<1
41.	6,000	<1	9	20	<1	<1
42.	6,000	1	3	230	<1	<1
43.	9,000	26	2	70	<1	<1
44.	18,000	44	29	110	<1	<1
45.	20,000	55	33	70	<1	<1

Table 6 (Continued)

Sample No.	Raw			Pasteurized		
	Standard Plate	Entero-coccus	Coliform	Standard Plate	Entero-coccus	Coliform
			count/ml			
46.	13,000	3	3	440	< 1	< 1
47.	25,000	150	24	40	< 1	< 1
48.	34,000	14	6	250	< 1	< 1
Average	34,000	110	61	460	< 1	< 1
% reduction in counts after pasteurization				98.6	100	100

These results show that more than 98% of the total bacteria and all of the enterococci and coliform bacteria were destroyed during the pasteurization treatment. This was further confirmed by failure of both enterococcus and coliform colonies to appear on plates made from milk held for 7 days at 7 C from all laboratory-pasteurized samples.

Manufacturing grade raw milk

The Standard Plate, enterococcus and coliform counts of the manufacturing grade raw milk samples are presented in Table 7. The data clearly show that variations between individual counts of manufacturing grade raw milk were far wider than with grade A raw milk. The counts in each of the three bacterial numerical groupings were usually much higher than in grade A milk. However, in many samples the counts of each type also were quite low. The average Standard Plate Count was 2,000,000/ml, the average enterococcus count, 5,600/ml, and the average coliform count, 6,400/ml. The enterococcus and coliform counts were 0.28 and 0.32%, respectively, of the

total bacterial count.

The frequency distribution of the bacterial content of samples is given in Table 8. While more than two-fifths of the samples had total counts of 200,000 or less/ml, more than one-third of the samples had total counts of more than 1,000,000/ml. Less than one-third of the samples had enterococcus counts of 100 or less/ml, and 45% of the samples had coliform counts of 100 or less/ml.

Table 7. Standard Plate, enterococcus and coliform counts of manufacturing grade raw milk

Sample No.	Standard Plate	Entero-coccus	Coliform	Sample No.	Standard Plate	Entero-coccus	Coliform
	Count/ml				Count/ml		
1.	84,000	72	220	21.	6,000,000	20,000	88,000
2.	320,000	170	41	22.	460,000	1,100	49
3.	110,000	78	13	23.	27,000	2	4
4.	110,000	300	77	24.	500,000	95	1
5.	34,000	350	370	25.	17,000	160	1
6.	59,000	13	33	26.	83,000	7	1
7.	340,000	370	46	27.	14,000	190	90
8.	99,000	320	11	28.	430,000	2,600	690
9.	92,000	370	78	29.	3,700,000	7,600	30,000
10.	29,000	46	68	30.	5,800,000	6,000	5,000
11.	3,500,000	600	23,000	31.	1,000,000	2,200	70
12.	400,000	350	56	32.	1,800,000	810	180
13.	7,000	7	11	33.	3,600,000	36,000	1,700
14.	130,000	60	1,100	34.	270,000	10	70
15.	3,800,000	3,000	800	35.	430,000	340	1,800
16.	2,500,000	1,300	1	36.	440,000	120	310
17.	3,000,000	10	6	37.	81,000	3,200	2,400
18.	400,000	32,000	500	38.	2,800,000	30,000	40
19.	30,000	35	7	39.	3,100,000	28,000	1,300
20.	350,000	600	13	40.	250,000	17,000	1,100

Table 7 (Continued)

Sample No.	Standard Plate Count/ml	Entero-coccus Count/ml	Coliform	Sample No.	Standard Plate Count/ml	Entero-coccus Count/ml	Coliform
41.	190,000	1	2,100	76.	1,500,000	2,400	1,500
42.	2,100,000	2,100	45,000	77.	400,000	30	10
43.	6,000,000	30,000	40,000	78.	31,000,000	130	59,000
44.	30,000	950	10	79.	440,000	800	9,800
45.	560,000	3,500	1,300	80.	600,000	540	100
46.	2,800,000	3,200	200	81.	3,000,000	6,300	5,400
47.	3,900,000	9,000	16,000	82.	50,000	10	10
48.	3,100,000	28,000	40	83.	2,000,000	760	930
49.	1,200,000	9,500	18,000	84.	340,000	2,400	600
50.	81,000	240	2,700	85.	30,000	10	10
51.	150,000	50	80	86.	1,700,000	8,700	37,000
52.	3,200,000	18,000	120	87.	30,000	10	10
53.	1,800,000	10,000	14,000	88.	50,000	10	20
54.	540,000	42,000	16,000	89.	460,000	170	1,900
55.	40,000	9,400	370	90.	40,000	30	30
56.	130,000	1,200	100	91.	4,000,000	7,000	970
57.	60,000	350	40	92.	1,400,000	34,000	35,000
58.	30,000	390	350	93.	30,000	10	10
59.	40,000	160	90	94.	2,300,000	260	5,900
60.	110,000	600	70	95.	900,000	190	10
61.	20,000	10	10	96.	40,000	30	10
62.	80,000	4,900	360	97.	1,500,000	36,000	20,000
63.	30,000	70	10	98.	1,100,000	460	190
64.	120,000	3,700	60	99.	5,300,000	26,000	45,000
65.	22,000,000	36,000	34,000	100.	280,000	5,600	7,500
66.	9,400,000	32,000	12,000	101.	50,000	1,100	1,400
67.	80,000	160	240	102.	1,800,000	270	110
68.	1,300,000	6,600	1,200	103.	38,000,000	16,000	29,000
69.	40,000	20	10	104.	200,000	20	30
70.	60,000	10	1,000	105.	60,000	340	590
71.	500,000	490	28,000	106.	40,000	70	20
72.	800,000	3,200	11,000	107.	40,000	10	10
73.	40,000	10	30	108.	6,800,000	11,000	14,000
74.	30,000	20	20	109.	3,500,000	5,900	33,000
75.	1,100,000	20,000	3,000	110.	470,000	3,300	3,700

Table 7 (Continued)

Sample No.	Standard Plate Count/ml	Entero-coccus Count/ml	Coliform	Sample No.	Standard Plate Count/ml	Entero-coccus Count/ml	Coliform
111.	430,000	90	20	116.	240,000	10	30
112.	30,000	10	10	117.	120,000	280	120
113.	12,000,000	35,000	36,000	118.	300,000	10	20
114.	3,900,000	80	1,800	119.	6,500,000	90	12,000
115.	60,000	320	900	120.	670,000	20	60
				Average	2,000,000	5,600	6,400
				% Total Count	100	0.28	0.32

Table 8. Frequency distribution of the bacterial content of 120 samples of manufacturing grade raw milk

Standard Plate Count/ml	Per cent samples in range: Enterococcus count/ml		Coliform count/ml	
	< 1-100	> 100	< 1-100	> 100
< 1-200,000	23.4	18.3	29.2	12.5
200,000-1,000,000	5.8	18.4	12.5	11.7
> 1,000,000	2.5	31.6	3.3	30.8
Total	31.7	68.3	45.0	55.0

The variations in Standard Plate Counts and the corresponding variations in enterococcus and coliform counts are presented in Table 9.

Table 9. Variations in the Standard Plate Counts of 120 samples of manufacturing grade raw milk corresponding to variations in the enterococcus and coliform counts

	Enterococcus count/ml		Coliform count/ml	
	< 1-100	> 100	< 1-100	> 100
No. of samples	38	82	54	66
Average SPC/ml	470,000	2,700,000	380,000	2,800,000
Ratio of SPC between groups	1	5.8	1	7.4

The results show that the total counts of the samples with higher enterococcus counts were considerably larger than the total counts of the samples with lower enterococcus counts. Similar increases were observed in total counts of samples with high coliform counts. The average total count of samples with 100 coliforms or less/ml was lower than the average total count of samples with 100 enterococci or less/ml. But the ratio was wider than the ratio of total counts of samples with high and low enterococcus counts.

The analysis of variance of the Standard Plate, enterococcus and coliform counts is presented in Table 10.

The F values for the enterococcus and the coliform counts were 2.29 and 33.09. This suggests that the relationship between the coliform count and the total count was highly significant. On the other hand, a significant relationship between the enterococcus and the total count did not exist in the manufacturing grade raw milk.

Table 10. Analysis of Variance of the Standard Plate, enterococcus and coliform counts of samples of manufacturing grade raw milk

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F Value[1]
Standard Plate Count	119	3,084,604,971		
Enterococcus and coliform counts	2	963,948,111		
Coliform count	1	599,793,782	599,793,782	33.09
Enterococcus count	1	41,611,119	41,611,119	2.29
Deviations	117	2,120,656,860	18,125,272	

[1]F Value = variance ratio.

Relation of Coliform and Yeast and Mold Counts to the Enterococcus Count of Butter

Experimental butter

Experimental butter was prepared in nine separate churnings from cream inoculated with eight different combinations of an enterococcus and a coliform culture; one was inoculated with an enterococcus, a coliform and a flavor culture. Half of the butter was salted and half was left unsalted; out of each half, one portion was properly worked and the other was insufficiently worked. Ten samples were taken from each of the 36 portions thus made. They were kept at 3, 10 and -20 C as stated under experimental methods, and were examined at intervals of 4 hr, 24 hr, 3 days and 1, 2, 3, 4, 5, 6 and 8 weeks.

The effects of salting, working, storage temperature and time on the enterococcus counts of butter are summarized in Table 11 and on the coliform counts in Table 12. The chemical composition of the experimental butter is presented in Table 13. Averages of the enterococcus and coliform counts, as presented in Tables 11 and 12, are summarized in Table 14.

The data presented in Table 13 indicate that the butter had a fairly uniform composition.

The data presented in Tables 11 and 14 indicate that the viable counts of enterococci decreased on storage. However, the decline was gradual and slow (Figures 1 and 2). A fairly large number of these organisms were able to withstand the micro-environment of butter as well as the frozen storage. Salt has a somewhat detrimental effect on these organisms, but, nevertheless, a large number of them persisted during the 8-week storage period. There was multiplication of S. durans in unsalted, poorly worked butter, the highest count being in the fourth week when the temperature of storage was increased to 10 C. No such increase in the numbers of S. faecalis was observed.

The coliform organisms also persisted in unsalted butter (Tables 12 and 14). The E. coli strains gradually decreased in numbers (Figures 3 and 4). Both strains of A. aerogenes multiplied in large numbers during storage. The largest increase in numbers was registered during 10 C storage (Figures 5 and 6). Salt has a pronounced effect on the survival of coliforms, most of which were killed during the initial 4 hr. preparation period. The salt-resistant strains persisted longer than the sensitive strains. A. aerogenes can tolerate salt better than E. coli. The

Table 11. Effect of salting, working, storage temperature and time on the

Churning No.	Inoculum	Treatment given % Salt	Working	Storage 3C 4 hr	24 hr	3 days	1 wk	
1.	S. faecalis	-	Proper	160,000	140,000	170,000	120,000	13
	+	-	Poor	89,000	73,000	91,000	54,000	6
	E. coli(SS)[1]	2.00	Proper	64,000	37,000	35,000	39,000	4
		1.95	Poor	110,000	41,000	34,000	56,000	7
2.	S. faecalis	-	Proper	81,000	73,000	35,000	57,000	7
	+	-	Poor	89,000	61,000	68,000	75,000	11
	E. coli(SR)[2]	2.00	Proper	71,000	68,000	26,000	27,000	2
		1.95	Poor	43,000	30,000	33,000	17,000	4
3.	S. faecalis	-	Proper	49,000	51,000	91,000	110,000	7
	+	-	Poor	50,000	28,000	74,000	91,000	8
	A. aerogenes (SS)[1]	2.00	Proper	27,000	22,000	21,000	21,000	3
		1.95	Poor	53,000	28,000	32,000	32,000	4
4.	S. faecalis	-	Proper	46,000	72,000	75,000	66,000	8
	+	-	Poor	61,000	110,000	61,000	92,000	9
	A. aerogenes (SR)[2]	2.00	Proper	32,000	31,000	60,000	55,000	3
		2.05	Poor	30,000	40,000	62,000	63,000	5
5.	S. durans	-	Proper	26,000	19,000	21,000	13,000	1
	+	-	Poor	19,000	21,000	15,000	27,000	2
	E. coli(SS)[1]	2.00	Proper	10,000	3,400	2,900	3,100	
		1.95	Poor	60,000	7,700	4,400	3,600	
6.	S. durans	-	Proper	5,400	8,100	12,000	7,300	
	+	-	Poor	5,900	9,900	16,000	14,000	3
	E. coli(SR)[2]	2.00	Proper	2,300	2,100	3,400	3,000	
		2.05	Poor	5,400	4,600	3,000	5,700	
7.	S. durans	-	Proper	9,400	16,000	7,200	11,000	
	+	-	Poor	6,900	16,000	12,000	19,000	1
	A. aerogenes (SS)[1]	2.00	Proper	2,300	2,700	3,300	3,800	
		2.00	Poor	5,400	7,000	6,200	6,300	

[1]SS = salt sensitive.

[2]SR = salt resistant.

e temperature and time on the enterococcus count of experimental butter

Storage temperature and time								
3C					10C			-20C
24 hr	3 days	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	8 wk
count/ml								
140,000	170,000	120,000	130,000	70,000	81,000	83,000	69,000	56,000
73,000	91,000	54,000	60,000	70,000	95,000	49,000	65,000	23,000
37,000	35,000	39,000	41,000	28,000	25,000	26,000	24,000	14,000
41,000	34,000	56,000	74,000	55,000	40,000	30,000	31,000	33,000
73,000	35,000	57,000	76,000	69,000	43,000	35,000	31,000	17,000
61,000	68,000	75,000	110,000	85,000	70,000	60,000	45,000	38,000
68,000	26,000	27,000	27,000	24,000	22,000	22,000	6,000	25,000
30,000	33,000	17,000	49,000	33,000	27,000	30,000	16,000	30,000
51,000	91,000	110,000	75,000	81,000	76,000	48,000	35,000	24,000
28,000	74,000	91,000	88,000	59,000	44,000	77,000	41,000	22,000
22,000	21,000	21,000	30,000	20,000	17,000	8,000	11,000	17,000
28,000	32,000	32,000	41,000	25,000	15,000	13,000	17,000	20,000
72,000	75,000	66,000	87,000	64,000	72,000	34,000	57,000	20,000
110,000	61,000	92,000	95,000	98,000	65,000	48,000	49,000	26,000
31,000	60,000	55,000	38,000	38,000	34,000	22,000	22,000	16,000
40,000	62,000	63,000	50,000	57,000	39,000	32,000	34,000	16,000
19,000	21,000	13,000	11,000	16,000	13,000	9,900	10,000	2,700
21,000	15,000	27,000	23,000	22,000	18,000	14,000	26,000	27,000
3,400	2,900	3,100	5,400	3,500	3,300	1,200	800	2,100
7,700	4,400	3,600	6,900	4,500	3,800	1,900	900	2,300
8,100	12,000	7,300	8,200	11,000	6,200	3,700	5,000	700
9,900	16,000	14,000	37,000	58,000	36,000	6,900	19,000	9,300
2,100	3,400	3,000	2,800	1,600	1,500	900	500	200
4,600	3,000	5,700	3,700	2,500	2,800	1,700	800	300
16,000	7,200	11,000	8,800	5,800	5,900	12,000	4,000	2,400
16,000	12,000	19,000	14,000	13,000	41,000	21,000	29,000	6,000
2,700	3,300	3,800	3,500	2,800	1,700	700	400	1,100
7,000	6,200	6,300	4,600	3,600	2,400	1,200	800	5,300

Table 11 (Continued)

Churning No.	Inoculum	Treatment given % Salt	Working	Storage 3C 4 hr	24 hr	3 days	1 wk	
8.	S. durans	-	Proper	9,400	6,200	14,000	6,900	
	+	-	Poor	7,500	8,400	14,000	16,000	1
	A. aerogenes	2.00	Proper	2,400	2,800	2,600	1,700	
	(SR)^2	1.95	Poor	3,400	5,400	3,200	3,200	
9.	S. faecalis	-	Proper	43,000	100,000	94,000	100,000	10
	+	-	Poor	110,000	120,000	110,000	110,000	10
	A. aerogenes	2.00	Proper	38,000	47,000	39,000	42,000	3
	+ (SR)^2	1.95	Poor	69,000	64,000	62,000	53,000	3
	Starter							

Storage temperature and time									
	3C					10C			-20C
r	24 hr	3 days	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	8 wk
				count/ml					
00	6,200	14,000	6,900	5,700	7,200	5,400	3,800	2,500	2,300
00	8,400	14,000	16,000	14,000	20,000	69,000	35,000	6,300	9,700
00	2,800	2,600	1,700	1,200	800	500	300	300	1,200
00	5,400	3,200	3,200	1,900	1,200	800	600	600	2,400
00	100,000	94,000	100,000	100,000	82,000	77,000	65,000	38,000	15,000
00	120,000	110,000	110,000	100,000	120,000	100,000	95,000	77,000	16,000
00	47,000	39,000	42,000	37,000	36,000	29,000	28,000	27,000	14,000
00	64,000	62,000	53,000	35,000	35,000	37,000	37,000	24,000	15,000

Table 12. Effect of salting, working, storage temperature and time on the

Churning No.	Inoculum	Treatment given % Salt	Working	Storage 3C 4 hr	24 hr	3 days	1 wk	
1.	E. coli(SS)[1] + S. faecalis	-	Proper	10,000	12,000	9,800	3,300	1
		-	Poor	9,700	10,000	8,000	1,000	
		2.00	Proper	5	<1	<1	<1	
		1.95	Poor	50	<1	<1	<1	
2.	E. coli(SR)[2] + S. faecalis	-	Proper	35,000	30,000	19,000	23,000	2
		-	Poor	43,000	33,000	37,000	35,000	2
		2.00	Proper	30	12	5	6	
		1.95	Poor	80	20	60	16	
3.	A. aerogenes + (SS)[1] S. faecalis	-	Proper	9,500	5,300	4,800	180,000	49
		-	Poor	2,300	850	11,000	600,000	1,80
		2.00	Proper	2	2	2	14	
		1.95	Poor	10	8	60	470	
4.	A. aerogenes + (SR)[2] S. faecalis	-	Proper	82,000	130,000	130,000	100,000	8
		-	Poor	140,000	170,000	140,000	130,000	11
		2.00	Proper	500	570	740	260	
		2.05	Poor	1,700	1,200	650	480	
5.	E. coli(SS)[1] + S. durans	-	Proper	20,000	23,000	23,000	8,400	
		-	Poor	35,000	18,000	18,000	13,000	
		2.00	Proper	46	150	42	6	
		1.95	Poor	64	60	22	4	
6.	E. coli(SR)[2] + S. durans	-	Proper	19,000	34,000	35,000	29,000	2
		-	Poor	37,000	44,000	49,000	39,000	3
		2.00	Proper	620	330	260	120	
		2.05	Poor	1,200	210	190	92	
7.	A. aerogenes + (SS)[1] S. durans	-	Proper	31,000	55,000	41,000	120,000	12
		-	Poor	18,000	25,000	51,000	990,000	3,20
		2.00	Proper	44	86	6	6	
		2.00	Poor	58	420	260	450	

[1]SS = salt sensitive.

[2]SR = salt resistant.

ge temperature and time on the coliform count of experimental butter

Storage temperature and time								
3C					10C			-20C
24 hr	3 days	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	8 wk
count/ml								
12,000	9,800	3,300	12,000	7,200	12,000	7,500	1,900	<1
10,000	8,000	1,000	4,100	4,500	6,900	7,200	3,700	<1
<1	<1	<1	<1	<1	<1	<1	<1	<1
<1	<1	<1	<1	<1	<1	<1	<1	<1
30,000	19,000	23,000	27,000	22,000	7,700	6,900	4,200	680
33,000	37,000	35,000	26,000	24,000	30,000	77,000	38,000	1,300
12	5	6	6	6	2	<1	<1	2
20	60	16	60	10	4	<1	<1	2
5,300	4,800	180,000	490,000	230,000	600,000	810,000	470,000	46
850	11,000	600,000	1,800,000	1,200,000	2,100,000	1,100,000	180,000	3,700
2	2	14	16	2	2	<1	<1	<1
8	60	470	1,900	270	2,700	26	22	<1
30,000	130,000	100,000	84,000	82,000	720,000	550,000	1,600,000	12
70,000	140,000	130,000	110,000	100,000	130,000	410,000	1,200,000	40
570	740	260	16	6	36	16	62	2
1,200	650	480	34	10	32	48	280	<1
23,000	23,000	8,400	7,400	7,300	3,700	3,500	1,800	<1
18,000	18,000	13,000	8,000	8,000	4,000	4,300	2,500	40
150	42	6	6	2	<1	<1	<1	<1
60	22	4	2	2	2	<1	<1	<1
34,000	35,000	29,000	24,000	17,000	17,000	14,000	6,600	900
44,000	49,000	39,000	34,000	21,000	22,000	76,000	61,000	3,500
330	260	120	2	2	2	<1	<1	<1
210	190	92	8	2	22	460	240	2
55,000	41,000	120,000	120,000	980,000	920,000	1,200,000	350,000	12
25,000	51,000	990,000	3,200,000	9,500,000	8,300,000	1,100,000	1,400,000	12,000
86	6	6	1,200	3,400	830	600	90	<1
420	260	450	4,500	13,000	19,000	19,000	2,600	<1

Table 12 (Continued)

Churning No.	Inoculum	Treatment given % Salt	Working	Storage t 30 4 hr	24 hr	3 days	1 wk	2
								cc
8.	A. aerogenes + (SR)[2]	-	Proper	140,000	110,000	150,000	94,000	100,
		-	Poor	170,000	180,000	300,000	160,000	130,
	S. durans	2.00	Proper	450	270	150	32	
		1.95	Poor	900	1,700	290	130	
9.	A. aerogenes + (SR)[2]	-	Proper	77,000	78,000	42,000	370,000	660,
		-	Poor	480,000	740,000	550,000	430,000	620,
	S. faecalis + Starter	2.00	Proper	58	34	18	26	
		1.95	Poor	26	35	14	12	

Storage temperature and time									
	3C					10C			-20C
r	24 hr	3 days	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	8 wk
				count/ml					
00	110,000	150,000	94,000	100,000	85,000	78,000	260,000	370,000	22
00	180,000	300,000	160,000	130,000	180,000	1,500,000	2,800,000	1,700,000	230
50	270	150	32	2	2	2	2	2	< 1
00	1,700	290	130	4	36	2,000	380	6,300	< 1
00	78,000	42,000	370,000	660,000	610,000	2,200,000	2,400,000	3,500,000	4
00	740,000	550,000	430,000	620,000	710,000	1,600,000	4,800,000	4,600,000	6
58	34	18	26	6	10	170	70	2	< 1
26	35	14	12	6	15	190	64	40	< 1

Table 13. Chemical composition of experimental butter

Churning No.	Inoculum	Treatment given: Salt	Working	Moisture %	Fat %	Curd %	Salt %
1.	*S. faecalis* + *E. coli*(SS)[1]	Unsalted	Proper	17.50	81.40	1.00	-
	" "	"	Poor	17.00	81.90	1.10	-
	" "	Salted	Proper	16.50	80.50	1.00	2.00
	" "	"	Poor	16.70	80.30	1.05	1.95
2.	" + *E. coli*(SR)[2]	Unsalted	Proper	17.10	81.90	1.00	-
	" "	"	Poor	17.50	81.50	1.00	-
	" "	Salted	Proper	16.90	80.10	1.00	2.00
	" "	"	Poor	16.90	80.10	1.05	1.95
3.	" + *A. aerogenes*(SS)[1]	Unsalted	Proper	16.60	82.40	1.00	-
	" "	"	Poor	17.00	82.00	1.00	-
	" "	Salted	Proper	17.00	80.05	0.95	2.00
	" "	"	Poor	16.90	80.15	1.00	1.95
4.	" + *A. aerogenes*(SR)[2]	Unsalted	Proper	16.80	82.20	1.00	-
	" "	"	Poor	16.60	82.40	1.00	-
	" "	Salted	Proper	16.60	80.40	1.00	2.00
	" "	"	Poor	16.90	80.10	0.95	2.05
5.	*S. durans* + *E. coli*(SS)[1]	Unsalted	Proper	16.15	82.80	1.05	-
	" "	"	Poor	16.35	82.65	1.00	-
	" "	Salted	Proper	17.00	80.00	1.00	2.00
	" "	"	Poor	16.50	80.50	1.05	1.95

[1] SS = salt sensitive.

[2] SR = salt resistant.

Table 13 (Continued)

Churning No.	Inoculum	Treatment given: Salt	Treatment given: Working	Moisture %	Fat %	Curd %	Salt %
6.	S. durans + E. coli(SR)[2]	Unsalted	Proper	16.40	82.60	1.00	-
	" "	"	Poor	16.60	82.40	1.00	-
	" "	Salted	Proper	16.20	80.80	1.00	2.00
	" "	"	Poor	16.70	80.25	1.00	2.05
7.	" + A. aerogenes(SS)[1]	Unsalted	Proper	17.00	82.00	1.00	-
	" "	"	Poor	17.10	81.90	1.00	-
	" "	Salted	Proper	16.80	80.20	1.00	2.00
	" "	"	Poor	17.00	80.05	0.95	2.00
8.	" + A. aerogenes(SR)[2]	Unsalted	Proper	16.90	82.10	1.00	-
	" "	"	Poor	17.00	82.00	1.00	-
	" "	Salted	Proper	16.70	80.30	1.00	2.00
	" "	"	Poor	16.50	80.50	1.05	1.95
9.	S. faecalis + A. aerogenes(SR)[2] + Flavor Culture	Unsalted	Proper	16.00	82.65	1.35	-
	" "	"	Poor	16.10	82.60	1.30	-
	" "	Salted	Proper	16.50	80.25	1.25	2.00
	" "	"	Poor	16.00	80.80	1.25	1.95

[1]SS = salt sensitive

[2]SR = salt resistant

Table 14. Average enterococcus and coliform counts in experimental butter

Inoculum	No. of Churnings Aver.	Treatment given % Salt	Working	Storage temperature 3C 4 hr	24 hr	3 days	1 wk	2 wk	
									count/ml
S. faecalis	4	-	Proper	84,000	85,000	92,000	88,000	93,000	7
"	4	-	Poor	72,000	68,000	73,000	78,000	88,000	7
"	4	2.00	Proper	49,000	40,000	36,000	36,000	34,000	2
"	4	2.00	Poor	59,000	35,000	40,000	42,000	54,000	4
S. durans	4	-	Proper	13,000	12,000	14,000	9,600	8,400	1
"	4	-	Poor	9,800	14,000	14,000	19,000	22,000	2
"	4	2.00	Proper	4,300	2,800	3,100	2,900	3,200	
"	4	2.00	Poor	5,500	6,200	4,200	4,700	4,300	
E. coli(SS)[1]	2	-	Proper	15,000	17,000	16,000	6,000	9,800	
"	2	-	Poor	22,000	15,000	13,000	7,000	6,000	
"	2	2.00	Proper	26	75	21	3	3	
"	2	1.95	Poor	57	30	11	2	1	
E. coli(SR)[2]	2	-	Proper	27,000	32,000	27,000	26,000	25,000	2
"	2	-	Poor	40,000	39,000	43,000	37,000	30,000	2
"	2	2.00	Proper	330	170	130	63	4	
"	2	2.05	Poor	650	120	130	54	34	
A. aerogenes (SS)[1]	2	-	Proper	20,000	30,000	23,000	150,000	300,000	60
"	2	-	Poor	10,000	13,000	31,000	800,000	2,500,000	5,00
"	2	2.00	Proper	23	44	4	10	590	
"	2	2.00	Poor	34	210	160	460	3,200	
A. aerogenes (SR)[2]	2	-	Proper	110,000	120,000	140,000	97,000	94,000	8
"	2	-	Poor	160,000	170,000	230,000	150,000	120,000	14
"	2	2.00	Proper	500	420	440	150	9	
"	2	1.95	Poor	1,300	1,500	470	300	19	

[1]SS = salt sensitive.

[2]SR = salt resistant.

ounts in experimental butter

Storage temperature and time								
3C					10C			-20C
hr	3 days	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	8 wk
count/ml								
,000	92,000	88,000	93,000	71,000	68,000	50,000	48,000	39,000
,000	73,000	78,000	88,000	78,000	69,000	59,000	50,000	27,000
,000	36,000	36,000	34,000	27,000	25,000	20,000	16,000	18,000
,000	40,000	42,000	54,000	43,000	30,000	26,000	25,000	25,000
,000	14,000	9,600	8,400	10,000	7,600	7,400	5,400	2,000
,000	14,000	19,000	22,000	28,000	41,000	19,000	20,000	13,000
,800	3,100	2,900	3,200	2,200	1,800	800	500	1,200
,200	4,200	4,700	4,300	3,000	2,500	1,300	800	2,600
,000	16,000	6,000	9,800	7,300	7,600	5,500	1,900	<1
,000	13,000	7,000	6,000	6,300	5,500	5,800	3,100	20
75	21	3	3	1	<1	<1	<1	<1
30	11	2	1	1	1	<1	<1	<1
,000	27,000	26,000	25,000	20,000	13,000	11,000	5,400	800
,000	43,000	37,000	30,000	23,000	26,000	77,000	50,000	2,400
170	130	63	4	4	2	<1	<1	1
120	130	54	34	6	13	230	120	2
,000	23,000	150,000	300,000	600,000	760,000	980,000	410,000	29
,000	31,000	800,000	2,500,000	5,000,000	5,000,000	1,000,000	780,000	8,000
44	4	10	590	1,700	810	300	45	1
210	160	460	3,200	6,800	12,000	9,000	1,300	<1
000	140,000	97,000	94,000	84,000	400,000	410,000	1,000,000	17
000	230,000	150,000	120,000	140,000	800,000	1,600,000	1,400,000	140
420	440	150	9	4	19	8	32	1
500	470	300	19	43	1,000	420	3,300	<1

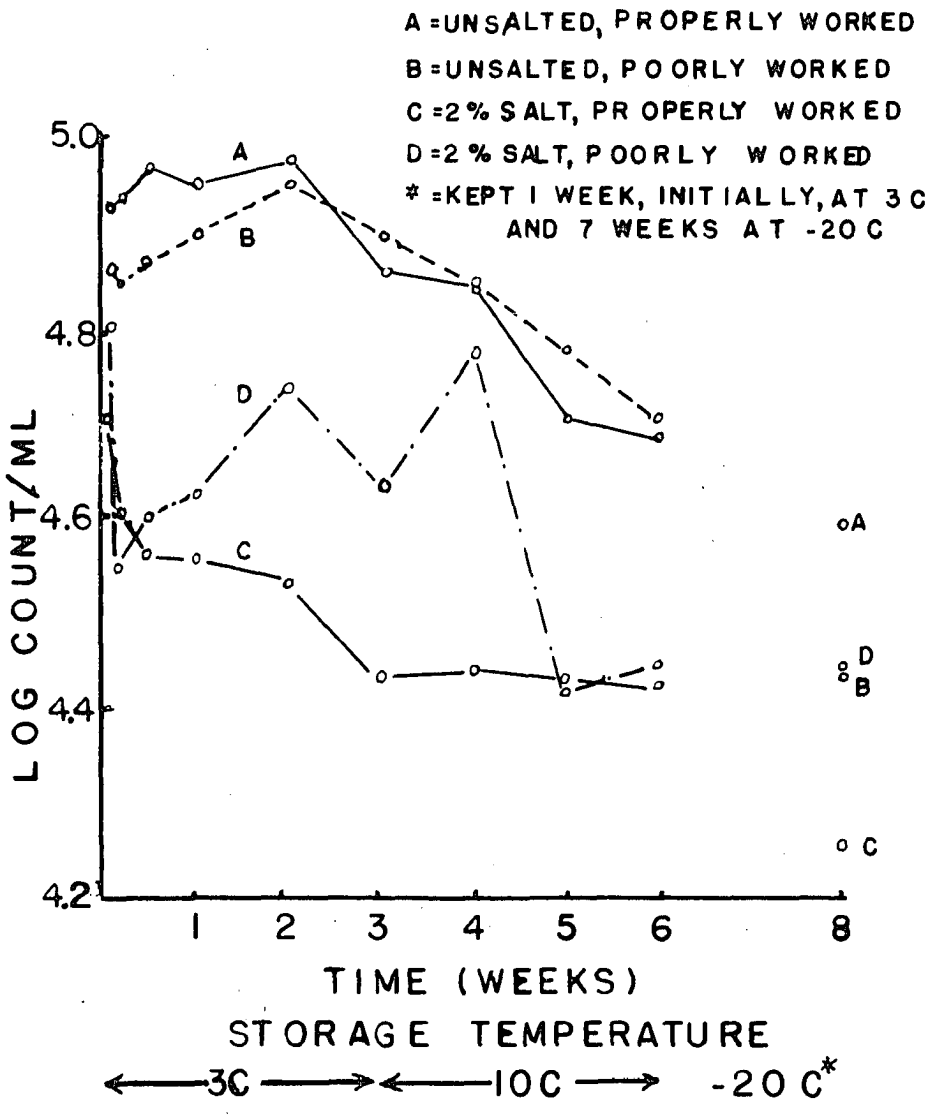

Figure 1. Effect of salting, working, storage temperature and time on S. faecalis in experimental butter

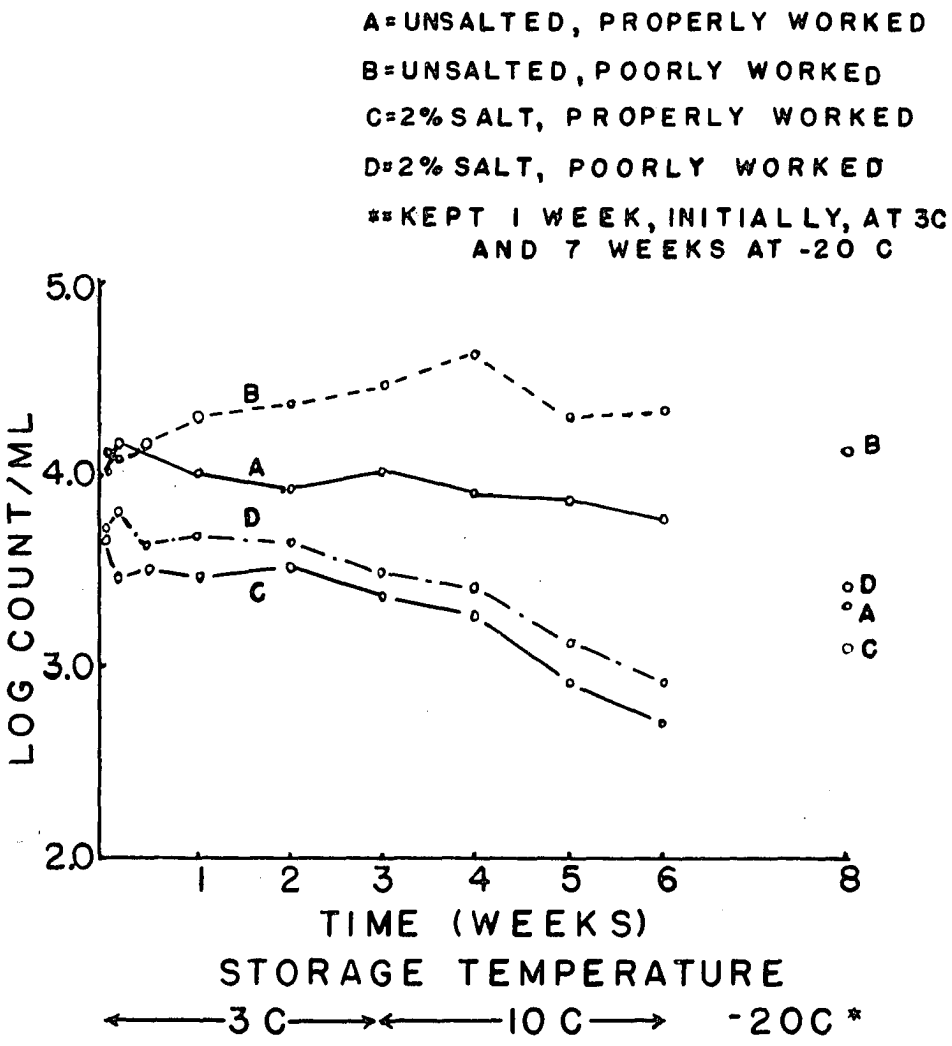

Figure 2. Effect of salting, working, storage temperature and time on S. durans in experimental butter

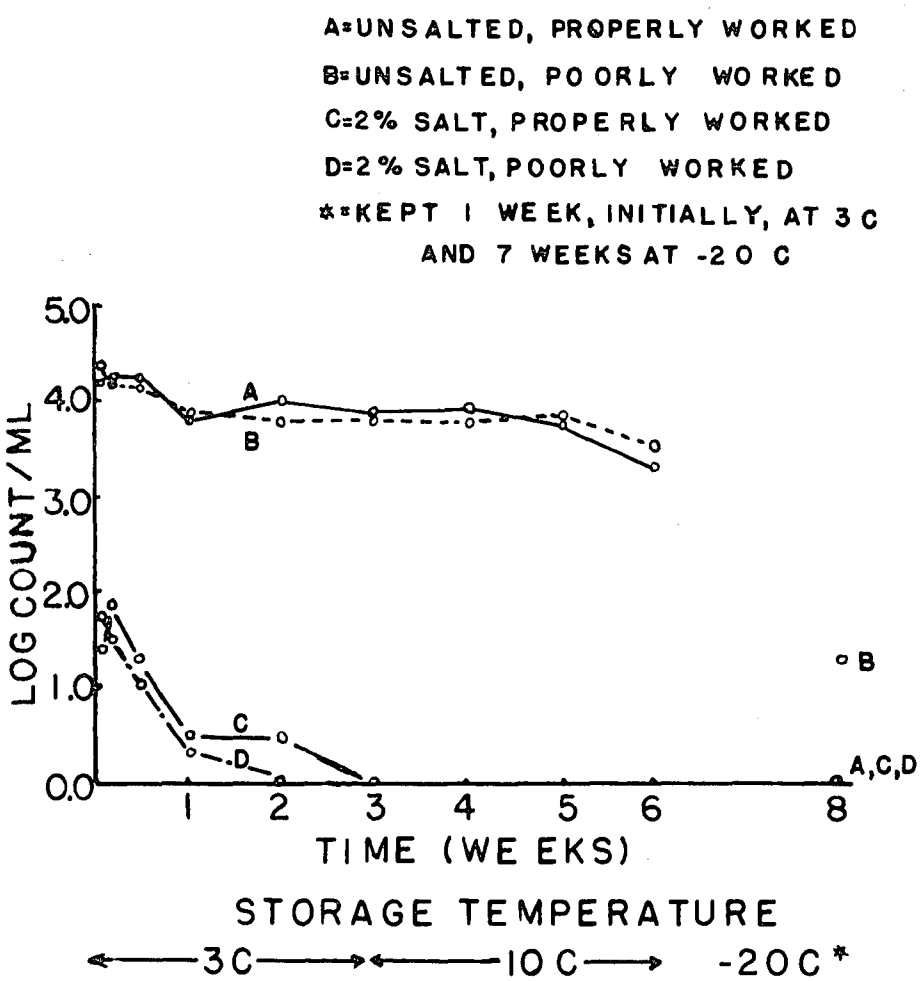

Figure 3. Effect of salting, working, storage temperature and time on E. coli (salt-sensitive) in experimental butter

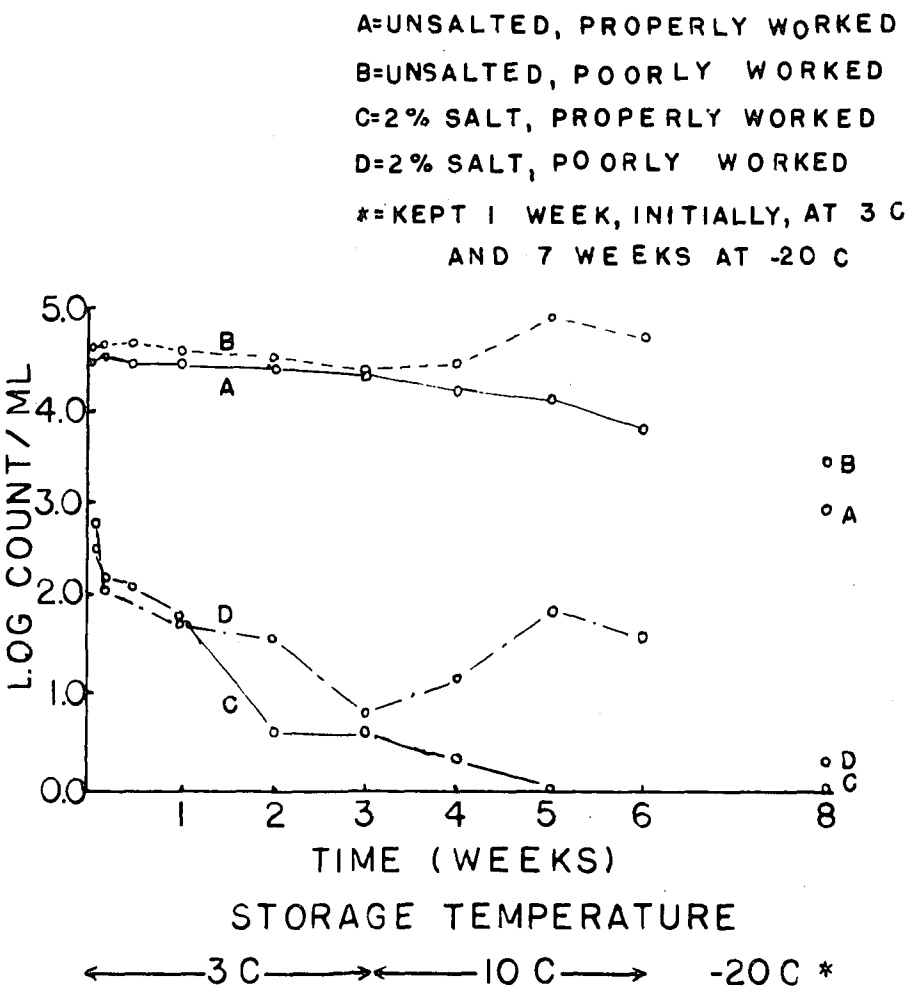

Figure 4. Effect of salting, working, storage temperature and time on E. coli (salt-resistant) in experimental butter

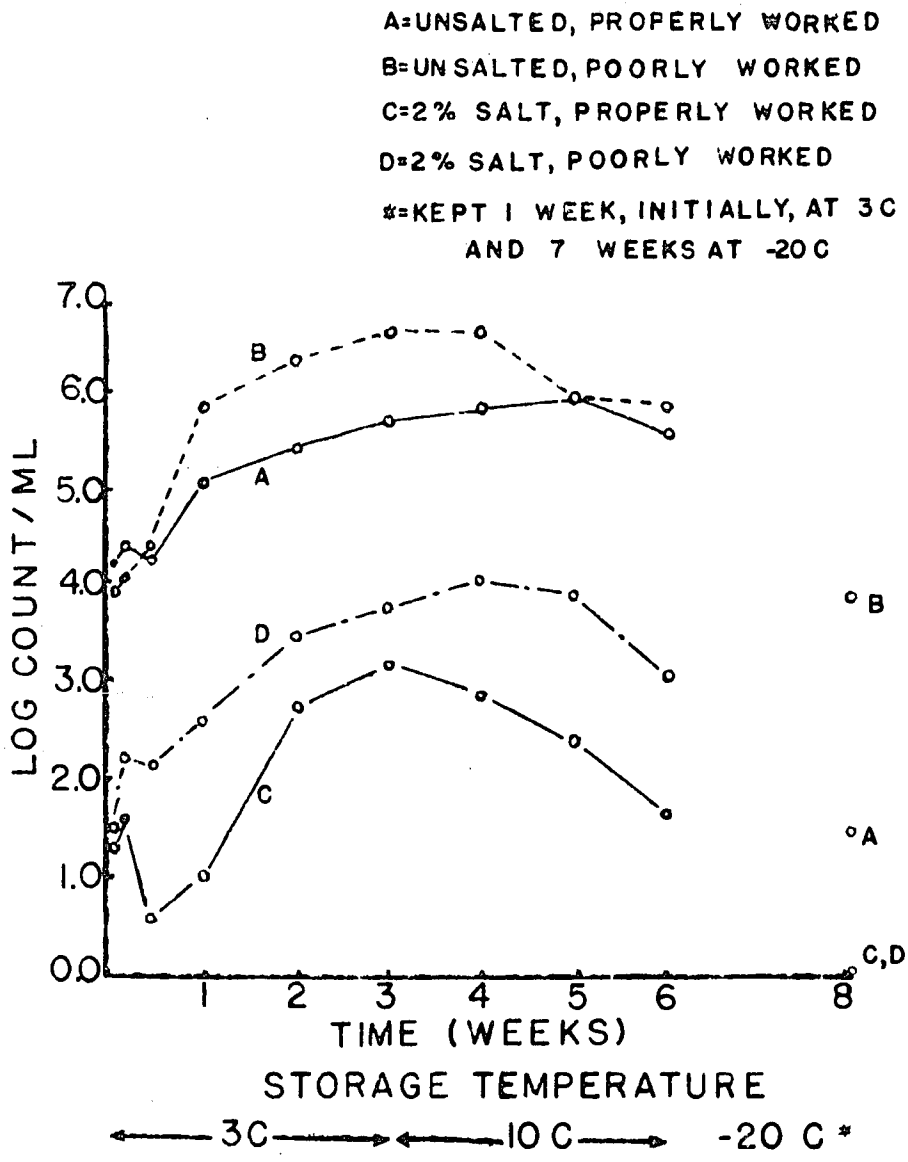

Figure 5. Effect of salting, working, storage temperature and time on A. aerogenes (salt-sensitive) in experimental butter

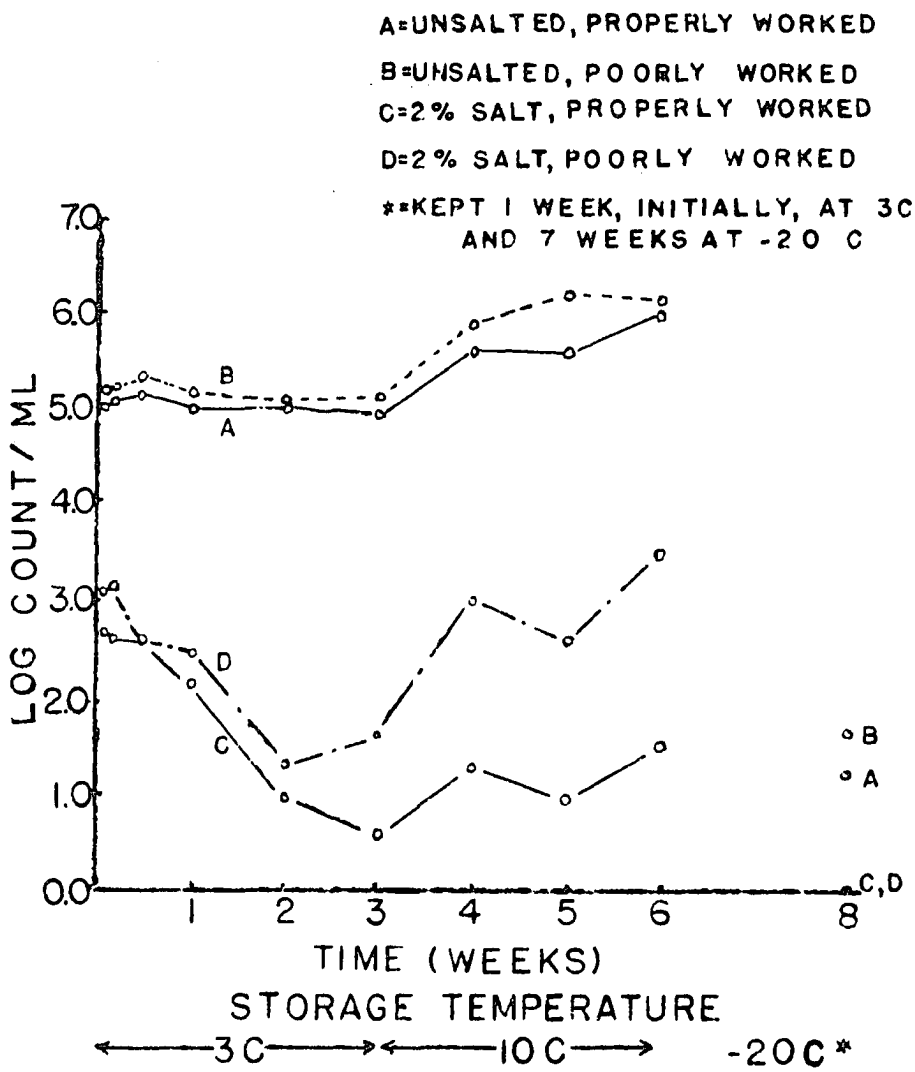

Figure 6. Effect of salting, working, storage temperature and time on A. aerogenes (salt-resistant) in experimental butter

Table 15. Recovery of enterococcus and coliform organisms in butter made from inoculated cream

Inoculum	Treatment % Salt	Working	No. inoculated in cream	No. recovered in butter	% recovery
			count/g	count/ml	
S. faecalis	-	Proper	760,000	84,000	11.00
	-	Poor	"	72,000	9.50
	2.00	Proper	"	49,000	6.40
	2.00	Poor	"	59,000	7.70
S. durans	-	Proper	120,000	13,000	10.30
	-	Poor	"	9,800	8.00
	2.00	Proper	"	4,300	3.50
	2.00	Poor	"	5,500	4.50
E. coli (SS)[1]	-	Proper	220,000	15,000	7.00
	-	Poor	"	22,000	10.00
	2.00	Proper	"	26	0.01
	1.95	Poor	"	57	0.03
E. coli (SR)[2]	-	Proper	380,000	27,000	7.00
	-	Poor	"	40,000	10.00
	2.00	Proper	"	330	0.09
	2.05	Poor	"	650	0.17
A. aerogenes (SS)[1]	-	Proper	250,000	20,000	8.00
	-	Poor	"	10,000	4.00
	2.00	Proper	"	23	0.01
	2.00	Poor	"	34	0.01
A. aerogenes (SR)[2]	-	Proper	860,000	110,000	12.80
	-	Poor	"	160,000	18.40
	2.00	Proper	"	500	0.06
	1.95	Poor	"	1,300	0.15

[1]SS = salt sensitive

[2]SR = salt resistant

Table 16. Effect of frozen storage on the enterococcus and coliform counts of experimental butter

Inoculum	Treatment given % Salt	Working	Temperature and time of storage 3 C for 7 days	-20 C for 7 wk.	% Recovery
			count/ml		
S. faecalis	-	Proper	88,000	39,000	44.30
	-	Poor	78,000	27,000	34.60
	2.00	Proper	36,000	18,000	50.00
	2.00	Poor	40,000	25,000	62.50
S. durans	-	Proper	9,600	2,000	20.80
	-	Poor	19,000	13,000	68.00
	2.00	Proper	2,900	1,200	41.40
	2.00	Poor	4,200	2,600	61.90
E. coli (SS)[1]	-	Proper	6,000	<1	0
	-	Poor	7,000	20	0.28
	2.00	Proper	3	<1	0
	1.95	Poor	2	<1	0
E. coli (SR)[2]	-	Proper	26,000	800	3.10
	-	Poor	37,000	2,400	6.50
	2.00	Proper	63	1	1.60
	2.05	Poor	54	2	3.70
A. aerogenes (SS)[1]	-	Proper	150,000	29	0.02
	-	Poor	800,000	8,000	1.00
	2.00	Proper	10	<1	0
	2.00	Poor	460	<1	0
A. aerogenes (SR)[2]	-	Proper	97,000	17	0.02
	-	Poor	150,000	140	0.10
	2.00	Proper	150	1	0.66
	1.95	Proper	300	<1	0

[1]SS = salt sensitive

[2]SR = salt resistant

former organism also showed some growth during storage of the salted butter. Frozen storage killed most of the coliforms in butter. The working of butter had a marked effect on the counts of both enterococci and coliforms. In poorly worked butter, the organisms showed noticeably irregular trends in survival or destruction.

Recovery of enterococci and coliforms in butter made from inoculated cream is presented in Table 15. From 3.5 to 11.0% of all of the enterococci inoculated into cream were recovered in the butter. The coliforms were recovered from 4.0 to more than 18.0% in unsalted and less than 0.2% in salted butter.

The effect of frozen storage on the enterococcus and coliform counts of butter is summarized in Table 16. Approximately 21.0 to 68.0% of the enterococcus members present initially survived the 7-week storage at -20 C. Less than 7.0% of the coliforms survived frozen storage. The salt-resistant strains of the coliform bacteria showed better survival under frozen condition in butter. There was a tendency toward better survival when the initial coliform counts were large. The percentage of surviving coliforms was larger in poorly worked than in properly worked butter.

The flavor culture added to butter did not have an effect on the enterococcus or coliform count of butter as stored in this study.

Line-run samples of butter

Line-run samples of butter were obtained from 20 different churnings at eight creameries. Samples were collected at ten consecutive points along the processing line. Results are presented in Table 17. Examination of the data shows that raw cream contained the highest number of

Table 17. Enterococcus, coliform and yeast and mold counts of line-run samples

Churning No.	Plant No.	Enterococcus count/ml: Raw cream (1)	Pasteurized cream (2)	Cream from holding vat (3)	Cream from churn (4)	Cream after 2 min churning (5)	Buttermilk (6)	Unwashed butter granules (7)	Washed butter (8)	Salted butter (9)	Salted butter after 7 days at 7 C (10)	(1)
1.	1	400,000	1	100	90	87	220	6	3	2	<1	8,000,
2.	1	460,000	8	17	7	27	46	3	2	1	2	3,800,
3.	1	1,100,000	1	22	24	20	40	3	2	1	<1	4,700,
4.	2	1,200	<1	<1	<1	<1	<1	<1	-	<1	<1	42,
5.	2	1,000	<1	<1	<1	<1	<1	<1	-	<1	<1	220,
6.	3	360,000	1	-	-	21	250	38	17	13	150	750,
7.	4*	-	2,800	2,900	2,900	3,100	950	100	-	59	40	
8.	4*	-	440	700	740	1,200	480	120	-	31	14	
9.	4*	-	25	21	65	180	74	23	-	3	2	
10.	5	45,000	<1	2	1	3	2	2	41	2	1	43,
11.	5	350	-	<1	-	2	15	11	1	<1	<1	1,
12.	5	-	-	57	50	60	160	9	13	10	6	
13.	6	850,000	<1	100	130	140	310	27	13	72	70	1,500,
14.	7	64,000	<1	66	96	160	340	20	-	26	32	37,
15.	8	-	-	1,000	1,000	1,100	1,800	200	140	74	67	
16.	6	-	-	250	350	330	380	68	55	39	30	
17.	7	-	-	250	340	600	1,100	100	-	67	34	
18.	1	-	<1	900	1,000	800	1,200	120	100	30	12	
19.	5	50,000	<1	<1	<1	8	13	7	2	1	<1	540,
20.	5	50,000	<1	<1	<1	7	12	4	4	3	2	
**Average		280,000	1	170	210	200	290	30	30	20	24	1,800,

*Cream was separated from pasteurized milk and no further heat treatment was given

**Average of churnings using pasteurized cream only, excludes churnings no. 7, 8 an

Coliform count/ml										Yeast and mold count/ml									
Line-run samples										Line-run samples									
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
00,000	1	1,300	86	75	55	9	7	<1	<1	12,000	<1	36	7	9	22	1	<1	9	7
00,000	4	130	120	140	450	20	28	<1	<1	17,000	<1	1	2	4	29	4	2	1	2
00,000	<1	38	51	210	500	27	19	<1	<1	17,000	<1	3	17	27	39	8	9	10	6
12,000	<1	<1	<1	<1	<1	<1	-	<1	<1	100	<1	<1	<1	<1	<1	<1	-	<1	<1
20,000	<1	<1	<1	<1	<1	<1	-	<1	<1	5	<1	<1	<1	<1	<1	<1	-	<1	<1
50,000	<1	-	-	3	380	37	27	21	41	12,000	1	-	-	1	460	480	370	330	270
-	3,600	4,200	5,300	6,500	4,100	470	-	<1	<1	-	44	42	39	38	280	25	-	320	300
-	9,700	8,700	11,000	16,000	12,000	230	-	<1	<1	-	44	22	50	74	240	33	-	230	170
-	4,500	2,200	2,000	2,000	3,600	200	-	<1	<1	-	9	10	12	28	280	130	-	190	120
13,000	<1	<1	<1	2	2	1	2	1	<1	140	<1	<1	1	<1	<1	9	5	<1	<1
1,700	-	6	-	2	2	<1	<1	<1	<1	13	-	<1	-	<1	<1	<1	<1	<1	<1
-	-	43	21	18	90	13	11	<1	<1	-	-	<1	<1	<1	1	<1	5	<1	<1
10,000	<1	110	330	450	1,700	73	39	6	1	1,300	<1	4	3	22	120	15	27	180	240
17,000	<1	1	1	17	17	1	-	<1	<1	170	<1	<1	<1	2	1	<1	-	<1	<1
-	-	190	400	390	500	39	14	<1	<1	-	-	<1	<1	<1	<1	<1	<1	<1	<1
-	-	1,300	1,600	1,900	2,600	110	33	14	6	-	-	<1	210	150	240	28	19	56	23
-	-	1,100	1,600	2,000	2,900	130	-	<1	<1	-	-	2	2	94	110	63	-	2	2
-	<1	3,000	3,400	3,500	13,000	600	420	<1	<1	-	<1	7	11	21	32	26	16	4	4
0,000	<1	<1	<1	1	2	<1	<1	<1	<1	120	<1	1	<1	<1	<1	<1	14	1	<1
-	<1	1	<1	5	110	3	2	<1	<1	-	<1	<1	<1	<1	<1	<1	<1	<1	<1
10,000	<1	450	480	510	1,300	63	46	2	2	5,500	<1	8	17	19	62	37	36	35	33

n to the cream.

and 9.

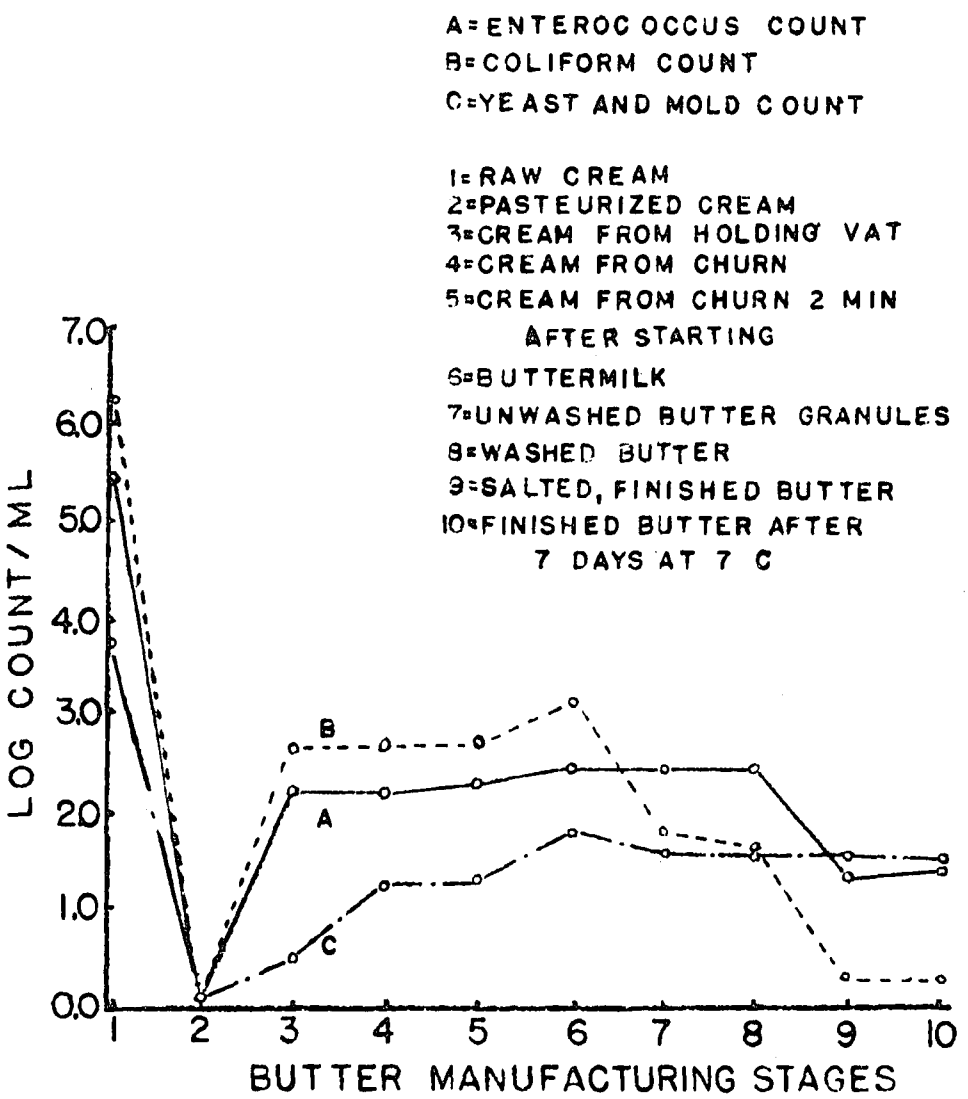

Figure 7. Enterococcus, coliform, and yeast and mold counts of line-run samples

organisms. Pasteurization of the cream eliminated almost all organisms of the three groups. In one of the plants, pasteurized whole milk was separated to obtain cream for churning. This cream was not given any additional heat-treatment. As a result, relatively high numbers of enterococci, coliforms, and yeasts and molds were present in the cream and the unsalted butter made from the cream.

The most important single source of contamination in the line was the holding vat. The largest numbers of the enterococcus and the coliform organisms were introduced at this point. Churns, particularly the wooden ones used at three of the eight plants surveyed, also contributed to the microbial flora of the butter, particularly the yeasts and molds. A large proportion of all three types of organisms was eliminated in the buttermilk, leaving the butter granules lower in the microbial content. Washing of the butter granules further reduced the number of organisms in the butter. In one instance, more enterococci were introduced into washed butter than were originally present in the unwashed granules. In another case, the salting operation, or, perhaps the water used to control the moisture level of the butter, added to the enterococcus count. Otherwise, salting, as well as the holding of butter at 7 C for 7 days, had an inhibitory effect on the enterococci, and completely eliminated the coliforms in most cases. In one case, both enterococci and the coliforms registered a substantial increase in numbers during the holding period. Some reduction in the yeast and mold count occurred during storage of salted butter at 7 C for 7 days. In two cases, however, the counts showed an increase on storage. The general pattern of microbial contamination of butter

along the processing line is graphically presented in Figure 7.

Contest butter samples

Samples of butter were obtained from several Iowa contests. The samples were taken from larger quantities of salted butter and were about one week old. The results are presented in Table 18. The average enterococcus, coliform, and yeast and mold counts were 25, 5 and 18/ml of butter, respectively. There appeared to be little relationship between the microbial content and the quality of butter. Of the 57 samples with judging scores of less than 90, high microbial content (more than ten enterococci or coliforms or more than 20 yeasts and molds/ml) was observed in 22 samples compared to 35 samples having low counts. Of 266 samples having scores of 93 or over, high microbial counts were present in 46 samples while 220 samples had low counts.

The frequency distribution of the contest butter samples according to microbial content is presented in Table 19.

Only a small number of samples had all three types of organisms present. Approximately one-third of the samples did not have any of these organisms. Enterococci were present in three-fifths of the samples, yeasts and molds in about one-fourth, while the coliforms were present only in a small number of samples. Enterococci alone were present in more than one-third of the samples, while only a small percentage of the samples had yeasts and molds alone. No samples of butter contained the coliform organisms alone. More than ten enterococci/ml were present in 21.3% of the samples and more than ten coliforms/ml in only 5.1% of the samples; 9.3% of the samples had more than 20 yeasts and molds/ml.

Table 18. Enterococcus, coliform and yeast and mold counts of butter samples

Sample No.	Entero-coccus	Coli-form	Yeast and mold	Judging score	Sample No.	Entero-coccus	Coli-form	Yeast and mold	Judging score
	count/ml					count/ml			
1.	<1	<1	<1	98	36.	1	<1	8	91
2.	2	<1	<1	96	37.	<1	<1	<1	98
3.	6	<1	<1	96	38.	<1	<1	<1	94
4.	8	<1	<1	96	39.	<1	<1	4	96
5.	<1	<1	<1	97	40.	2	4	<1	90
6.	2	<1	<1	98	41.	<1	<1	<1	97
7.	<1	<1	<1	97	42.	<1	<1	<1	97
8.	1	30	10	92	43.	<1	<1	<1	98
9.	6	<1	<1	90	44.	<1	<1	<1	95
10.	<1	<1	<1	98	45.	250	70	90	84
11.	290	270	120	85	46.	<1	<1	<1	98
12.	<1	<1	<1	97	47.	8	<1	<1	95
13.	26	<1	10	97	48.	38	<1	8	96
14.	2	<1	<1	97	49.	<1	<1	<1	98
15.	97	2	40	95	50.	<1	<1	<1	98
16.	17	<1	50	96	51.	67	4	<1	93
17.	2	<1	<1	98	52.	31	6	<1	89
18.	2	6	<1	96	53.	8	4	<1	95
19.	<1	<1	<1	97	54.	4	<1	12	93
20.	2	<1	<1	97	55.	<1	<1	8	98
21.	<1	<1	<1	97	56.	1	<1	<1	100
22.	44	4	40	93	57.	290	22	<1	95
23.	<1	<1	<1	94	58.	<1	<1	<1	98
24.	<1	<1	<1	95	59.	<1	<1	<1	94
25.	63	<1	<1	97	60.	1	<1	<1	97
26.	10	<1	<1	98	61.	2	<1	<1	97
27.	<1	<1	10	95	62.	<1	<1	<1	96
28.	<1	<1	<1	97	63.	2	<1	10	95
29.	4	<1	<1	96	64.	48	<1	<1	98
30.	<1	<1	4	95	65.	2	<1	<1	97
31.	3	<1	<1	97	66.	1	<1	<1	99
32.	<1	<1	10	98	67.	<1	<1	4	96
33.	<1	<1	20	96	68.	4	<1	<1	98
34.	1	<1	<1	97	69.	52	<1	<1	97
35.	<1	<1	30	95	70.	5	<1	<1	92

Table 18 (Continued

Sample No.	Entero-coccus count/ml	Coli-form count/ml	Yeast and mold count/ml	Judging score	Sample No.	Entero-coccus count/ml	Coli-form count/ml	Yeast and mold count/ml	Judging score
71.	26	<1	6	96	109.	4	<1	<1	95
72.	6	<1	<1	96	110.	<1	<1	<1	84
73.	8	<1	<1	81	111.	22	<1	<1	88
74.	1	<1	4	97	112.	<1	<1	<1	98
75.	4	<1	<1	97	113.	1	<1	<1	91
76.	42	4	80	93	114.	5	<1	<1	98
77.	4	<1	30	95	115.	<1	<1	<1	98
78.	2	<1	<1	98	116.	1	<1	<1	98
79.	<1	4	8	94	117.	<1	<1	<1	98
80.	<1	<1	<1	97	118.	75	34	1,000	74
81.	<1	<1	10	77	119.	2	<1	<1	97
82.	<1	<1	<1	92	120.	<1	320	20	92
83.	<1	<1	<1	99.6	121.	<1	<1	<1	91
84.	1	<1	4	96	122.	5	<1	<1	95
85.	1	<1	<1	97	123.	5	<1	<1	98
86.	50	<1	<1	99.8	124.	<1	<1	<1	99.4
87.	2	<1	40	95	125.	1	<1	<1	96
88.	15	<1	600	75	126.	1	<1	<1	98
89.	<1	<1	<1	99	127.	<1	<1	<1	96
90.	32	<1	<1	99	128.	31	4	<1	94
91.	<1	<1	<1	98	129.	2	<1	<1	97
92.	<1	<1	<1	98	130.	<1	<1	<1	92
93.	12	4	10	93	131.	<1	<1	<1	95
94.	3	<1	6	96	132.	2	4	4	84
95.	<1	<1	4	91	133.	<1	<1	900	85
96.	<1	<1	<1	98	134.	14	<1	8	95
97.	<1	<1	<1	97	135.	<1	<1	<1	95
98.	<1	<1	<1	100	136.	5	<1	<1	95
99.	<1	4	10	93	137.	<1	<1	<1	93
100.	1	<1	16	96	138.	<1	<1	<1	92
101.	<1	<1	8	96	139.	24	<1	4	96
102.	<1	<1	<1	98	140.	12	<1	<1	98
103.	<1	<1	<1	88	141.	7	<1	<1	96
104.	1	<1	<1	93	142.	<1	<1	<1	99
105.	2	<1	<1	89	143	400	80	4	92
106.	5	<1	4	88	144.	<1	<1	<1	98
107.	2	<1	<1	96	145.	1	<1	<1	99
108.	<1	<1	<1	98	146.	1	<1	<1	98

Table 18 (Continued)

Sample No.	Entero-coccus count/ml	Coli-form count/ml	Yeast and mold count/ml	Judging score	Sample No.	Entero-coccus count/ml	Coli-form count/ml	Yeast and mold count/ml	Judging score
147.	6	<1	<1	96	185.	1	<1	6	85
148.	<1	<1	<1	95	186.	62	16	<1	91
149.	46	<1	4	93	187.	9	6	<1	96
150.	2	<1	8	94	188.	16	2	<1	94
151.	17	<1	<1	98	189.	3	<1	<1	98
152.	<1	4	14	93	190.	3	78	60	87
153.	<1	<1	<1	99	191.	120	<1	160	85
154.	<1	<1	<1	99	192.	2	<1	<1	97
155.	<1	<1	40	94	193.	6	<1	24	92
156.	<1	<1	<1	97	194.	6	<1	<1	96
157.	2	<1	<1	98	195.	4	<1	54	85
158.	4	<1	10	87	196.	120	24	20	87
159.	<1	<1	<1	88	197.	6	<1	<1	96
160.	6	<1	4	84	198.	<1	<1	<1	99
161.	1	<1	<1	98	199.	8	<1	4	95
162.	54	6	100	91	200.	<1	<1	<1	97
163.	<1	<1	<1	99.2	201.	4	<1	<1	98
164.	7	<1	<1	97	202.	2	<1	<1	94
165.	10	<1	<1	92	203.	13	<1	<1	97
166.	1	6	8	95	204.	24	10	<1	94
167.	330	24	40	94	205.	500	<1	<1	80
168.	<1	<1	<1	88	206.	5	2	<1	96
169.	<1	170	20	90	207.	<1	<1	<1	85
170.	<1	30	8	94	208.	4	<1	<1	99
171.	1	<1	<1	96	209.	46	240	<1	82
172.	<1	<1	<1	98	210.	12	<1	16	90
173.	<1	<1	<1	96	211.	8	2	<1	96
174.	2	<1	<1	96	212.	<1	<1	14	85
175.	180	120	400	80	213.	<1	<1	<1	93
176.	2	<1	<1	97	214.	2	<1	<1	98
177.	<1	<1	<1	87	215.	210	<1	<1	97
178.	170	26	120	86	216.	<1	<1	<1	95
179.	2	<1	<1	90	217.	25	2	10	81
180.	2	<1	<1	99	218.	<1	<1	<1	97
181.	1	<1	<1	96	219.	<1	<1	4	94
182.	12	<1	<1	96	220.	<1	<1	<1	87
183.	6	<1	<1	96	221.	<1	<1	<1	97
184.	<1	<1	<1	96	222.	<1	<1	<1	88

Table 18 (Continued)

Sample No.	Entero-coccus count/ml	Coli-form count/ml	Yeast and mold count/ml	Judging score	Sample No.	Entero-coccus count/ml	Coli-form count/ml	Yeast and mold count/ml	Judging score
223.	<1	<1	<1	98	263.	<1	<1	<1	98
224.	1	<1	<1	94	264.	6	<1	<1	93
225.	<1	<1	<1	97	265.	1	<1	<1	97
226.	1	<1	4	92	266.	120	4	120	82
227.	6	<1	8	85	267.	880	<1	<1	92
228.	<1	<1	<1	93	268.	<1	<1	<1	81
229.	<1	<1	<1	97	269.	<1	<1	<1	88
230.	4	<1	<1	100	270.	3	<1	<1	88
231.	3	<1	<1	94	271.	<1	<1	10	93
232.	1	<1	<1	81	272.	1	<1	<1	99
233.	1	<1	<1	84	273.	1	<1	<1	98
234.	34	2	<1	95	274.	1	<1	<1	97
235.	<1	<1	<1	96	275.	2	<1	<1	96
236.	<1	<1	<1	99	276.	3	<1	<1	96
237.	3	2	<1	98	277.	<1	<1	18	98
238.	9	<1	<1	96	278.	<1	<1	<1	88
239.	160	140	12	89	279.	2	<1	<1	99
240.	15	2	<1	96	280.	2	<1	<1	96
241.	2	<1	<1	90	281.	<1	<1	<1	98
242.	92	18	<1	96	282.	2	<1	<1	89
243.	<1	<1	<1	96	283.	<1	<1	<1	100
244.	3	<1	<1	89	284.	<1	<1	<1	98
245.	6	<1	<1	83	285.	2	<1	<1	98
246.	<1	<1	<1	99	286.	<1	<1	<1	97
247.	<1	<1	<1	91	287.	<1	<1	<1	97
248.	6	2	<1	96	288.	<1	<1	<1	99
249.	3	<1	<1	97	289.	<1	<1	<1	88
250.	<1	<1	<1	97	290.	<1	<1	4	95
251.	<1	<1	<1	97	291.	<1	<1	<1	100
252.	<1	<1	<1	95	292.	3	<1	<1	99
253.	4	<1	4	96	293.	1	<1	<1	96
254.	<1	<1	<1	97	294.	2	<1	<1	90
255.	<1	<1	<1	96	295.	<1	<1	<1	90
256.	23	6	<1	94	296.	3	<1	<1	88
257.	<1	<1	<1	97	297.	660	<1	120	95
258.	100	48	<1	90	298.	66	<1	<1	89
259.	<1	<1	<1	97	299.	580	<1	<1	98
260.	2	<1	<1	99	300.	<1	<1	<1	94
261.	<1	<1	<1	96	301.	100	<1	400	80
262.	2	<1	<1	98	302.	1	<1	<1	97

Table 18 (Continued)

Sample No.	Entero-coccus count/ml	Coli-form count/ml	Yeast and mold count/ml	Judging score	Sample No.	Entero-coccus count/ml	Coli-form count/ml	Yeast and mold count/ml	Judging score
303.	55	<1	32	93	341.	14	<1	32	97
304.	<1	<1	<1	86	342.	<1	<1	<1	98
305.	<1	<1	<1	97	343.	730	<1	34	91
306.	<1	<1	<1	98	344.	46	<1	<1	95
307.	<1	<1	<1	96	345.	<1	<1	<1	98
308.	7	<1	<1	98	346.	1	<1	<1	89
309.	140	40	180	88	347.	1	<1	<1	97
310.	4	<1	20	86	348.	<1	<1	8	95
311.	<1	<1	<1	98	349.	<1	<1	<1	97
312.	1	<1	8	91	350.	11	<1	<1	98
313.	<1	<1	<1	94	351.	7	<1	<1	98
314.	2	<1	<1	83	352.	14	<1	<1	98
315.	2	<1	4	95	353.	<1	<1	<1	98
316.	1	<1	<1	97	354.	<1	<1	<1	98
317.	<1	<1	<1	97	355.	41	<1	<1	97
318.	10	<1	220	82	356.	<1	<1	7	-
319.	75	<1	<1	98	357.	1	<1	2	-
320.	2	<1	<1	94	358.	<1	<1	6	-
321.	1	<1	<1	96	359.	<1	<1	<1	-
322.	<1	<1	<1	98	360.	<1	<1	<1	-
323.	<1	<1	<1	96	361.	150	41	270	-
324.	100	<1	<1	94	362.	40	<1	300	-
325.	1	<1	8	94	363.	14	<1	170	-
326.	33	10	<1	92	364.	2	<1	120	-
327.	7	<1	<1	98	365.	1	<1	<1	-
328.	1	<1	<1	94	366.	<1	<1	<1	-
329.	89	<1	<1	95	367.	6	<1	<1	-
330	1	<1	<1	98	368.	70	1	240	-
331.	<1	<1	<1	89	369.	32	<1	<1	-
332.	1	<1	<1	100	370.	67	<1	<1	-
333.	110	4	<1	95	371.	30	6	23	-
334.	30	8	<1	86	372.	34	<1	2	-
335.	<1	<1	<1	97	373.	12	<1	4	-
336.	<1	<1	<1	90	374.	<1	<1	<1	-
337.	<1	<1	<1	98	375.	2	<1	<1	-
338.	<1	<1	<1	100					
339.	2	<1	<1	98	Average	25	5	18	
340.	<1	<1	<1	98					

Table 19. Frequency distribution of the microbial content of 375 samples of contest butter

Type of indicator organism present	Per cent of sample in range of Enterococcus count/ml 1-10	>10	Coliform count/ml 1-10	>10	Yeast and mold count/ml 1-20	>20	Total
1. Enterococcus, coliform, yeast and mold	0.8	5.6	2.9	3.5	2.1	4.3	6.4
2. Enterococcus and coliform	2.1	4.3	5.6	0.8	-	-	6.4
3. Enterococcus, yeast and mold	6.9	5.1	-	-	7.7	4.3	12.0
4. Coliform, yeast and mold	-	-	0.8	0.8	1.6	0	1.6
5. Enterococcus alone	28.8	6.4	-	-	-	-	35.2
6. Coliform alone	-	-	0	0	-	-	0
7. Yeast and mold alone	-	-	-	-	4.8	0.8	5.6
8. None	-	-	-	-	-	-	32.8 32.8
Total	38.7	21.3	9.3	5.1	16.3	9.3	32.8 100.0

Data showing the relationship of enterococcus and coliform counts to the keeping quality of butter are presented in Table 20. No relationship existed between the enterococcus and coliform count and the keeping quality of butter. Samples with high enterococcus and coliform counts had high keeping quality scores whereas samples with low counts also had low keeping quality scores. Enterococci showed both increase and decline in numbers in butter samples during the holding period, but the coliform organisms, with the exception of only a few cases, usually showed marked decline in numbers during the keeping quality test period.

Table 20. Relation of enterococcus and coliform counts to the keeping quality of butter

Sample No.	Initial		After 10 days at 21 C		Keeping quality score: 15 pts.
	Enterococcus	Coliform	Enterococcus	Coliform	
	count/ml				
1.	43	4	43	4	15
2.	63	<1	12	<1	15
3.	2	4	2	<1	15
4.	250	70	3,100	230	15
5.	38	<1	8	<1	15
6.	67	4	4	6	15
7.	31	6	5	<1	10
8.	8	4	7	<1	15
9.	290	22	79	3	15
10.	48	<1	9	<1	15
11.	52	<1	35	<1	15
12.	5	<1	<1	<1	13
13.	42	4	210	8	15
14.	<1	4	530	<1	15
15.	50	<1	450	36	14.8
16.	32	<1	3,000	<1	15
17.	<1	4	<1	<1	0
18.	75	34	57	130	15
19.	<1	320	<1	<1	15
20.	31	4	36	<1	15
21.	2	4	8	<1	15
22.	400	80	180	5	15
23.	46	<1	8	<1	15
24.	<1	4	<1	<1	15
25.	54	6	67	<1	15
26.	1	6	<1	<1	15
27.	330	24	970	<1	15
28.	<1	170	4	3,200	15
29.	<1	30	2	10	15
30.	180	120	150	48	15
31.	170	26	380	2,200	15
32.	62	16	59	6	15
33.	10	6	7	2	15
34.	4	78	1	61	13
35.	120	24	43	1	15
36.	24	10	15	4	15
37.	46	240	42	100	15
38.	160	140	58	120	15
39.	92	18	70	86	15
40.	24	6	130	4	13
41.	100	48	49	12	10
42.	120	4	410	5	15

Occurrence and Significance of Enterococci and Other Organisms in Cheddar Cheese

Total, enterococcus, and coliform counts were made on 72 samples of Cheddar cheese. Observations on the presence of gas in cheese were also made. The results are presented in Table 21. Averages were 21,000,000/g for the total count, 160,000/g for the enterococcus count, and 2,600/g for the coliform count. There was no relationship between the enterococcus and coliform counts and the presence of gas in cheese. Gassy cheese had low coliform counts, and both high as well as low enterococcus counts. Similarly, gas was absent in cheese with high enterococcus and coliform counts.

Samples of cheese kept at 3.3 C were examined for total, enterococcus, and coliform counts at monthly intervals over a 5-month period. The results are presented in Table 22. There was a progressive decline in the bacterial numbers of all groups, and a substantial decrease in the total count. In one case the total count was reduced to less than 1% of the initial count. The decline in the enterococcus counts was slow and less marked, being 60% on an average. The coliforms showed a more rapid decrease and none of these organisms was present in the cheese at the end of three months.

Table 21. Total, enterococcus and coliform counts and their relationship to the presence of gas in Cheddar cheese

Sample No.	*Total	Entero-coccus count/g	Coliform	Presence of gas	Sample No.	*Total	Entero-coccus count/g	Coliform	Presence of gas
1.	29,000,000	36,000	27,000	Slight	26.	17,000,000	4,100	<10	None
2.	7,800,000	560,000	2,700	Some	27.	14,000,000	88,000	20	Some
3.	87,000,000	2,200,000	30,000	Some	28.	6,500,000	41,000	20	None
4.	26,000,000	940,000	11,000	Slight	29.	11,000,000	51,000	<10	None
5.	32,000,000	2,600,000	100,000	Some	30.	13,000,000	46,000	<10	None
6.	130,000,000	6,000	10	None	31.	7,300,000	51,000	20	None
7.	11,000,000	78,000	10	None	32.	20,000,000	9,600	10	None
8.	8,800,000	24,000	780	Some	33.	2,300,000	790	<10	Some
9.	6,000,000	460	10	Some	34.	6,800,000	4,100	<10	None
10.	16,000,000	250,000	100	Some	35.	18,000,000	2,500	<10	Some
11.	12,000,000	30,000	50	Some	36.	16,000,000	1,600	<10	Slight
12.	10,000,000	410,000	4,600	None	37.	33,000,000	4,200	<10	Slight
13.	5,900,000	17,000	<10	Slight	38.	8,900,000	1,000	<10	None
14.	12,000,000	2,500	50	None	39.	-	50	<10	Some
15.	23,000,000	62,000	1,500	None	40.	-	1,800,000	450	Some
16.	21,000,000	69,000	110	None	41.	-	46,000	<10	Some
17.	14,000,000	470	<10	Slight	42.	-	38,000	<10	None
18.	12,000,000	200	10	Some	43.	-	100	<10	Some
19.	18,000,000	15,000	10	Slight	44.	-	47,000	60	Some
20.	11,000,000	10,000	<10	None	45.	-	700	<10	Some
21.	19,000,000	1,800	<10	None	46.	-	1,100	<10	None
22.	68,000,000	20,000	4,400	None	47.	-	300	30	Some
23.	7,500,000	60	<10	Some	48.	-	500,000	150	Some
24.	17,000,000	840	<10	Slight	49.	-	50,000	<10	Some
25.	14,000,000	1,200	<10	Some	50.	-	17,000	20	Some

*Plates incubated at 21 C for 5 days.

Table 21 (Continued)

Sample No.	*Total	Entero-coccus count/g	Coliform	Presence of gas	Sample No.	*Total	Entero-coccus count/g	Coliform	Presence of gas
51.	-	1,800	190	Some	63.	-	20	< 10	None
52.	-	7,000	< 10	Slight	64.	-	1,200	50	Some
53.	-	610,000	< 10	Some	65.	-	200,000	180	None
54.	-	1,800	< 10	None	66.	-	1,800	1,100	None
55.	-	240	< 10	None	67.	-	310,000	90	Much
56.	-	45,000	10	Some	68.	-	2,200	< 10	None
57.	-	1,600	< 10	Some	69.	-	930	< 10	None
58.	-	960	30	Some	70.	-	160	< 10	None
59.	-	710	< 10	Some	71.	-	170	< 10	None
60.	-	100	20	Much	72.	-	1,300	< 10	None
61.	-	2,900	< 10	None					
62.	-	2,300	< 10	None	Average	21,000,000	160,000	2,600	

*Plates incubated at 21 C for 5 days.

Table 22. Variations in total, enterococcus and coliform counts during ripening of Chedd

Sample No.	Initial			1 mo.			2 mo.	
	Total	Entero-coccus	Coli-form	Total	Entero-coccus	Coli-form	Total	Entero-C coccus f
								coun
1.	29,000,000	36,000	27,000	6,600,000	680,000	2,200	2,800,000	260,000
2.	7,800,000	560,000	2,700	5,700,000	540,000	1,200	2,800,000	220,000
3.	87,000,000	2,200,000	30,000	53,000,000	4,300,000	3,700	15,000,000	1,500,000
4.	26,000,000	940,000	11,000	16,000,000	430,000	2,400	2,100,000	140,000
5.	32,000,000	2,600,000	100,000	25,000,000	1,600,000	20,000	8,000,000	280,000<
6.	130,000,000	6,000	10	5,000,000	2,600	< 1	7,400,000	2,700<
Average	52,000,000	1,100,000	28,000	19,000,000	1,300,000	5,000	6,300,000	400,000

eddar cheese (3.3 C)

o-Coli- form	3 mo. Total	3 mo. Entero-Coli-coccus form	4 mo. Total	4 mo. Entero-Coli-coccus form	5 mo. Total	5 mo. Entero-Coli-coccus form
ount/g						
00 10	6,200,000	770,000<1	4,200,000	470,000<1	4,200,000	280,000<1
00 80	2,600,000	340,000<1	3,800,000	310,000<1	2,100,000	140,000<1
00 10	26,000,000	1,300,000<1	19,000,000	1,400,000<1	18,000,000	1,800,000<1
00 50	10,000,000	330,000<1	7,500,000	340,000<1	4,000,000	58,000<1
00<1	7,800,000	540,000<1	6,700,000	200,000<1	7,600,000	350,000<1
00<1	1,300,000	3,600<1	820,000	3,200<1	410,000	2,300<1
00 25	9,000,000	550,000<1	7,000,000	450,000<1	6,000,000	440,000<1

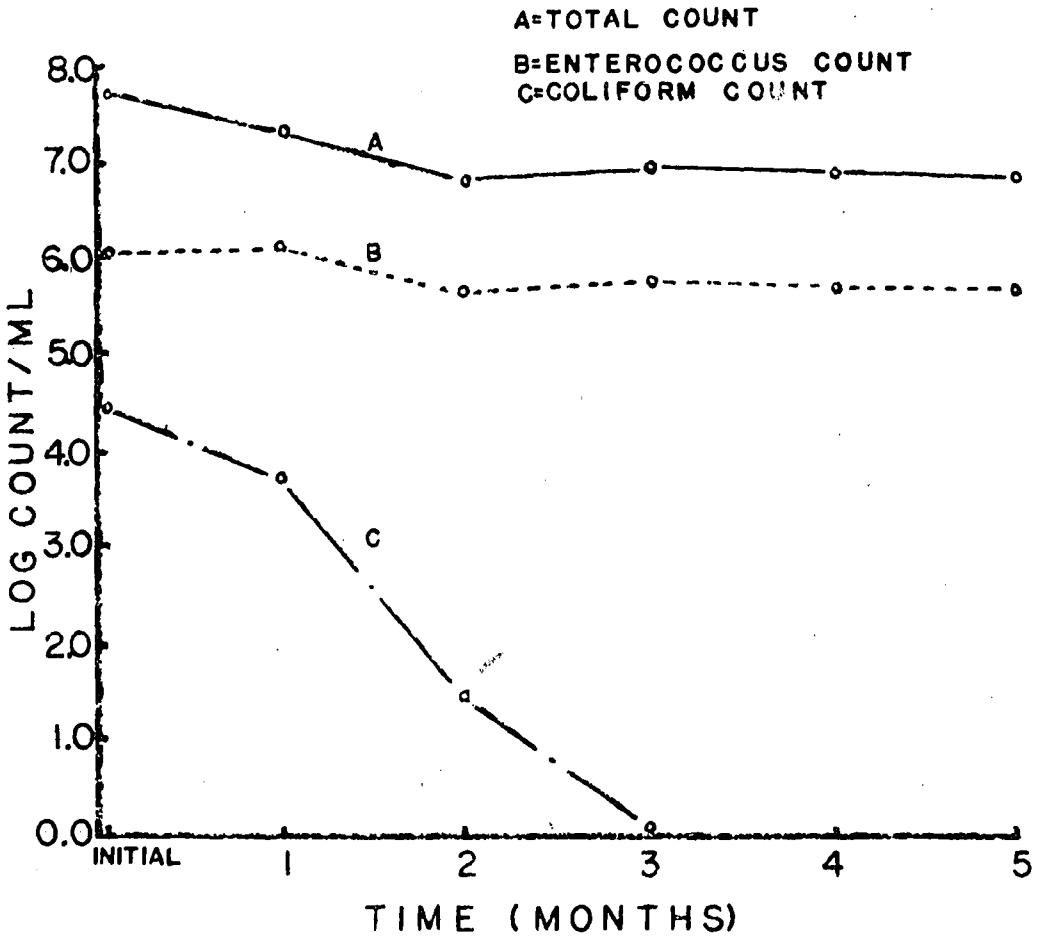

Figure 8. Variations in the total, enterococcus, and coliform counts during ripening of Cheddar cheese (3C)

DISCUSSION

Relation of Enterococcus and Coliform Counts to the Standard Plate Count of Milk

Grade A raw milk

The production and handling of milk is frequently difficult to control. As a result, wide variations in bacterial content occur. Gross contamination, use of inadequately cleaned utensils and improper cooling will substantially increase the total count of milk. On the other hand, rigid observance of general cleanliness reduces the total count. In some instances, however, milk produced under good, clean conditions, but improperly cooled, will have a high total count, although the number of some specific bacterial types considered indicative of poor sanitary quality may remain low. Badly contaminated milk, promptly cooled and held at a low temperature, may have a low total count but yet contain a significant number of organisms indicative of poor quality. Therefore, bacteriological test results must be interpreted with caution.

Total and coliform counts are generally made to test the quality of milk and milk products. The American Association of Medical Milk Commissioners, Inc. (3) recognizes a coliform count standard of not more than ten/ml for certified milk. Only the State of New Hampshire requires that, in raw milk to be pasteurized, the count of coliform organisms shall not exceed 100/ml (3). The Milk Ordinance and Code recommended by the U. S. Public Health Service (127) prescribes the coliform standard for both grade A and B pasteurized milk and milk products at not more than ten/ml.

According to this code, total plate counts shall not exceed 200,000 and 1,000,000/ml for grade A and B (manufacturing) grade raw milks. Recommendations for the enterococcus count of milk or milk products have not been given.

Wide variations in the total, enterococcus, and coliform counts of grade A raw milk have been observed in this investigation (Table 1). Enterococci constituted 0.20% and coliforms 0.13% of the average total count of a large number of samples. White and Sherman (172) reported that the enterococcus count formed 0.40% of the total count of raw milk studied by them. These authors considered this a low percentage. They also noticed wide variations in counts. Because of these observations, they thought it inadvisable to use the enterococcus count as a criterion of milk quality. This view is not substantiated by the present investigation.

In a limited number of raw milk samples, Higginbottom (60) found an average enterococcus count of 30/ml and a coliform count of less than 10/ml.

The present work shows that the average total bacterial count of the test samples of grade A raw milk was low, being only 100,000/ml. Only about 8% of the samples had total counts exceeding the limit of 200,000/ml prescribed for grade A raw milk (Table 2). Fewer samples in the low enterococcus category, than in the low coliform group, had high total counts. This indicates a closer relationship between total numbers and enterococci than between total numbers and coliform bacteria. The average coliform count of 130/ml was far in excess of the ten/ml recommended for certified

milk. This count was even greater than the 100/ml permitted by New Hampshire for raw milk for pasteurization. Only about 28% of the samples examined in this study met the requirements for total and coliform counts for certified milk. By raising the total count from 30,000 to 200,000/ml to include all grade A specifications, the percentage of acceptable samples rose only 8% from 28% to 36%. This indicates that most of the samples with ten coliforms or less/ml were found in the low total count group with counts of 30,000 or less/ml.

A similar pattern within narrower ranges was evidenced by the enterococci. The average enterococcus count at 200/ml for grade A raw milk was higher than the average coliform count. A relatively lower percentage of samples, 25%, with ten enterococci or less/ml were found in the 30,000 or less/ml total count group. A lower percentage, 5.5%, than in the case of coliforms, would be added to this group by including samples representing total counts between 30,000 to 200,000/ml.

The percentage of samples, 26%, with more than 100 organisms/ml was higher for enterococci than the 19% for the coliforms. The average total count of samples with enterococcus counts of ten or less/ml was lower than the average total count for samples with ten coliforms or less/ml (Table 3). This is significant; this fact also affected materially the ratios between total counts of samples with enterococcus and coliform counts within the ranges of ten or less/ml, more than ten to 100/ml, and more than 100/ml. The ratios of total counts within these three categories of samples were 1.0, 3.1, and 5.0 for enterococci and 1.0, 1.7, and 2.3 for the coliforms, respectively. There was a considerable difference between the F values

of the enterococcus and the coliform counts, as obtained from the analysis of variance of the total, enterococcus, and the coliform counts (Table 4). The findings suggest that the relationship between the enterococcus and total counts was highly significant. A significant relationship did not exist between the coliform and total counts of grade A raw milk.

Sherman and Wing (152) considered a coliform count of less than ten/cc for certified milk not unduly stringent. Similarly, a coliform standard of less than 100/cc was reasonable for high grade raw milk in the opinion of these authors. Fay (44) observed the standard of ten coliforms/ml for certified milk as rigid and impractical for market grades of milk produced under less exacting conditions. Finkelstein (46) reported that coliforms were present in raw milk to the extent of less than 100/cc where care was used, and averaged 588/cc where indifferent methods were used in production. Johns (70) found that milk produced under unclean conditions had a high total count, but a low coliform count. He suggested that the coliform test does not reflect udder cleanliness. Thom (166) traced coliform contamination to milking equipment on 17 occasions, to the farm tank on eight occasions, and to the cows only on four occasions, suggesting that the chances of coliform contamination were greater from the milking equipment than the bulk tank or the cow. He also noted rapid multiplication at 4 C when gram-negative rods predominated the milk flora.

In view of the findings of the present investigation, the enterococcus count seems to be a more sensitive test than the coliform test to evaluate the sanitary quality of grade A raw milk. Other workers have not reported on enterococci. However, their findings, as mentioned

previously, do suggest that the coliform test is not a suitable index for determination of the quality of raw milk. In the light of the observations of Sherman and Wing (152) and of Fay (44) concerning the coliform standards of certified and grade A raw milk, certain generalizations can be made on the use of the enterococcus count. Since about one-fourth of the samples examined in the present study had an enterococcus count of ten or less/ml and a corresponding total count of 30,000 or less/ml, it is proposed that a quality standard based on the enterococcus count should require a count of ten or less for certified raw milk. About 71% of the samples examined had total counts of 200,000 or less/ml and an enterococcus count of 100 or less/ml. It is, therefore, proposed that grade A raw milk should not have an enterococcus count exceeding 100/ml.

Manufacturing grade raw milk

Almost two-thirds of the manufacturing grade samples examined in this study had total counts of 1,000,000 or less/ml (Table 8). About one-third had enterococcus counts of 100 or less/ml and the remaining two-thirds of the samples had more than 100/ml. The coliform counts of 45% of the samples were 100 or less/ml and the remaining samples contained more than 100/ml. The averages were: total count, 2,000,000/ml; enterococcus count, 5,600/ml; and the coliform count, 6,400/ml (Table 7). The average total count of samples with 100 or less coliforms/ml was lower than the average total count of samples with 100 or less enterococci/ml (Table 9). The ratio of average total counts of samples with less than 100 indicator organisms/ml was lower for enterococci than for the coliform organisms. This suggests that in manufacturing grade milk, the coliforms represented

the quality better than the enterococci. The F value for the enterococcus count was considerably lower than that for the coliform count (Table 10). This suggests that the relationship between the coliform count and the total count was highly significant. A significant relationship between the enterococcus and total count, unlike the case of grade A raw milk, did not exist in manufacturing grade raw milk.

The present study, therefore, reveals that the coliform count is a better criterion of quality for manufacturing grade raw milk than the enterococcus count. In view of the limited number of samples examined in this study, and the considerable variations noticed in individual counts, it does not appear reasonable to propose any limits for either of these indicator organisms in manufacturing grade raw milk.

The grade A raw milk samples with an average total count of 120,000/ml, an enterococcus count of 170/ml and a coliform count of 70/ml, when held at 7 C for 7 days, showed increases of 250,000, 4,500, and 3,400,000%, respectively (Table 5). The fact that coliform organisms grow at a much faster rate at lower temperatures than enterococci is considered important. Because of modern technology, some milk may be held on the producing farm for up to three days before delivery to the plant. In view of these considerations, the coliform test cannot be relied upon as a suitable test for milk quality.

Samples of raw milk, with average counts of 110 enterococci/ml and 61 coliforms/ml, when laboratory pasteurized failed to show growth on selective media (Table 6). Coliforms generally do not survive proper pasteurization treatment. Survival of a heat treatment of 65 C for 30

min by S. faecalis was reported by Sherman et al. (149). Iyengar et al. (68) reported that both S. faecalis and its variety liquefaciens survived a heat treatment of 63 C for 30 min when the counts were more than 50,000/ml in skim milk. The maximum enterococcus count in the samples used in this study was 630/ml. Growth was not shown by either enterococci or the coliform bacteria in laboratory-pasteurized samples of milk held for 7 days at 7 C. It may be presumed that either the organisms did not survive the heat treatment because of their low numbers or that the selective medium failed to permit growth of the heat-treated cells.

Relation of Coliform and Yeast and Mold Counts to the Enterococcus Count of Butter

Experimental butter

Coliform and yeast and mold counts have long been employed as routine tests to determine the adequacy of pasteurization and sanitary conditions in the butter industry. The presence of these organisms in finished butter is considered to be due to inefficient pasteurization of cream or carelessness in the handling of the product. The coliform or the yeast and mold count run on butter does not accurately measure the quality of raw materials used or the keeping quality of butter.

Many uncontrolled factors affect the coliform count of commercial butter samples. As a result, many workers doubt the applicability of this test for quality control purposes. The strain of organism, the moisture and salt content, the degree of working, the storage temperature and time, all affect the coliform count of finished butter to a large extent.

The experimental butter that was prepared for the study of the effect of salting, working, storage temperature and time on enterococci and coliforms, had a fairly uniform composition (Table 13).

This study shows that the enterococcus strains gave a more gradual and lesser decline in butter during storage than the coliforms (Tables 11, 12, and 14; Figures 1 to 6). Salt had an effect in reducing the enterococcus count of butter to some extent. In poorly worked butter, S. durans even showed some growth, which was more marked during the storage period of butter at 10 C than at 3 C. The uneven distribution of moisture in poorly worked butter might cause accumulation of free moisture. This would permit the subsequent growth of organisms which the transfer of butter from 3 C to 10 C might stimulate. No storage growth was observed in the case of S. faecalis inoculated in experimental butter. S. durans seems to withstand the micro-environment of butter better than S. faecalis. As a result, it was able to show some growth in the poorly worked butter.

In unsalted butter the coliform organisms also persisted during the storage period. The E. coli strains, both salt sensitive as well as salt resistant, showed a gradual decline in numbers during the storage period. In the case of the A. aerogenes strains, there was appreciable growth from the first week in the salt-sensitive strain, and from the fourth week in the salt-resistant strain when the storage temperature was raised to 10 C. Salting killed most of the E. coli, although a small number of the salt-resistant strain persisted for a long period. Salting also greatly reduced the numbers of A. aerogenes strains. Only a few of the salt-sensitive organisms were present in butter in the first week of

storage, but they did grow later on to quite an extent. This was a fast growing strain as was shown by its growth in large numbers in unsalted butter during the corresponding storage period. The salt-resistant A. aerogenes survived salting in larger numbers than the salt-sensitive strain initially, but, in properly worked butter, eventually decreased on storage. However, in poorly worked butter, there was an appreciable increase in numbers, particularly at 10 C.

Although few coliform organisms can survive the salt treatment, certain strains are, nevertheless, capable of multiplying during storage if the butter has not been worked properly.

The recovery of both enterococcus and coliform organisms in butter from inoculated cream was relatively comparable in the case of unsalted butter (Table 15). In salted butter, however, the enterococci were recovered in lower numbers than from the unsalted butter, while the coliforms were nearly eliminated. Less than 0.2% coliforms inoculated in cream were recovered from the salted butter.

About one-fifth to two-thirds of the numbers of enterococci present in the butter initially, survived the frozen storage. Relatively few coliforms were able to survive 7-weeks storage at -20 C (Table 16). Less than 1% of the initial inoculum of both strains of A. aerogenes and the salt-sensitive strain of E. coli survived this storage period. The salt-resistant strain of E. coli survived up to 6.5% in unsalted and to 3.7% in salted butter.

There is little work reported on experimental butter made with coliform-inoculated cream. Information on enterococci is completely lacking.

Hammer and Yale (53) reported that, in butter made from inoculated cream, Escherichia species did not grow in 10 days at 7 C in salted butter, although, in unsalted butter, some could grow. Aerobacter species sometimes grew in salted butter and regularly grew in unsalted butter. They further reported that the Aerobacter species grew more rapidly and reached higher numbers than the Escherichia species. They also noted that only 2.0 to 2.9% of the numbers of the Escherichia-Aerobacter group initially present per ml of cream were retained per ml of fresh unsalted butter. Relatively higher percentages of coliform organisms than reported by Hammer and Yale (53), were retained in butter in the present study. Although these authors did not discuss the salt resistance or mention the species or organisms used in their work, the findings are in general agreement with those of the present work. Singh and Nelson (155) introduced three strains of E. coli and two strains of A. aerogenes into cream before churning. They reported that the strain of organism, the amount of salt, and the temperature of storage affected the coliform population of butter.

The various strains of coliform bacteria are affected, differently, by salting, working, and exposure to storage temperature and time. The fate of these organisms after contaminating a butter supply is highly unpredictable; depending on the effect of the factors mentioned above, a large portion of these organisms may be completely eliminated. On the other hand, a few organisms could grow to appreciable numbers. Therefore, the coliform count cannot be used as a satisfactory test for control of the sanitary quality of butter. This study has demonstrated that enterococci do not grow in properly worked butter, and do not decline in numbers

appreciably in salted butter during the ordinary course of storage. These organisms survive the frozen storage in far greater percentage, while the coliform organisms are almost eliminated. It is, therefore, suggested that the enterococcus count should be substituted for the coliform count as a more reliable test for measurement of the sanitary quality of commercial butter supplies.

Line-run samples of butter

The study on experimental butter has shown that the coliform test cannot be relied upon to measure the sanitary quality of either fresh or stored butter. It has been common practice for many years, however, to use the coliform test on line-run samples to detect sources of contamination.

Using the enterococci, coliform and yeasts and molds as indicator organisms, experiments were carried out to evaluate the sensitivity of these test organisms for detection of the sources of contamination in butter. This study indicated that raw cream had high counts of the three types of organisms (Table 17). They were almost completely destroyed during pasteurization of the cream, however (Figure 7). The largest contamination with both enterococci and coliforms occurred after the pasteurized cream was transferred to the holding vat. Wooden churns, particularly, contributed to the contamination. Yeasts and molds were introduced into the cream from the holding vat and from the churn in appreciable numbers.

A large proportion of these organisms was eliminated in the buttermilk, leaving the butter granules lower in count. In one of the plants using an old wooden churn, however, the number of both enterococci and

coliforms in the unwashed butter granules was larger than in the cream two min after the start of churning, even though a larger proportion of these organisms had already been eliminated in the buttermilk. This count increase may have been due to a continuous addition of these organisms from the churn during churning. Wooden churns are hard to clean and sanitize and provide ample chances for contamination.

Washing usually reduced the numbers of organisms, but in a few cases all three types actually increased after washing. A good supply of wash water is, therefore, essential for high quality butter. In one instance, because of the salting operation, or perhaps due to the water added to adjust the moisture level of the butter, the enterococcus count increased. Holding the salted, finished butter at 7 C for 7 days in one instance increased the enterococcus count more than ten-fold. This occurred in the sample involving the wooden churn previously mentioned. This increase could have been due to the development of a resistant flora in the churn, since the coliform bacteria in this butter also survived the salt treatment and later increased in number on holding at 7 C for 7 days. Otherwise, both salting and holding of butter at 7 C for 7 days partially reduced the enterococcus count and completely eliminated the coliforms in most cases. Frequently, more yeasts and molds than were formerly present in the unsalted butter were found in the salted butter. This suggests their introduction into butter either during or following the salting operation. Some reduction in the yeast and mold count occurred during storage of salted butter at 7 C for 7 days. In two cases, however, the counts showed an increase, indicating growth of these organisms in salted

butter.

Little work has been reported on the coliform determination of line-run samples in butter and information on enterococci is completely lacking. Crossly (32) reported the incidence of coliform bacteria in pasteurized cream, storage vats, churns before starting, washed butter granules, and salted butter at 5.0, 68.2, 73.3, 83.3, and 61.0% respectively. These results were based on the qualitative estimates of coliforms in line-run samples and did not include the numbers infecting the product at each stage as was done in the present study. The results of this study are in general agreement with those of Crossly, even though his data showed a relatively higher incidence of coliform bacteria in salted butter than in the present work. White and Smith (171) found that 5% of the washed and 10% of the non-washed butter that they studied, had high initial coliform counts exceeding ten/ml. This suggests a loss of organisms in the washing of butter. Corley and Hammer (30) reported that commercial butter, from plants using water commonly containing coliform organisms, regularly contained these organisms when not salted and sometimes contained them when salted. Singh and Nelson (155) reported that coliform counts detected contamination early in the processing operation more accurately than did yeast and mold or total plate counts. This study is again in general agreement with those of these earlier workers, although their reports did not cover all stages of the manufacturing process.

This work shows that coliforms were present in large numbers in the initial stages of butter making, but were considerably reduced on salting and storage. Yeast and mold counts were variable. On the other hand,

the enterococcus population showed a more consistent trend throughout the entire manufacturing process, even surviving the salting and storage periods. It is suggested that determination of their numbers would form a more reliable evaluation of the sanitary quality of butter than the coliform or yeast and mold counts. More than half of the churnings in this study had enterococcus counts of ten or less/ml in the finished salted butter. Most of the low count samples came from plants using metal churns and good, sanitary practices. In one plant using exceptionally clean practices, none of the three types of organisms could be detected at any stage of operation in two different churnings from pasteurized cream to salted butter. Although the number of churnings used in this study was small, they were representative of different plant conditions and practices. It is indicated that butter made with good, sanitary practices should not contain more than ten enterococci/ml.

Contest butter samples

This study involving fairly large numbers of contest butter samples representing many plants, shows that 60.0% of the samples contained enterococci while the coliform and the yeasts and molds were present in only 14.4 and 25.6% of the samples (Tables 18, 19). Enterococci were the sole indicator organism in 35.2% of the samples while yeasts and molds were found in 5.6% and coliforms in none of the samples. About one-third of the samples had no indicator organisms.

This study also revealed little relationship between the initial quality or the keeping quality of butter and the enterococcus, coliform or yeast and mold counts (Table 18, 20). Of the 57 samples with scores of less than 90, high counts (more than ten enterococci or coliforms or

more than 20 yeasts and molds/ml) were observed in 40% of the samples. Of 266 samples having scores of 93 or more, high counts were present in 17% of the samples. A possible relationship, on a numerical basis, may exist between poor quality of butter and high indicator organism count. These results should be interpreted with caution since samples of butter containing unusually high counts had both poor as well as excellent judging scores.

Similar results were previously obtained by Parfitt (123) who found no relationship between the yeast and mold count of butter and the presence of coliform organisms or between the keeping quality and the presence or absence of the coliform group. After examining a large number of samples from 49 Australian plants, Roughley and McLeod (137) reported that 76.9% had ten coliform bacteria or less/ml and 64.5% of the butter samples had 20 yeast and molds or less/ml. The results of the present study are in general agreement with the findings of these workers.

Because of the large percentage of samples containing enterococci with or without coliforms or yeasts and molds, and in view of the results from experimental butter and line-run samples, it may be concluded that the enterococcus count is a better sanitary quality test for butter than the coliform or yeast and mold counts. Since a much larger number of samples had ten enterococci or less/ml, it may be concluded that butter made under good sanitary conditions should not have more than ten enterococci/ml. Also, a vast majority of the samples had 20 yeasts and molds or less/ml. The present standard (3) of not more than 20 yeasts and molds for good quality butter appears to be reasonable.

Occurrence and Significance of Enterococci and Other Organisms in Cheddar Cheese

Enterococci are adaptable to the conditions that exist in ripening Cheddar cheese. These organisms enter the milk as natural contaminants, multiply during the cheese making process, and persist as one of the dominant bacterial groups throughout the ripening process. They have been associated with the development of flavor in cheese and have even been regarded as being involved in the production of gassy cheese.

This study shows that enterococci were present in appreciable numbers whereas coliform bacteria were present in small numbers (Table 21). Although the samples were collected from plants reported to have gas trouble, there appeared to be no relationship between the enterococcus and the coliform counts and the presence of gas in the cheese. Only two samples out of the 72 examined, which had an appreciable amount of gas, had relatively lower enterococcus and coliform counts than others which had little or no gas, but had higher enterococcus counts with or without high coliform counts.

In the small number of samples taken and examined during the ripening period of cheese, a substantial decline occurred in the total count. The total count was reduced to less than 1% of the initial count in one case (Table 22). The decline in the enterococcus count was less and slow, 40% of the initial flora remaining at the end of 5 months. The coliforms showed a more rapid decrease after the first month; none of them were present in cheese at the end of 3 months (Figure 8).

Yale (178) observed that the rate of the disappearance of coliforms in cheese was quite variable. The coliform count of cheese a few days or a few weeks old was not an accurate index of the initial coliform content. Rasic (133) reported active multiplication of coliform bacteria during the manufacture of white cheese and rapid decline in their numbers during ripening. They practically disappeared after one month.

The data presented in this study are in general agreement with the findings of other workers. However, little work is reported on the presence of enterococci in cheese. Comparative studies such as those reported in the present study, are lacking in the literature. This study may serve to provide a small portion of the much needed information on the presence, significance, and survival of this important group of bacteria in Cheddar cheese. Indeed, this simple survey raises more questions than answers.

SUMMARY AND CONCLUSIONS

This study was undertaken to assess the sanitary significance of enterococci in raw grade A and manufacturing grade milk, butter, and other dairy products.

A total of 330 samples of grade A raw and 120 samples of manufacturing grade raw milk were examined using Standard Plate, enterococcus, and coliform counts. Enterococcus, coliform, and yeast and mold counts were run on 360 samples of experimentally made butter, 200 line-run samples collected at ten points from 20 churnings at eight Iowa creameries and on 375 exhibit butter samples collected at several Iowa contests. Seventy-two samples of Cheddar cheese, including six taken during a 5-month ripening period at monthly intervals, were examined for total, enterococcus and coliform counts.

The Citrate Azide agar of Reinbold et al. (134), modified by increasing the sodium azide concentration to 0.4 g/liter, was used as described by Saraswat et al. (139).

Wide variations between the Standard Plate, enterococcus and coliform counts of individual samples of raw milk were observed. The averages for the grade A samples were: Standard Plate Count, 100,000/ml; enterococcus count, 200/ml; and coliform count, 130/ml. The percentages of the average enterococcus and coliform counts of the average total count were 0.20 and 0.13. Only 8% of the samples had total counts of more than 200,000/ml, nearly 70% had more than ten enterococci/ml and 62% samples had more than ten coliform organisms/ml. The F values obtained from the analysis of

variance of the three bacterial counts, were 10.28 for the enterococcus and 1.79 for the coliform count, suggesting that the relationship between the enterococcus and the total count was highly significant. The enterococcus count was considered a more sensitive test than the coliform count when used to evaluate the sanitary quality of high grade raw milk. A quality standard of ten or less enterococci/ml is suggested for certified raw milk and an enterococcus count not to exceed 100/ml for grade A raw milk.

The averages for manufacturing grade raw milk were: Standard Plate Count, 2,000,000/ml; enterococcus count, 5,600/ml; and coliform count, 6,400/ml. The percentages of the average enterococcus and coliform counts of the average total count were 0.28 and 0.32. About two-thirds of the samples had total counts of 1,000,000 or less/ml, about one-third had enterococcus counts of 100 or less/ml and 45% of the samples had coliform counts of 100 or less/ml. The F values for the enterococcus and coliform counts were 2.29 and 33.09, indicating a highly significant relationship between the coliform count and the total count. Contrary to the findings for grade A raw milk, the coliform count is a better criterion for evaluating the sanitary quality of manufacturing grade raw milk than is the enterococcus count.

Samples of raw milk with average counts of 110 enterococci/ml and 61 coliforms/ml were laboratory pasteurized; the enterococcus and coliform organisms did not survive. There was no growth on selective media when pasteurized samples held at 7 C for 7 days were again plated. It is believed that enterococci do not usually survive pasteurization if present

in small numbers in raw milk.

Average increases of 250,000% in the Standard Plate Count, 4,500% in the enterococcus count and 3,400,000% in the coliform count were shown in grade A raw milk held at 7 C for 7 days. Because of the high rate of growth of coliforms at low temperatures, it is suggested that determination of their numbers is of little value in raw milk.

In experimental butter made from inoculated cream, the enterococci showed a more gradual decline in numbers during storage than did the coliforms. Growth was not observed in the case of S. faecalis, while S. durans showed some growth in poorly worked unsalted butter during the storage period. Salt had some effect in reducing the enterococcus count of butter. In unsalted butter, the E. coli strains showed a gradual decline in numbers, while the A. aerogenes strains grew appreciably during storage. Salting eliminated most of the E. coli, although a small number of salt-resistant strains persisted for a longer period. Salting also greatly reduced the numbers of A. aerogenes, but they were capable of growing during the storage period, particularly in poorly worked salted butter when the storage temperature was increased from 3 C to 10 C.

From 3.5 to 11.0% of the enterococci inoculated in cream were recovered in butter; from 4.0 to more than 18.0% of the coliforms were recovered in unsalted butter and less than 0.2% in salted butter.

About two-fifths to two-thirds of the enterococci survived frozen storage at -20 C for 7 weeks in butter. Less than 1% of the A. aerogenes and salt-sensitive E. coli survived frozen storage; the salt-resistant E. coli survived up to 6.5% in unsalted and 3.7% in salted butter,

indicating better durability of this strain among the coliforms for frozen storage.

Almost all of the enterococci, coliforms, and yeasts and molds were eliminated during pasteurization in commercial buttermaking. Most contamination in the line came from the holding vats and churns, particularly the wooden ones. Washing reduced the microbial numbers in most cases, but the largest portion of the microbial flora in butter was eliminated in the buttermilk. Salting and holding of butter at 7 C for 7 days had some effect on the enterococcus count, but produced almost complete destruction of the coliforms. There was also some reduction in the yeast and mold count. They occasionally showed some subsequent growth.

The average microbial counts in contest butter samples were: enterococci, 25/ml; coliforms, 5/ml; and yeasts and molds 18/ml. About 17% of samples with quality scores of 93 or above, had high microbial counts, while 40% of samples with scores of below 90 had high microbial counts. There appears to be at least some relationship between the high enterococcus, coliform, and yeast and mold counts and poor quality butter.

Enterococci were found in 60%, coliforms in 14.4%, and yeasts and molds in 25.6% of the samples examined. There was found to be no relationship between the microbial counts of the three types of organisms and the keeping quality of butter.

Since the enterococci are affected much less by the strain differences, salting, working, and storage temperature and time, persist more durably in the manufacturing line and are found more frequently in commercial butter than coliform organisms, it is suggested that the

enterococcus count is a more meaningful test than the coliform count as a criterion of the sanitary quality of butter. A standard of ten enterococci or less/ml is suggested for good quality butter.

The average initial bacterial counts in young Cheddar cheese were: total count, 21,000,000/g; enterococcus count, 160,000/g; and coliform count, 2,600/g. No relationship was observed between the enterococcus and coliform counts and the presence of gas in cheese. The total counts showed a substantial decrease during the ripening period. The decline in the enterococcus count was slow and less marked. The coliform bacteria showed a more rapid decrease; none of them were present in the cheese at the end of three months.

LITERATURE CITED

1. Abd-El-Malek, Y. and Gibson, T. Studies in the bacteriology of milk. I. The streptococci of milk. J. Dairy Research, 15: 233. 1948.

2. Allen, C. H. and Fabian, F. W. Comparison of Escherichia coli and Streptococcus faecalis as a test organism to determine the sanitary quality of food. Part II. J. Milk and Food Technol., 17: 237. 1954.

3. American Public Health Association. Standard methods for the examination of dairy products. 11th ed. New York, N. Y. Am. Publ. Health Assn. 1960.

4. Anderson, I. Emmental cheese from pasteurized milk. Sve. Mejer., 43: 253. 1951. Original not available for examination; abstracted in Dairy Sci. Abstr., 13: 336. 1953.

5. Anderson, L. and Storgards, T. Coliform organisms in milk. 15th Intern. Dairy Congr., 3: 1341. 1959.

6. Andrews, F. W. and Horder, T. J. A study of the streptococci pathogenic for man. Lancet, 2: 708. 1906.

7. Auld, W. C. and Parker, E. An outbreak of acute mastitis due to Streptococcus faecalis. Vet. Record, 59: 281. 1947.

8. Barnes, E. M. Differential and selective media for the faecal streptococci. J. Sci. Food Agric., 12: 656. 1959.

9. _____ Methods for the isolation of faecal streptococci (Lancefield Group D) from bacon factories. J. Appl. Bacteriol., 19: 193. 1956.

10. _____ Tetrazolium reduction as a means of differentiating Streptococcus faecalis from Streptococcus faecium. J. Gen. Microbiol., 14: 57. 1956.

11. _____ and Ingram, M. The identity and origin of faecal streptococci in canned hams. Ann. Inst. Pasteur Lille, 7: 115. 1955.

12. _____, _____, and Ingram, G. C. The distribution and significance of different species of faecal streptococci in bacon factories. J. Appl. Bacteriol., 19: 204. 1956.

13. Bartley, C. H. and Slanetz, L. W. Types and sanitary significance of fecal streptococci isolated from feces, sewage, and water. Am. J. Publ. Health, 50: 1545. 1960.

14. Bartram, M. T. and Black, L. A. The detection and significance of Escherichia-Aerobacter in milk. III. Correlation of total count and presence of the coli-aerogenes group. J. Dairy Sci., 20: 105. 1937.

15. Bellamy, W. D. and Gunsalus, I. C. Tyrosine decarboxylation of streptococci: growth requirements for active cell production. J. Bacteriol., 48: 191. 1944.

16. Breed, R. S., Murray, E. G. D., and Smith, N. R. Bergey's manual of determinative bacteriology. 7th ed. Baltimore, Md. The Williams and Wilkins Co. 1957.

17. _____ and Norton, J. F. Nomenclature for the colon group. Am. J. Publ. Health, 27: 560. 1937.

18. Buchbinder, L., Osler, A. G., and Steffen, G. I. Studies in enterococcal food poisoning. I. The isolation of enterococci from foods implicated in several outbreaks of food poisoning. Public Health Repts. (U. S.) 63: 109. 1948.

19. Burman, N. P. Some observations on coli-aerogenes bacteria and streptococci. J. Appl. Bacteriol., 24: 368. 1961.

20. Burton, M. O. Comparison of coliform and enterococcus organisms as indices of pollution in frozen foods. Food Research, 14: 434. 1949.

21. Buttiaux, R. Les streptocoques fécaux dans les eaux d'alimentation. Recherche. Espèces rencontrées. Signification. Ann. Inst. Pasteur, 95: 142. 1958.

22. _____ Les streptocoques fécaux des intestins humains et animaux. Ann. Inst. Pasteur, 94: 778. 1958.

23. _____ and Mossel, D. A. A. The significance of various organisms of fecal origin in foods and drinking water. J. Appl. Bacteriol., 24: 353. 1961.

24. Cataldi, M. S. and Montagna, C. P. The intestinal microflora in infants fed on human milk. Rev. Assoc. Argent. Dietol., 2: 36. 1944. Original not available for examination; abstracted in Dairy Sci. Abstr., 8: 23. 1946.

25. Chesbro, W. R. and Evans, J. B. Factors affecting the growth of enterococci in highly alkaline media. J. Bacteriol., 78: 858. 1959.

26. Childs, E. and Allen, L. A. Improved methods for determining the Most Probable Number of Bacterium coli and Streptococcus faecalis. J. Hyg., 51: 468. 1953.

27. Colobert, L. and Blondeau, H. L'espece Streptococcus faecalis. I. E'tude de l'homogeneite par la methode Adansonnienne. Ann. Inst. Pasteur, 103: 345. 1962.

28. _____ and _____ L'espece Streptococcus faecalis. III. La repartition differente des divers biotypes dams l'intestin de l'homme et du porc permet-elle de reveler l'origine humaine d'une contamination des semi-conserves de viande porc? Ann. Inst. Pasteur, 103: 707. 1962.

29. Cooper, K. E. and Ramadan, F. M. Studies in the differentiation between human and animal pollution by means of fecal streptococci. J. Gen. Microbiol. 12: 180. 1955.

30. Corley, R. T. and Hammer, B. W. Bacteriological studies on creamery water supplies. J. Dairy Sci., 25: 723. 1942.

31. Cowan, S. T. A taxonomist's view of the coli-aerogenes bacteria. J. Appl. Bacteriol., 19: 279. 1956.

32. Crossley, E. L. The coliform flora of milk and dairy products. J. Dairy Research, 14: 233. 1946.

33. _____ and Johnson, W. A. Bacteriological aspects of the manufacture of spray-dried milk and whey powders, including some observations concerning moisture content and solubility. J. Dairy Research, 13: 5. 1942.

34. Czulak, J. and Hammond, L. A. The use of Streptococcus durans as a as a cheese starter. 14th Intern. Dairy Congr., 2: 158. 1956.

35. Dack, G. M. Significance of enteric bacilli in foods. Am. J. Publ. Health, 45: 1151. 1955.

36. _____, Niven, C. F., Jr., Kirsner, J. B., and Marshall, H. Feeding tests on human volunteers with enterococci and tyramine. J. Infect. Dis., 85: 313. 1949.

37. Dahlberg, A. C. and Kosikowsky, F. V. The development of flavor in American Cheddar cheese made from pasteurized milk with Streptococcus faecalis starter. J. Dairy Sci., 31: 275. 1948.

38. Dangler, G. and Steffen, G. I. Studies on enterococcal food poisoning: A study of the incidence of enterococci and staphylococci in suspected food in outbreaks of food poisoning. J. Milk and Food Technol., 11: 242. 1948.

39. Defayolle, M. and Colobert, L. L'espece Streptococcus faecalis. II. E'tude de l'homogeneite par l'analyse factorielle. Ann. Inst. Pasteur, 103: 505. 1962.

40. Dible, H. The enterococcus and the fecal streptococci: their properties and relations. J. Pathol. and Bacteriol., 24: 3. 1921.

41. Eaves, G. N. and Mundt, J. O. Distribution and characterization of streptococci from insects. J. Insect Pathol., 2: 289. 1960.

42. Elliott, S. D. Teichoic acid and the group antigen of group D streptococci. Nature, 193: 1105. 1962.

43. Evans, A. C. and Chinn, A. L. The enterococci: with special reference to their association with human disease. J. Bacteriol., 54: 495. 1947.

44. Fay, A. C. Our industry today: Reappraisal of the quality control of milk supplies from farm bulk tanks. J. Dairy Sci., 43: 116. 1960.

45. Feagan, J. Isolation of a malty-flavor producing strain of Streptococcus faecalis. Australian J. Dairy Technol., 13: 79. 1958.

46. Finkelstein, R. Occurrence of the colon-aerogenes group of organisms in raw and in pasteurized milk and its significance. J. Dairy Sci., 2: 460. 1919.

47. Fleming, A. On the specific anti-bacterial properties of penicillin and potassium tellurite. J. Path. Bacteriol., 35: 831. 1932.

48. Geldreich, E. F., Bordener, R. H., Huff, C. B., Clark, H. F., and Kabler, P. W. Type distribution of coliform bacteria in the feces of warm-blooded animals. J. Water Pollut. Contr. Fed., 34: 295. 1962.

49. Geldreich, E. F., Huff, C. B., Bordener, R. H., Kabler, P. W., and Clark, H. F. The fecal coli-aerogenes flora of soils from various geographical areas. J. Appl. Bactériol., 25: 87. 1962.

50. Gopalkrishna, B. N. and Laxminarayana, H. Studies on the coliform bacteria in milk. I. Source, incidence and distribution. Indian J. Dairy Sci., 2: 135. 1949.

51. Gunsalus, I. C., Niven, C. F., Jr., and Sherman, J. M. The identification of "Streptococcus lactis R" as a strain of Streptococcus faecalis. J. Bacteriol., 48: 611. 1944.

52. Hajna, A. A. and Perry, C. A. Comparative study of presumptive and confirmatory media for bacteria of the coliform group and for fecal streptococci. Am. J. Publ. Health, 33: 550. 1943.

53. Hammer, B. W. and Yale, M. W. Development of the Escherichia-Aerobacter group of bacteria in butter. J. Dairy Sci., 15: 199. 1932.

54. Hartman, P. A. Enterococcus: coliform ratios in frozen chicken pies. Appl. Microbiol., 8: 114. 1960.

55. Hartmann, G. Ein Beitrag zur Reinzüchtung von Mastitisstreptokokken aus verunreiningtem Material. Milchw. Forsch., 18: 116. 1937.

56. Hartsell, S. E. and Caldwell, J. H. Lysozyme and the differentiation of group D streptococci. Proc. 2nd Intern. Sympos. on Fleming's Lysozyme, Milan, Italy. 1961.

57. Henriksen, S. D. Comparison of coliform organisms from feces and from water in Norway. Acta Path. et Microbiol. Scand., 35: 75. 1954.

58. Herschdoerfer, S. M. and Ward, P. S. Testing of ice cream for coliforms. 16th Intern. Dairy Congr., C: 75. 1962.

59. Higginbottom, C. The associated growth of the coli-aerogenes group and other bacteria in milk. 15th Intern. Dairy Congr., 3: 1349. 1959.

60. _____ Bacteriological studies on raw milk before and after the adoption of bulk milk cooling on the farm. 16th Intern. Dairy Congr., A: 426. 1962.

61. _____ Bacteriological studies of roller-dried milk powders, roller-dried butter milk and of roller- and spray-dried whey. J. Dairy Research, 13: 308. 1944.

62. Hiscox, E. R. and Briggs, C. A. E. Reviews of the progress of dairy science. Section B: Bacteriology and mycology as applied to dairying. J. Dairy Research, 22: 391. 1955.

63. _____ and _____ Reviews of the progress of dairy science. Section B: Bacteriology and mycology as applied to dairying. J. Dairy Research, 24: 387. 1957.

64. Horrock, S. An introduction to the bacteriological examination of water. London, England. J. and A. Churchill Publishers. 1901.

65. Hugh, R., Klopp, C. T., and Ryschenkow, E. The increased incidence of enterococci in the buccal cavity in the presence of disease. Med. Ann. District of Columbia, 28: 61. 1959.

66. Hunter, O. W. The colon-aerogenes group of milk. J. Dairy Sci., 2: 108. 1919.

67. Iya, K. K. and Frazier, W. C. Associative growth of Streptococcus lactis and Aerobacter aerogenes in milk. J. Dairy Sci., 34: 879. 1951.

68. Iyengar, M. K. K., Laxminarayana, H., and Iya, K. K. Studies on the heat resistance of some streptococci. Indian J. Dairy Sci., 10: 90. 1957.

69. Jarchovská, H. and Müller, V. Presence of enterococci in dried milk. Veterinařstvi, 10: 66. 1960. Original not available for examination; abstracted in Dairy Sci. Abstr., 23: 86. 1961.

70. Johns, C. K. The coliform count of raw milk as an index of udder cleanliness. 16th Intern. Dairy Congr., C: 365. 1962.

71. Jones, D. and Shattock, P. M. F. The location of the group antigen of group D streptococci. J. Gen. Microbiol., 23: 335. 1960.

72. Kalshoven, H. The applicability of the violet red-bile-agar plate for the quantitative estimation of bacteria of the coli-aerogenes group. Neth. Milk Dairy J., 7: 83. 1953.

73. Kampe, A. On coliform bacteria in raw milk. 13th Intern. Dairy Congr., 2: 148. 1953.

74. Kenner, B. A., Clark, H. F., and Kabler, P. W. Fecal streptococci. I. Cultivation and enumeration of streptococci in surface waters. Appl. Microbiol., 9: 15. 1961.

75. _____, _____, and _____ Fecal streptococci. II. Quantification of streptococci in feces. Am. J. Publ. Health, 50: 1553. 1960.

76. Kereluk, K. Studies on the bacteriological quality of frozen meat pies. III. Identification of enterococci isolated from frozen meat pies. Appl. Microbiol., 7: 324. 1959.

77. Kjellander, J. Enteric streptococci as indicators of fecal contamination of water. Acta Path. et Microbiol. Scand. Suppl. 136, 48: 38. 1960.

78. _____ and Nygren, B. On the occurrence of fecal streptococci in industrial food products. Acta Path. et Microbiol. Scand. Suppl. 154: 323. 1962.

79. Kosikowsky, F. V. The manufacture of Mozzarelle cheese from pasteurized milk. J. Dairy Sci., 34: 641. 1951.

80. _____ and Dahlberg, A. C. The growth and survival of Streptococcus faecalis in pasteurized milk American Cheddar cheese. J. Dairy Sci., 31: 285. 1948.

81. Larkin, E. P., Litsky, W., and Fuller, J. E. Fecal streptococci in frozen foods. I. A bacteriological survey of some commercially frozen foods. Appl. Microbiol., 3: 98. 1955.

82. _____, _____, and _____ Fecal streptococci in frozen foods. II. Effect of freezing storage on Escherichia coli and some fecal streptococci inoculated onto green beans. Appl. Microbiol., 3: 102. 1955.

83. _____, _____, and _____ Fecal streptococci in frozen foods. III. Effect of freezing storage on Escherichia coli, Streptococcus faecalis and Streptococcus liquefaciens inoculated into orange concentrate. Appl. Microbiol., 3: 104. 1955.

84. _____, _____, and _____ Fecal streptococci in frozen foods. IV. Effect of sanitizing agents and blanching temperatures on Streptococcus faecalis. Appl. Microbiol., 3: 107. 1955.

85. _____, _____, and _____ Incidence of fecal streptococci and coliform bacteria in frozen fish products. Am. J. Publ. Health, 46: 464. 1956.

86. Laxminarayana, H. and Iya, K. K. Studies on the reduction of tetrazolium by lactic acid bacteria. I. Dye reducing activities of different species. Indian J. Dairy Sci., 6: 75. 1953.

87. Leclerc, H. and Catsaras, M. Utilisation des membranes filtrantes dans la recherche des streptocoques fècaux des eaux d'alimentation. Ann. Inst. Pasteur Lille, 10: 193. 1958.

88. Lethem, W. A. The principles of milk legislation for safety, quality and freshness. 14th Intern. Dairy Congr., 3: 218. 1956.

89. Litsky, W., Mallmann, W. L., and Fifield, C. W. Comparison of the Most Probable Numbers of Escherichia coli and enterococci in river waters. Am. J. Publ. Health, 45: 1049. 1955.

90. _____, _____, and _____ A new medium for the detection of enterococci in water. Am. J. Publ. Health, 43: 873. 1953.

91. London, J. and Appleman, M. D. Oxidative and glycerol metabolism of two species of enterococci. J. Bacteriol., 84: 597. 1962.

92. Madsen, F. Investigations of coliforms in order to control the hygienic quality of butter. Arch. Esc. Vet. Minas Yerais, 22: 9, 1959. Original not available for examination; abstracted in Dairy Sci. Abstr., 25: 547. 1963.

93. Malaney, G. W., Weiser, H. H., Turner, R. O., and Van Horn, M. Coliforms, enterococci, thermodurics, thermophiles and psychrophiles in untreated farm pond waters. Appl. Microbiol., 10: 44. 1962.

94. Malcolm, J. F. The enrichment of aerogenes-cloacae types in milk held at low temperatures: with observations on the relative rates of growth of aerogenes-cloacae and B. coli types in milk at different temperatures. J. Dairy Research, 10: 410. 1939.

95. _____ The occurrence of coliform bacteria in milk. J. Dairy Research, 5: 15. 1933.

96. _____ The types of coliform bacteria in bovine feces. J. Dairy Research, 6: 383. 1935.

97. Mallmann, W. L. A new yardstick for measuring sewage pollution. Sew. Works J., 12: 875. 1940.

98. _____ and Kereluk, K. A new medium for the determination of enterococci in water. Proc. Soc. Am. Bacteriol. 1957: 142. 1957.

99. _____ and Seligmann, E. B. A comparative study of media for the detection of streptococci. Am. J. Publ. Health, 40: 286. 1950.

100. McKenzie, D. A. The use of thallium acetate glucose broth in the diagnosis of streptococcal mastitis. Vet. Record, 53: 473. 1941.

101. Medrek, T. F. and Barnes, E. M. The distribution of group D streptococci in cattle and sheep. J. Appl. Bacteriol., 25: 159. 1962.

102. _____ and _____ The physiological and serological properties of Streptococcus bovis and related organisms isolated from cattle and sheep. J. Appl. Bacteriol., 25: 169. 1962.

103. _____ and Litsky, W. Comparative incidence of coliform bacteria and enterococci in undisturbed soil. Appl. Microbiol., 8: 60. 1960.

104. Mieth, H. Untersuchungen über das Vorkommen von Enterokokken bei Tieren und Menschen. I. Ihr Vorkommen im Darm von gesunden Schlachtschweinen. Zentralbl. Bakteriol., Parasitenk., Infekt. und Hyg., Abt. I, Orig., 179: 456. 1960.

105. _____ Untersuchungen über das Vorkommen von Enterokokken bei Tieren und Menschen. II. Ihr Vorkommen in Stuhlproben von gesunden Menschen. Zentralbl. Bakteriol., Parasitenk., Infekt. und Hyg., Abt. I, Orig., 183: 68. 1961.

106. _____ Untersuchungen über das Vorkommen von Enterokokken bei Tieren und Menschen. III. Die Enterokokkenflora in den Faeces von Rindern. Zentralbl. Bakteriol. Parasitenk., Infekt. und Hyg., Abt. I, Orig., 185: 47. 1962.

107. Ministry of Health. The bacteriological examination of water supplies. Rept. Publ. Health Med. Subj. No. 71. London, England: H. M. S. O. 1934. Original not available for examination; cited in Crossley, E. L. The coliform flora of milk and dairy products. J. Dairy Research, 14: 233. 1946.

108. Morelis, P. and Colobert, L. Un milieu selectif permettant l'identification et le dencombrement rapides de Streptococcus faecalis. Ann. Inst. Pasteur, 95: 667. 1958.

109. Morris, C. S. and Edwards, M. A. Further investigations on the presence in raw milk of a bactericidal substance specific for certain strains of coliform organisms. J. Dairy Research, 17: 253. 1950.

110. Morris, R. L. and Cerny, J. Significant abnormalities in the violet red bile technique for coliforms in milk. J. Milk and Food Technol., 17: 185. 1954.

111. Mundt, J. O. Occurrence of enterococci: Bud, blossom and soil studies. Appl Microbiol., 9: 542. 1961.

112. _____, Coggin, J. H., Jr., and Johnson, L. F. Growth of Streptococcus faecalis var. liquefaciens on plants. Appl. Microbiol., 10: 552. 1962.

113. _____ and Johnson, A. H. Physiological properties of group D streptococci isolated from plants. Food Research, 24: 218. 1959.

114. _____, _____, and Khatchikian, R. Incidence and nature of enterococci on plant materials. Food Research, 23: 186. 1958.

115. Murray, J. G. A comparison of 30° and 37° as incubation temperatures in the presumptive coli-aerogenes tests for raw and pasteurized milk. Proc. Soc. Appl. Bacteriol., 16: 24. 1953.

116. _____ Incidence of pathogenic serotypes of Escherichia coli in milk for human consumption. 16th Intern. Dairy Congr., C: 372. 1962.

117. Olsen, Malling E. Use of penicillin in liquid and solid coliform media. 13th Intern. Dairy Congr., 3: 1302. 1953.

118. Olsen, S. J. Investigations into the occurrence of coliform bacteria in raw milk. 13th Intern. Dairy Congr., 2: 276. 1953.

119. Orla-Jensen, S. The lactic acid bacteria. Copenhagen. A. F. Host and Son. 1919.

120. Osler, A. G., Buchbinder, L., and Steffen, G. I. Experimental enterococcal food poisoning in man. Proc. Soc. Exptl. Biol., N. Y., 67: 456. 1948.

121. Ostrolenk, M. and Hunter, A. C. The distribution of enteric streptococci. J. Bacteriol., 51: 735. 1946.

122. Papavassiliou, J. Species differentiation of group D streptococci. Appl. Microbiol., 10: 65. 1962.

123. Parfitt, E. H. Frequency of the Escherichia-Aerobacter species in in commercial butter. J. Dairy Sci., 19: 496. 1936.

124. _____ Proposed standard for the yeast and mold count of salted butter made from sour cream. J. Dairy Sci., 20: 447. 1937.

125. Parr, L. W. Coliform bacteria. Bacteriol. Rev., 3: 1. 1939.

126. Pette, J. W. Lactic acid bacteria producing hydrogen sulphide in Gouda cheese. Neth. Milk Dairy J., 9: 291. 1955.

127. Public Health Service. Milk Ordinance and Code: 1953 Recommendations. U. S. Dept. Health, Education and Welfare, Publ. Health Serv. Publ. No. 229, Washington, D. C. 1953.

128. Raadsveld, C. W. Bitter compounds from cheese. 13th Intern. Dairy Congr., 2: 676. 1953.

129. Raj, H. and Liston, J. Detection and enumeration of fecal indicator organisms in frozen sea foods. I. Escherichia coli. Appl. Microbiol. 9: 171. 1961.

130. _____, Wiebe, W. J. and Liston, J. Detection and enumeration of fecal indicator organisms in frozen sea foods. II. Enterococci. Appl. Microbiol., 9: 295. 1961.

131. Ramadan, F. M. and Abd-Elnaby, H. A. Bacteriological examination of unbottled soft drinks. Appl. Microbiol., 10: 311. 1962.

132. Rao, R. S. and Dudani, A. T. Bacteriological tests for judging the quality of ice cream. 16th Intern. Dairy Congr., C: 94. 1962.

133. Rasic, J. Trends of bacterial population during manufacture and ripening of white cheese. 16th Intern. Dairy Congr., B: 840. 1962.

134. Reinbold, G. W., Swern, M., and Hussong, R. V. A plating medium for the isolation and enumeration of enterococci. J. Dairy Sci., 36: 1. 1953.

135. Report of the Coli-Aerogenes (1956) Sub-Committee of the Society for Applied Bacteriology. The nomenclature of coli-aerogenes bacteria. J. Appl. Bacteriol., 19: 108. 1956.

136. Ross, A. D. and Thatcher, F. S. Bacteriological content of marketed precooked frozen foods in relation to public health. Food Technol., 12: 369. 1958.

137. Roughley, R. J.and McLeod, R. W. Microbiological survey of butter in New South Wales. Australian J. Dairy Technol., 15: 190. 1960.

138. Sadek, G. M. and Eissa, A. M. The incidence of coliform organisms in Damietta cheese and its relation to the salt content and acidity. Indian J. Dairy Sci., 10: 184. 1957.

139. Saraswat, D. S., Clark, W. S., Jr., and Reinbold, G. W. Selection of a medium for the isolation and enumeration of enterococci in dairy products. J. Milk and Food Technol., 26: 114. 1963.

140. Sasaki, R., Tsugo, T., and Nakae, T. On the bacteriological properties of raw milk in Japan. 15th Intern. Dairy Congr., 1: 275. 1959.

141. Sharpe, M. E. Occurrence of a common type antigen in streptococci of groups D and N. J. Gen. Microbiol., 7: 192. 1952.

142. _____ and Fewins, B. G. Serological typing of strains of Streptococcus faecium and unclassified group D streptococci isolated from canned hams and pig intestines. J. Gen. Microbiol., 23: 621. 1960.

143. _____ and Shattock, P. M. F. The serological typing of group D streptococci associated with outbreaks of neonatal diarrhoea. J. Gen. Microbiol., 6: 150. 1952

144. Shattock, P. M. F. Enterococci: Chemical and biological hazards in food. Ames, Iowa. Iowa State University Press. 1962.

145. _____ The faecal streptococci. 12th Intern. Dairy Congr., 2: 598. 1949.

146. _____ The identification and classification of Streptococcus faecalis and some associated streptococci. Ann. Inst. Pasteur Lille, 7: 95. 1955.

147. Sherman, J. M. The enterococci and related streptococci. J. Bacteriol., 35: 81. 1938.

148. _____ The streptococci. Bacteriol. Rev., 1: 3. 1937.

149. _____, Mauer, J. C., and Stark, P. Streptococcus faecalis. J. Bacteriol., 33: 275. 1937.

150. _____, Smiley, K. L., and Niven, C. F., Jr. The identity of a streptococcus associated with food poisoning from cheese. J. Dairy Sci., 26: 321. 1943.

151. _____ and Stark, P. The differentiation of Streptococcus lactis from Streptococcus faecalis. J. Dairy Sci., 17: 525. 1934.

152. _____ and Wing, H. U. The significance of colon-bacteria in milk, with special reference to standards. J. Dairy Sci., 16: 165. 1933.

153. _____ and _____ Streptococcus durans. J. Dairy Sci., 20: 165. 1937.

154. Simonart, P. and Lambert, R. Suggestions pour le controle microbiologique des laits. 13th Intern. Dairy Congr., 2: 317. 1953.

155. Singh, R. N. and Nelson, F. E. Coliform bacteria in butter. J. Dairy Sci., 31: 726. 1948.

156. Skaudhauge, K. Studies on enterococci with special reference to the serological properties. Copenhagen. Einar Munkogaards. 1950. Original not available for examination; cited in Shattock, P. M. F. The identification and classification of Streptococcus faecalis and some associated streptococci. Ann. Inst. Pasteur Lille, 7: 95. 1955.

157. Slade, H. D. and Shockman, G. D. The protoplast membrane and the D antigen of Streptococcus faecalis. Proc. Am. Soc. Microbiol. 1963: 46. 1963.

158. Slanetz, L. W. and Bartley, C. H. Numbers of enterococci in water, sewage and feces determined by the membrane filter technique. J. Bacteriol., 74: 591. 1957.

159. _____, Bent, M. S., and Bartley, C. H. Use of the membrane filter technique to enumerate enterococci in water. Public Health Repts. (U. S.), 70: 67. 1955.

160. Smillie, D. M. Coliform contamination of farm milks. Dairy Inds., 18: 580. 1953.

161. Smit, J., Krol, B. M. and Van Wijk, A. J. The B. coli test in the routine analysis of raw milk. A. Van Leeuwenhoek J. Microbiol. and Serol., 6: 1. 1939.

162. Smith, D. G. and Shattock, P. M. F. The serological grouping of Streptococcus equinus. J. Gen. Microbiol., 29: 731. 1962.

163. Smith, F. R. The occurrence of Streptococcus zymogenes in the intestines of animals. J. Dairy Sci., 22: 201. 1939.

164. Smith. H. W. The development of the bacterial flora of the feces of animals and man: The changes that occur during aging. J. Appl. Bacteriol., 24: 235. 1961.

165. Thiercelin, M. E. Sur un diplocoque saprophyte de l'intestin susceptible de devenir pathogène. Compt. Rend. Soc. Biol., 5: 269. 1899.

166. Thom, V. M. The influence of production methods on the hygienic quality of farm tank milk. 16th Intern. Dairy Congr., A: 409. 1962.

167. Thomas, S. B., Druce, R. G., and Elson, K. An ecological study of the coli-aerogenes bacteria of surface soil. J. Appl. Bacteriol., 23: 169. 1960.

168. Thomson, G. I. Coliform bacteria in New Zealand butter. J. Dairy Research, 17: 72. 1950.

169. Tittsler, R. P., Sanders, G. P., Lochry, H. R., and Sager, O. S. The influence of various lactobacilli and certain streptococci on the chemical changes, flavor development and quality of Cheddar cheese. J. Dairy Sci., 31: 716. 1948.

170. Walter, H. E., Sadler, A. M., Malkames, J. P., and Mitchell, C. D. Method of manufacturing cheese. U. S. Pat. 2,796,351. 1957. Original not available for examination; abstracted in Dairy Sci. Abstr., 20: 207c. 1958.

171. White, A. H. and Smith, K. N. Washed and non-washed butter. III. Microbiological aspects. J. Dairy Sci., 39: 1359. 1956.

172. White, J. C. and Sherman, J. M. Occurrence of enterococci in milk. J. Bacteriol., 48: 262. 1944.

173. Wilkerson, W. B., Ayers, J. C., and Kraft, A. A. Occurrence of enterococci and coliform organisms on fresh and stored poultry. Food Technol., 15: 286. 1961.

174. Williams, D. J. The associative growth of some thermoduric bacteria in pasteurized milk. J. Appl. Bacteriol., 19: 185. 1956.

175. Wilson, G. S., Twigg, R. S., Wright, R. C., Hendry, C. B., Cowell, M. P., and Maier, I. The bacteriological grading of milk. Spec. Rept. Ser. Med. Res. Coun., Lond., No. 206. London, England: H. M. S. O. 1935. Original not available for examination; cited in Report of the Coli-Aerogenes (1956) Sub-Committee of the Society for Applied Bacteriology. The nomenclature of coli-aerogenes bacteria. J. Appl. Bacteriol., 19: 108. 1956.

176. Winter, C. E. and Sandholzer, L. A. Isolation of enterococci from natural sources. J. Bacteriol., 51: 588. 1946.

177. Wolin, M. J., Manning, G. B., and Nelson, W. O. Ammonium salts as a sole source of nitrogen for the growth of Streptococcus bovis. J. Bacteriol., 78: 147. 1959.

178. Yale, M. W. Significance of the coliform group of bacteria in American Cheddar cheese. J. Dairy Sci., 26: 766. 1943.

179. Zaborowski, H., Huber, D. A., and Rayman, M. M. Evaluation of microbiological methods used for the examination of precooked frozen foods. Appl. Microbiol., 6: 97. 1958.

180. Zubrzycke, L. and Spaulding, E. H. Studies on the stability of the normal human fecal flora. J. Bacteriol., 83: 868. 1962.

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