SANITARY STATUS OF PRECOOKED FROZEN DESSERT-TYPE FOOD

by

NAGENDRA NATH SHARAN VERMA

Graduate Bihar Veterinary College, Patna, 1947
Post-Graduate (Vet. Sci.), Indian
Veterinary Research Institute, U. P., 1953

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements of the degree

MASTER OF SCIENCE

Department of Bacteriology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1964

Approved by: V. D. Foltz
Major Professor

TABLE OF CONTENTS

INTRODUCTION

Food preservation by freezing has been practiced by the people dwelling in the colder regions of the world since time unknown. Mechanical refrigeration as a mode of preservation in the warmer climates has been a comparatively recent achievement. With the development of modern freezing and storage facilities people were introduced to various varieties of commercial frozen food products. The most recent entry to this market has been precooked frozen foods.

Precooked frozen foods have gained great popularity in the modern food service operation because of qualities of less labor and time in preparation, little or no waste, year round availability, and long storage life along with the maintenance of freshness. Consequently, numerous varieties of the so-called "heat and eat" foods are available in the present day market.

Before freezing, these foods are cooked at high temperatures. The process usually destroys most, if not all, microorganisms. However, cooking also softens the structure of the constituents of these foods, making them more liable to contamination by microorganisms. If extreme sanitary care and cleanliness of the plants are not maintained during the subsequent various stages of manufacturing, it may lead to disastrous results. The process of freezing and storage at low temperature may destroy some, but not all, contaminants.

Precooked frozen foods are highly perishable. They are excellent substrates for the multiplication of microorganisms.

Some of the constituents of these foods also offer considerable protection to the contaminants against the lethal effects of low temperature. Several varieties of these foods, such as, creamed fish, beef and poultry pies, chicken a la king and frozen sea foods, have been found to be highly contaminated. It has been shown that such contaminated and grossly mishandled precooked frozen products can be truly dangerous, and are potential public health hazards.

Staphylococcal food intoxication is recognized as the commonest of food poisoning of bacterial origin. In the United States alone, out of 162 total food-borne diseases reported in the year 1960, 54 were staphylococcus food poisoning (Daucer, 1961). Coagulase positive staphylococci, responsible for these food poisonings, occur normally on the skin, hands, mouth, nasal passages, clothes, cuts and wounds of men. Thus, the precooked frozen foods, during their various stages of processing, can readily be contaminated by these organisms due to the slightest carelessness of the workers involved in food handling and processing.

Many of the precooked frozen foods are heated before serving and possibly a majority of the microorganisms present may be destroyed by this treatment, but sterility is seldom achieved. The chances of destroying food poisoning organisms from contaminated frozen desserts and pies are remote because these are usually served frozen, or after thawing at room temperature for 1 to 1½ hours, or after being in the oven at

350° F for 20 to 25 minutes. Moreover, poisoning from the heat-stable enterotoxin formed by staphylococci always remains a possibility even though they themselves may be destroyed.

This study was undertaken with the objective of assessing the sanitary status of the precooked frozen dessert type foods available at the consumer level. In order to achieve this objective, total bacterial population, and densities of coliform and staphylococci, in these foods were determined and their correlations were studied. Attempts were also made to determine the presence of enterotoxin producing strains of staphylococci, and coliforms of possible fecal origin and significant from a public health standpoint.

REVIEW OF LITERATURE

Bacteriological History of Precooked Frozen Food. According to Trassler and Evers (1957) people were introduced to commercial precooked frozen foods in the early thirties although it was only in the early fifties that the sale of these gained popularity in the common markets. Information regarding the various microbiological problems and the sanitary status of these foods, however, began to accumulate in the literature in the late forties.

Proctor and Phillips (1947) were among the earliest workers to have done an extensive microbiological survey of commercially prepared precooked frozen foods. They determined total bacterial counts, taxonomic composition of the bacterial flora, and the frequency of coliforms. They reported that for most products

the plate counts were between 10,000 and 50,000 per gram. However, none of the experimentally prepared, frozen precooked foods, showed counts in excess of 15,000 per gram. They also observed considerable variations in the plate counts of different products of the same manufacturer and in products of the same character made by different manufacturers, as well as in the samples purchased at different seasons from different retail outlets.

In general, the pastry products were found to exhibit the lowest bacterial population whereas the highest counts were found in creamed fish products (Proctor and Phillips, 1948). A majority of the samples had coliform counts less than 50 per gram, and the highest counts were obtained from creamed fish products. They found a predominance of Micrococcus species in meat products, Flavobacterium species in fish products and Bacillus species in soups. Appreciable numbers of Sarcina, Achromobacter and Staphylococcus species were contained in many of the various products.

Gunderson and Rose (1948) reported, in contrast to the above findings, exceptionally high numbers of bacteria in commercially produced chicken chow mein and chicken salad despite storage at -12° to -19° F for a period up to 5½ months. Five of eight samples of chicken salad contained potential food poisoning hemolytic Staphylococcus aureus. Buchbinder et al (1949) stated that staphylococci of the food poisoning type were present in 12 out of 39 samples of chicken a la king studied, and that eight of

these gave counts between 1000 and 100,000, 2 gave 200,000 to 400,000, and in 1 case, more than 2,000,000 per gram. More than half of the samples yielded plate counts in excess of 1,000,000 per gram and 7.7 percent of all samples yielded coliform counts greater than 100,000 but less than 500,000 per gram. However, they found most of the other frozen precooked product samples in a comparatively superior sanitary status. Similar results in chicken a la king were obtained by Hussemann (1951) and Logan et al (1951).

According to Canala-Parola and Ordal (1957) the baking time and temperatures specified for some of the brands of precooked frozen chicken and turkey pies were not sufficient to cause destruction of nonspore-forming organisms like coliforms, enterococci, staphylococci and salmonellae. Out of 40 unbaked pies examined by them, 20 had total counts above 100,000 per gram and 18 had coliform counts above 10 per gram; 37 of these yielded coagulase positive staphylococci whereas enterococci were present in all of the samples. Five Salmonella cultures were isolated from these unbaked pies.

Litsky et al (1957) examined 132 commercially packed tuna, chicken and turkey and beef frozen pies on a nationwide scale. Over 70 percent of the pies had total counts under 50,000 per gram and 60 percent had counts lower than 25,000 per gram. Coliform counts varied from 0 to 154 per gram and enterococci from 0 to 2000 per gram. They found no apparent correlation between the three determinations since the highest total count

was accompanied by zero enterococci MPN while the highest coliform count was accompanied by zero enterococci MPN and one of the lowest total counts. On similar studies of 188 meat pies Kereluk and Gunderson (1959a) reported that 93 percent of the samples had total bacterial counts under 100,000 per gram, about 83 percent had under 50,000 per gram and 75 percent of the samples had the counts under 25,000 per gram. Huber et al (1958) studied the microbiological quality of 1282 samples of various precooked meat products and 274 samples of vegetable products and reported that 86 percent of the meat samples and 88 percent of the vegetable samples had standard plate counts of less than 50,000 per gram and in both the products the distribution curves were skewed well towards the low count side (below 10,000 per gram). Over 94 percent of the meat products and 96.8 percent of the vegetable products examined were coliform-free or had less than 10 organisms of the coliform group per gram. In plant studies of the production of precooked frozen meals done by these workers, in which samples were taken at various stages throughout the processing procedure, from the raw material to the finished products, they found a definite correlation between plant sanitation and the presence of coliforms, enterococci, staphylococci and the total bacterial count. Accompanying the higher coliform index, a corresponding increase in enterococci and staphylococci were noted.

Bacteriological examination of 117 specimens of various precooked frozen foods, such as chicken and turkey dinners, pot pies, pumpkin and coconut pies, purchased from retail outlets

revealed that a large proportion of these foods were contaminated (Ross-Doreen and Thatcher, 1958). Certain specimens were shown to contain E. coli, coagulase positive staphylococci and enterococci in large numbers. Members of the Bathesda-Bellerup and Providence groups of the paracolon organisms were also found in a few of the samples. Pumpkin pies and coconut pies showed comparatively low average plate counts and were found free from coagulase positive staphylococci. According to Machala (1961) 26 percent of the 192 assorted frozen food samples surveyed had a total plate count exceeding 100,000 per gram and 40 percent of these had more than 10 coliforms per gram. Six of the samples showed the presence of coagulase positive staphylococci with counts ranging from 1000 to 18,000 per gram.

Kachikian et al (1959) reported the results of the bacteriological survey of 144 samples of frozen breaded shrimp. The total number of bacteria varied from a minimum of 22,500 per gram to a maximum of 54 million per gram. Ninety-eight samples contained less than 100 coliform bacteria per gram and all the samples contained fecal streptococci. Their number varied from very few bacteria per gram to 13,500 per gram. On analysis of 91 samples of commercial frozen raw and cooked shrimp, Silverman et al (1961a) reported that the total bacterial count of these samples ranged from 50 to 9,000,000 organisms per gram; 75 percent of the samples contained coagulase positive staphylococci as a small percentage of total microbial flora.

Survival of Pathogens. Although it was commonly believed

that bacteria died when frozen (Keith, 1913), McClesky and Christopher (1941) proved that Staphylococcus aureus as well as some Salmonella species could survive in unsliced strawberries for 14 months at -18° C (0° F). They concluded that "it is fallacious for a producer to assume that deep-freeze storage will 'pasteurize' a contaminated product."

Phillips and Proctor (1947) examined a variety of commercial frozen precooked meat, poultry and fish products for their ability to support the growth and survival of experimentally inoculated cultures of Staphylococcus aureus and reported that creamed food products such as chicken a la king, creamed tuna, creamed salmon were good substrates for Staphylococcus aureus and they contained large numbers of these organisms even after storage at -18° C for 9 months. In their subsequent studies Proctor and Phillips (1948) observed that a notable decrease in the viable organisms occurred in the first few months of storage at 0° F, but the products were not sterile even after storage for over 30 months. They also reported that when food poisoning strains of Staphylococcus aureus, alpha type Streptococcus and Salmonella enteritidis were inoculated into various cooked foods and then frozen and stored at 0° F at least 10 percent of these pathogenic bacteria survived storage for six months in some of the foods.

Gunderson and Rose (1948) studied the death of six strains of Salmonella stored in sterile chicken chow mein at -25.5° C (-14° F) and reported that the rate of death was essentially

proportional to the number of viable bacteria present during the early stages of storage. They found 20 percent of the initial inoculum of Salmonella typhimurium survived storage for nine months. The mortality of Escherichia coli, Aerobacter aerogenes, Streptococcus faecalis and Streptococcus faecalis var. liquefaciens also has been observed under similar conditions (Kereluk and Gunderson, 1959b). These survived storage for 481 days at -6° F, but Aerobacter aerogenes and Streptococcus faecalis var. liquefaciens appeared to be more resistant than Escherichia coli and Streptococcus faecalis. Subsequently Raj and Liston (1961a) observed the survival of Salmonella typhimurium, Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli and Clostridium perfringens for 393 days in processed frozen fish homogenates stored at 0° F.

Hartsell (1951) observed greater destruction of pathogens on frozen beef and on peas at -9° C than -17° C. The order of resistance, from greatest to least of cultures on stored frozen beef or peas, was Staphylococcus aureus, Salmonella oranienburg, S. typhosa and S. dysenteriae. A vast number of papers prior to this finding, and subsequently, have given overwhelming proof that low freezing temperatures such as -20° C are less harmful to microorganisms than a medium range of temperatures such as -10° C (Berry, 1934; Haines, 1938; McFarlane, 1940; Weiser, 1951; Hucker et al, 1952).

Considerable concentration of sugars and fat generally diminish the mortality of microorganisms in frozen foods

(McFarlane, 1942). Woodburn and Strong (1960) showed that the addition of waxy rice flour (cooked), corn sirup and egg white gave protection to potentially food poisoning organisms, like S. typhimurium, Staphylococcus aureus and Streptococcus faecalis, from the lethal effects of freezing. Studies of the effects of multiple cyclic defrosting have showed that sea food materials had a more protective effect in comparison to cultures suspended in broth (Raj and Liston, 1961). Clement (1961) found a minimum survival of 65% of Escherichia coli when they were suspended in glycerol, frozen and stored in frozen state at -18° C, -23° C and -40° C.

In general, the effect of freezing on bacteria of frozen foods depends on various factors such as: rate and type of freezing, temperature and duration of storage, initial numbers, types and stages of growth phase of microorganisms involved, thawing processes, number of cyclical defrostings, and the physical protection offered by the food itself or its components (Ingram, 1951; Borgstrom, 1955).

Bacteriological Methods of Analysis. In the bacteriological studies of frozen foods there are essentially three types of microbes, namely: saprophytic, pathogenic and toxigenic which are of significance (Borgstrom, 1955). The latter two are of particular importance and the methods of bacteriological examination of these foods are primarily aimed at detecting these organisms. Straka and Stokes (1956) suggested that foods containing large numbers of microorganisms were not wholesome,

whether or not disease producing organisms were present. Heller (1951) reported that in most cases where bacterial counts were very high in a food, the process of manufacturing was such that it would permit the entry into the food of both spoilage types and food poisoning types of organisms. Huber et al (1958) showed that precooked frozen foods prepared under proper sanitary conditions and technical supervision had standard plate counts much below the maximum limit of 100,000 per gram. They also stated that high bacterial plate counts on frozen foods may be taken as proof of poor sanitary quality. Several others have confirmed this view (Abrahamson, 1960; Dack et al, 1960). Goresline (1959) stated that the presence of pathogens in precooked frozen meals was a remote possibility, provided that the standard aerobic plate count of 100,000 per gram and a coliform level of 10 per gram were enforced. In support of his statement he mentioned that no food poisoning had ever occurred from products purchased by the Quartermaster Corps under such contracts.

Viable Count. Heller (1951) employed various counting methods in estimating the number of microorganisms in 10,000 samples in a wide variety of food stuffs. He noted that the viable count gave a convenient method for following bacterial contamination at various stages of manufacturing. Standard Methods for the Examination of Dairy Products (APHA, 1960) states "The agar plate method has been used officially longer than other methods for estimating bacterial populations

. . . it is especially suited to determinations where bacterial densities are low."

Borgstrom (1955) reviewed the various microbiological problems of frozen foods and stated that the results of determination of microbiological content of these foods may be considerably influenced by the sampling technique employed and the media used. A sample which contained too much water would give erroneous results on thawing if a proportionate amount of the water was not included. He stated that sampling of unthawed products was preferred in such cases.

Jones and Ferguson (1951) made a comparative study of the bacterial counts obtained from samples prepared in a Waring blender and shaking by machine and by hand and reported that 47% of the samples gave higher counts, 21% gave lower counts in comparison to counts of machine or hand shaken samples whereas 32% of the samples showed no significant difference between the three different methods. They also reported that sometimes the product in the process of comminuting in the Waring blender was reduced to so fine a state that difficulty was experienced in pipetting a portion of the product for analysis. In similar studies Zaborowski et al (1958) found that the use of the blender method resulted in slightly higher counts. Recommended Methods for the Microbiological Examinations of Foods has suggested the use of a "blender" for the preparation of samples, and tryptone glucose agar medium for plating. Hartman and Hunterberger (1961) made extensive studies of the various factors which might affect

the enumeration of bacteria in precooked frozen food. They observed a considerable foaming in frozen cream pies after homogenization. According to them a blending time of 30 sec. to 1 min. appeared to be sufficient for these foods which already had a well dispersed consistency. They also showed that the total and enterococci counts were not significantly affected by the two different sample sizes of 50 grams and 100 grams.

Indicator Organisms: Coliform. A vast amount of research has been carried out to develop methods for the detection of indicator organisms which could be used in determining the sanitary quality of foods. According to Buttiaux and Mossel (1961) E. coli was chosen as the indicator organism originally by Scherdinger as early as 1892. They suggested that ideally the indicator organism occurring in the intestine should possess four properties: 1) specificity, i.e., it should occur only in an intestinal environment; 2) its density in faeces should be very high; 3) it should possess a high resistance to the extra-enteral environment, the pollution of which is to be indicated; and 4) it should permit relatively easy and fully reliable detection even in low numbers.

Thomson (1955) mentioned that out of the normal inhabitants of the intestinal tracts of men, E. coli, the most characteristic organism of human faeces, is commonly present in numbers up to 5×10^8 per gram.

According to the Recommended Methods of Microbiological Examinations of Foods (APHA, 1958) "out of the suggested indicators

of sanitary quality of foods, three have been given the most consideration: E. coli, coliforms and enterococci." It has also been pointed therein that the presence of large numbers of these organisms in a food may not necessarily cause an immediate health hazard but it acts as a flag of warning, indicating the possibility of spoilage, loss of quality or even a source of a health hazard.

Zaborowski et al (1958) mentioned that since the coliform group of organisms cannot survive the time-temperature conditions of normal cookery, the coliform count has been a useful indicator of post-cooking contamination in precooked frozen foods.

Buttiaux (1959) after his extensive investigations has shown that the specificity of E. coli is unquestionable, but the use of antibiotics active against these organisms has greatly modified this situation; Klebsiella, being resistant to the antibiotics has become so preponderant that the Klebsiella : E. coli ratio may exceed 100:1. The investigations of Buttiaux and Mossel (1961) have so far revealed that Enterobacteriaceae species (Aerobacter aerogenes and Aerobacter cloacae) occur only rarely in the human intestine whereas the Citrobacter species were definitely found in faeces but they also occur in soil which may be only slightly polluted or not at all.

Some workers have also stated that the presence of coliform bacteria in cooked frozen food may not be considered more undesirable than other innocuous microorganisms unless their fecal origin or other sources considered "dangerous" is proved

(Hartman, 1960; Machala, 1961).

Enterococci. The presence of enteric streptococci has almost always been found in human (Ostroleuk and Hunter, 1946; Bartley and Slanetz, 1960) and animal faeces (Brigg et al, 1954). However, their presence in other environments have also been shown (Mundt et al, 1958).

Some workers have suggested that enterococci are better indicators for detecting contamination in frozen foods than the coliform organisms as the former survive freezing and storage at low temperatures for comparatively longer periods (Burton, 1949; Larkin et al, 1955; Raj et al, 1961b). Ingram (1961) questioned the use of the resistant enterococci to measure a potential hazard, since the coliform dies out at a rate more nearly comparable with that of the Salmonella.

Media for Coliform Count. Many types of media have been proposed for isolation and enumeration of the coliform group of organisms from milk, dairy products and different varieties of food. Bartram and Black (1936) did a comparative study of five liquid and nine solid media for their efficiency in determining the presence of coliforms in milk, and reported that neutral red bile and violet red bile agar proved to be the most satisfactory solid media for isolation purposes. They further noted that violet red bile agar proved slightly better than neutral red bile agar in ease with which the coliforms were recognized and gave higher counts in some instances. In conformity with the above finding several other workers have shown that desoxycholate

agar and/or violet red bile agar gave as good or better results than liquid media for the determination of the coliform content of milk and dairy products (Yale, 1937; Miller and Prickett, 1938; Tiedeman and Smith, 1945) and precooked frozen foods (Buchbinder et al, 1949; Ross and Thatcher, 1958; Kereluk and Gunderson, 1959c). Hartman (1960) reported that the accuracy of recovery of E. coli from violet red bile agar plates was greater if only red colonies surrounded by a zone of precipated bile were isolated and the incubation period of 24 hours or less was adhered to. Use of desoxycholate agar or violet red bile agar for isolation and enumeration of coliform has also been recommended in Standard Methods (APHA, 1960) which states "solid media procedures are recommended over the multiple tube method because of the increased reproducibility and greater confidence . . . It also permits confirmation of any doubtful colonies."

Enterotoxin-producing Staphylococci. Barber (1914) for the first time reported gastro-intestinal upsets due to Staphylococcus albus isolated from milk. However, Dack et al were probably the first workers to give conclusive evidence that staphylococci may produce a filterable poison in food. Presence of enterotoxin in the filtrates of Staphylococcus cultures was further proved by animal inoculation tests (Dolman et al, 1936; Dolman and Wilson, 1940). Although methods for detecting the gastro-enterotoxic properties of staphylococci came to be known, the problem of distinguishing the food poisoning strains from other strains of staphylococci and micrococci in vitro remained

unsolved.

After popularization of the coagulase test in the United States by Chapman et al (1934) and in Britain by Cruickshank (1937), it was possible to differentiate pathogenic strains of staphylococci from the non-pathogenic ones. Chapman et al (1937), after their extensive studies of the properties of food poisoning strains of staphylococci, reported that the typical food poisoning staphylococci, in common with pathogenic staphylococci, produce yellow or orange pigment, hemolyse rabbit-blood, coagulate human and rabbit plasma, produce orange or deep violet growth on crystal violet agar, grow luxuriantly on bromthymol blue agar, and ferment mannitol.

Hussemann and Tanner (1949) studied the characteristics of 28 strains of staphylococci recovered from the foods accepted as the causative agent of food poisoning and 40 strains isolated from those foods which were not implicated with entoxication. In their studies they employed the technique of the Stone reaction (Stone, 1935), the series of tests outlined by Chapman et al (1937) and the kitten test of Dolman and Wilson (1940). They reported that none of the tests yet devised seemed to be wholly successful in separating the two forms. However, they did find that most enterotoxin formers were coagulase positive strains; fermented mannitol with the formation of acid and produced an orange pigment on Loeffler's medium. According to Thatcher and Simon (1956) enterotoxin may be produced by coagulase negative strains, although such a relationship may

be encountered very infrequently.

In a comparative study of physiological characters of 114 known food-poisoning strains of staphylococci and related varieties, Evans and Niven (1950) reported that all enterotoxigenic cultures were coagulase positive and physiologically comprised an extremely homogenous group. They appeared to be identical with other toxigenic coagulase-positive strains, which included eight cultures that failed to give evidence of enterotoxin production.

Baird-Parker (1962) stated that due to a lack of a simple and conclusive test for enterotoxin production it is safer to regard all coagulase positive staphylococci as potentially able to cause food poisoning.

Isolation of enterotoxigenic staphylococci from food is frequently made difficult by the presence of large numbers of other closely related saprophytic organisms. In order to overcome this difficulty many studies have been made and various types of selective media and selective enrichment procedures have been devised. Chapman (1945) reported that the addition of 75 grams of NaCl to 1 liter of Bacto phenol red mannitol agar provided an improved isolation medium for coagulase positive staphylococci. A year later Chapman (1946) suggested a single medium for the selective isolation of plasma-coagulating staphylococci and for improved testing of chromogenesis, plasma-coagulation, mannitol fermentation and the Stone reaction. He reported that the ingredients of the culture medium have a

profound effect upon the cultural reactions of staphylococci and the most satisfactory combination, in grams per liter, was mannitol 10; agar 15; gelatine 30; tryptone 10; anhydrous K_2HPO_4 5; lactose 2; yeast extract 2.5; and NaCl 75. After 48 hours incubation at 37° C no other bacteria except staphylococci (albus and aureus) grew on this medium.

Hartsell (1951) reported that for getting higher numbers of viable staphylococci from frozen foods, staphylococcus medium number 110 was as effective as yeast extract veal infusion agar.

A medium containing tellurite and lithium chloride as selective agents was described by Ludlam (1949) for the isolation of Staphylococcus. Zebovitz et al (1955) made modification in Ludlam's media by the addition of glycine and agar and described it as tellurite glycine agar (TGA). They claimed this medium to be superior to all other media currently in use. However, both of these media were found inhibitory to certain strains of coagulase positive staphylococci and they failed to inhibit all coagulase negative strains (Chapman, 1949; Zebovitz et al, 1955; Innes, 1960).

Gillespie and Alder (1952) reported that most strains of coagulase positive staphylococci produced opacity in egg yolk broth, whereas the coagulase negative strains failed to do so. He suggested that it may provide a simple means of subdividing the strains into two groups. However, the finding awaits confirmation (Innes, 1960). Several media containing egg yolk have been described (Innes, 1960; Hopton, 1961) with varied

results. Baird-Parker (1962) developed a selective and diagnostic medium (ETGPA) for isolating coagulase positive staphylococci from foods. He reported that the performance of this medium has been found to be better than Chapman's Staphylococcus 110 medium (S-110), Zabovitz et al medium; Carter's and Innes' medium. He, however, stated that the main drawback with this medium was that the four solutions (egg yolk, tellurite, glycine and pyruvate) had to be added to the medium before use and that plates of the medium could not be stored for more than 24 hours. Since coagulase positive staphylococci were present in frozen foods, as a small percentage of the microbial flora, selective enrichment in trypticase soy broth containing 10 percent NaCl was found to be a more successful isolation technique (Zaborowski et al, 1958; Silverman et al, 1961b). On the contrary Carter (1960) reported that the total number of coagulase positive staphylococci isolated from frozen food by the three methods (direct plating on S-110; plating on S-110 with egg yolk; and streaking on S-110 with or without egg yolk from 10 percent NaCl containing enrichment media) were essentially the same.

MATERIALS AND METHODS

Collection of Samples. Different varieties of precooked frozen pies and desserts produced by various manufacturers and available for sale in the local market (Manhattan, Kansas) were randomly selected and purchased. Care was taken to select only

intact packages showing no evidence of leakage or tampering. Immediately after purchase they were transported to the laboratory and kept in a freezer cabinet at 0° F until examined; the bacteriological examinations were carried out within one week from the date of purchase. For the present study a total of 102 samples of fifteen different varieties of seven different brands were examined, and were obtained in several lots. The various varieties of the products examined during this study are listed in Table 1.

Preparation of samples. In order to facilitate sampling of the frozen products, specimens were transferred to a refrigerator at 5° to 8° C for about two hours to allow partial thawing. The outer side of the packages were cleaned and sanitized with sterile cotton soaked in 70 percent alcohol. Packages were opened using aseptic technic to avoid external contamination. The internal metal container of the packages along with its paper covering were withdrawn aseptically. Sterile forceps were used to lift the upper paper covering and with the help of a sterilized wooden tongue depressor 11 grams of the material were aseptically removed from a representative midportion of the packaged material and transferred into sterile screw-capped, flat-bottomed 350 ml bottles (6" x 2") containing 99 ml sterile distilled water. Prior to use, these dilution blanks were chilled to 4°-5° C in the refrigerator. After transferral of the material, the bottle along with its contents was vigorously agitated in a shaking machine at an oscillation of 250 (± 10)

per minute for 5 to 6 minutes. This made a homogenous 1:10 suspension of the material. From this, one ml was transferred aseptically to 9 ml sterile dilution blanks made up in screw capped test tubes (150 x 16 mm). These were thoroughly mixed by twenty complete up and down movements in about one foot arc. Similarly 1:1000 dilutions were made.

Comparative studies with blenderized samples showed no discernable difference, and due to practical difficulties, the blender method was not used.

Wooden tongue depressors used for sample transfer were sterilized in a hot air oven. The dilution blanks were sterilized in the autoclave at 120° C for 15 min.

Viable Count. Plate count agar (Difco) was used for the purpose of estimating the bacterial population of the samples. One ml aliquot of 1:100 and 1:1000 dilutions of the samples were transferred aseptically to duplicate petri dishes (100 mm x 15 mm), into which was poured 20 ml of melted media (at 45° C) and the contents mixed thoroughly by 10 to 12 gentle rotations. The plates when cooled and set were inverted and incubated at 37° C for 48 hr. The colonies were counted with the aid of a Quebec colony counter.

Preliminary trials on five samples to determine the dilution needed to get counts within 30 to 300 colonies per plate were made. Counting of colonies and computation of standard plate counts were done according to the procedure described in Standard Methods for the Examination of Dairy Products (APHA,

1960).

Coliform Count. For the coliform count violet red bile agar (Difco) was used as per manufacturer's direction.

One ml of the 1:10 dilution of the homogenous suspension of each of the materials was transferred aseptically into duplicate petri dishes (100 mm) and mixed thoroughly with 15 ml of the melted medium cooled to 43°-45° C. After solidification of the medium another 5 ml of melted medium was overlayed to cover the first layer and allowed to harden. Incubation of these plates was done at 37° C for 20 to 24 hrs, after which the growths were examined by transmitted light for the appearance of the typical subsurface coliform colonies of about 0.5 mm or more in diameter having purplish-red color surrounded by a reddish zone of precipitated bile. The counting of the colonies and recording of the coliform counts were done according to Standard Methods (APHA, 1960).

Staphylococcus Count. Staphylococcus medium No. 110 (Difco) (S-110), a selective medium for the isolation of staphylococci, was used for primary isolation and enumeration. In order to check the sterility and also to have a perfectly dry surface, the plates were poured 36 hrs before use, incubated for 24 hrs at 37° C and then left at room temperature over night.

Then one ml of 1:10 dilution of the material was aseptically transferred to each of two plates and spread evenly over the surface with a sterile "L" shaped glass rod. The plates were examined after 48 hr incubation at 37° C for the presence of

typical colonies resembling staphylococci. These colonies were circular, entire, smooth, glistening in appearance, with white, orange or yellow pigmentation. If present, smears were made from such colonies, fixed, Gram stained and examined microscopically. Colonies yielding Gram positive spherical cells arranged in irregular clusters, the typical morphological character of staphylococci, were then counted and the staphylococcal content per gram of the test material was computed and recorded.

With a sterile inoculating needle the center of a colony was gently touched and transferred to proteose peptone agar slants for further study. After picking up the colonies preliminary observations for mannitol fermentation and gelatinase activity were made on the S-110 plating medium. One to two drops of bromocresol purple indicator were added to the area from which the colonies were picked, and any change in color of the added indicator to yellow, compared with that of the uninoculated medium, was taken to indicate the fermentation of mannitol. The plate was then flooded with 5 to 6 ml Frazier's gelatine developer solution and allowed to stand for 10 minutes and then observed for the development of a clear zone around the areas from which the colonies were picked (Frazier, 1926). Any clear zone was taken as indicative of gelatinase positive reaction.

Isolation, Purification and Identification: Coliform. Violet red bile agar medium used for the enumeration of the coliform group of organisms was screened to check the color and

size of the colonies as well as the area of red zone of precipitated bile around the respective colonies. For the sake of description purplish-red subsurface colonies, 1 mm or more in diameter and surrounded by a reddish zone of precipitated bile (Difco, 1953) were termed "typical" colonies while those less than 1 mm in diameter having a similar appearance but a smaller reddish zone, were termed "small" colonies. Two colonies of each type were transferred from violet red bile agar to bromthymol blue lactose broth fermentation tubes and incubated at 37° C for 24 to 48 hrs. As soon as acid and/or gas were produced, loopfuls were streaked on eosin methylene blue agar (Difco) plates which were poured at least 24 hrs before use and checked for sterility. These plates were examined after 24 hr incubation for the presence of coliform colonies with or without a metallic sheen and having a dark nucleated or brown center and a light colored periphery. The center of the typical, well isolated single colony was gently touched with the tip of a sterile inoculating needle and stroked onto proteose peptone agar slants. The isolated agar slant cultures were incubated for 24 hr at 37° C, Gram stained and their morphology and purity checked. Pure isolated cultures of Gram negative, non-spore-bearing rods were properly indexed with a serial number, date of isolation and the specimen number from which they were isolated.

Composition of bromthymol blue lactose broth per liter was as follows:

Beef extract	1.0 gram
Proteose peptone No. 3	10.0 gram
Sodium chloride	5.0 gram
Lactose	5.0 gram
Brom-thymol blue (1.6% alcoholic sol.)	1 ml

The final pH of the medium was 7.0.

Dehydrated Eosin methylene blue agar (EMB) was prepared as per manufacturer's instructions.

Each of the isolates was studied for its colonial character on EMB agar and proteose peptone agar slants after 24 hr incubation. Proteose peptone agar slants were further incubated for 48 hrs at 30° C for evidences of pigment production. All cultures were tested for indole production, methyl red reaction, Voges-Proskauer reaction, citrate utilization (IMViC pattern), nitrate reduction, gelatin hydrolysis, urease activity, H_2S production and motility. In the above studies young cultures (18 to 20 hr) grown on proteose peptone agar slants and emulsified in sterile distilled water were used along with known positive and negative controls. In general, the methods of procedure, composition of media and reagents and the interpretation of results as described by Lord (1959) were followed. Koser's citrate medium was replaced by Simmon's citrate agar (Difco) in citrate utilization test and Kovac's reagent, as recommended by Edwards and Ewing (1962) was used for indole test.

Differentiation of the coliform group of organisms into

different species was based on the interpretation of IMViC reactions given in Standard Methods for the Examination of Water and Waste water (APHA, 1960). Other biochemical tests were done as an adjunct to the IMViC tests so as to determine the biochemical behavior of the isolates.

Staphylococcus. All the staphylococcal colonies picked from S-110 medium and transferred to proteose peptone agar slants were purified on proteose peptone agar plates. After 24 hr incubation of these plates at 37° C, well isolated single colonies were marked. The center of the colony was touched with an inoculating needle and stroked on two proteose peptone agar slants and incubated for 24 and 48 hrs respectively. They were Gram stained and examined for purity, staining reaction and morphology. The cultures so isolated from different specimens were indexed as mentioned previously.

The proteose peptone agar used had the following composition:

Proteose peptone (Difco)	20.0 grams
Beef extract	3.0 grams
Sodium chloride	5.0 grams
Agar (Difco)	20.0 grams
Distilled water	1000 ml

The pH was adjusted to 7.2.

Catalase Test. For the catalase test 48-hr-old cultures grown on proteose peptone agar slants were used. One ml of 3 percent hydrogen peroxide was poured onto the growth and was

observed for liberation of bubbles of oxygen from the surface of the medium.

Anaerobic growth. Tubes (16 mm. dia.) containing 8 ml. volume of glucose yeast extract agar (pH 7.0) were prepared. The medium had the following composition:

Peptone	10.0 grams
Yeast extract	5.0 grams
Glucose	1.0 grams
Agar (Difco)	15.0 grams
Distilled water	1000 ml.

Before use the tubes were melted in running steam for 10 min. and cooled to 45° C. They were inoculated with one loopful of a turbid suspension of young culture (24 hr) and immediately cooled to speed solidification, then incubated at 37° C for 48 hrs. Cultures showing uniform growth throughout the depth of medium were considered as facultatively anaerobic and were subjected to further studies.

In order to determine the possible existence of potential enterotoxin producing strains of staphylococci the isolates were tested for pigment production, gelatine hydrolysis, hemolysis reaction, anaerobic fermentation of mannitol and the coagulase reaction.

Chromogenesis and Gelatin Hydrolysis. For these studies Frazier's gelatin agar (1926) medium was used. Isolates were streaked as a 3 to 4 cm. straight line on Frazier's gelatin agar plates, which were poured 24 hrs before use and checked for

sterility. After 48 hrs incubation at 37° C the growth was scraped off the plates onto white filter paper and the chromogenesis observed. Later the plates were observed for gelatin hydrolysis by flooding them with 5 to 6 ml of Frazier's gelatin developer and observing after a 10 min development for a clear zone around or under the streak.

Hemolysis. The pattern of hemolysis caused by the isolates was studied on sterile agar plates containing five percent sterile defibrinated sheep blood. Four isolates were streaked on each plate as per procedure described above. The plates were incubated for 48 hrs at 37° C and observed for a zone of clearing around the growth. The plates were later left overnight in the refrigerator at 5°-8° C and again observed to detect any changes in the pattern of hemolysis.

Mannitol Fermentation. Tubes containing 8 ml of brom-cresol purple mannitol agar were used for this test. The medium was prepared by adding mannitol, 5 grams, and 1 ml of 4% alcoholic solution of brom cresol purple to each liter of nutrient agar.

The medium was melted and cooled to 45° C and inoculated with young cultures by needle stab method and was immediately cooled in ice-water. The tubes were incubated for 7 days and examined every 24 hours for changes in the color of the indicator (towards yellow) to determine anaerobic fermentation of mannitol.

Coagulase Test. Bacto-coagulase plasma (Difco) was used

for detecting coagulase activity. The desiccated plasma was reconstituted as per directions of the manufacturer.

A small loopful of 20 to 24 hr growth on proteose peptone agar slants was added to 0.5 ml of the reconstituted plasma and readings made at the end of 1, 3 and 24 hr incubations.

In all the tests mentioned above, known coagulase positive and negative reference strains were used as control.

RESULTS AND DISCUSSION

Bacteriological examination of 102 precooked frozen dessert-type food samples were made. The products comprised fifteen different varieties in five brands. Flavor, texture, color and general appearance examinations were made on each sample before the bacteriological examination. No abnormalities were detected.

Standard Plate Count. Standard plate count determinations were made after 48 hr incubation at 37° C. The data of the bacteriological population of the various food varieties examined are presented in Table 1, from which it would appear that out of 102 samples examined, 100 (98%) had plate counts below 100,000 per gram. Only two of the samples (1.96%), one each from banana cream pies and cream cheese cakes, had counts exceeding 100,000 per gram. None of the products examined showed counts above 200,000 per gram. Over 90 percent of the samples had counts below 41,000 per gram and 75 percent had counts varying in between 3,000 to 20,000 per gram whereas

Table 1. Standard plate count from precooked frozen pies and desserts.

Varieties	No. of Samples of Brand. Brand.	No.	No. of Samples Showing SPC/gram (in thousands) <3	3-20	21-40	41-60	61-80	81-100	101-200	Average SPC/g in thousands	Total Samples of different varieties	Average SPC/g (in thousands) of each variety
Strawberry cream pie	A	10		8			1	1		24.60	16	19.18
	B	6	1	4	1					10.16		
Banana cream pie	A	8		7				1		19.82		
	B	3		1	1				1	81.66	12	34.41
	C	1		1						11.00		
Lemon cream pie	A	8	1	6	1					7.00	12	5.62
	B	4	1	3						3.00		
Coconut cream pie	A	4		4						11.75		
	B	6		4	1	1				21.00	11	16.81
	C	1		1						12.00		
Coconut custard pie	A	10	1	9						10.0	10	10.00
Chocolate cream pie	A	7		4	2	1				23.43	9	20.33
	B	2		2						9.5		
Pecan pie	A	6	1	3	2					16.16	8	13.87
	B	2		2						14.00		
Neapolitan cream pie	A	7		7						7.85	7	7.85
Caramel cream pie	A	5		5						11.00	5	11.00
Cream cheese cake	D	5			1		1	2	1	92.00	5	92.00
Pumpkin pie	A	1		1						3.0	2	3.00
	C	1		1						3.0		
Pineapple cheese cake	D	2			2					30.00	2	30.00
Southern pecan pie	B	1		1						3.00	1	3.00
Mince pie	A	1		1						5.00	1	5.00
Apple crisp	E	1		1						4.00	1	4.00
Total		102	5	76	11	2	2	4	2			
Percent		100	4.9	74.48	10.78	1.96	1.96	3.92	1.96			
Cumulative %			4.9	79.38	90.16	92.12	94.08	98.0	100			

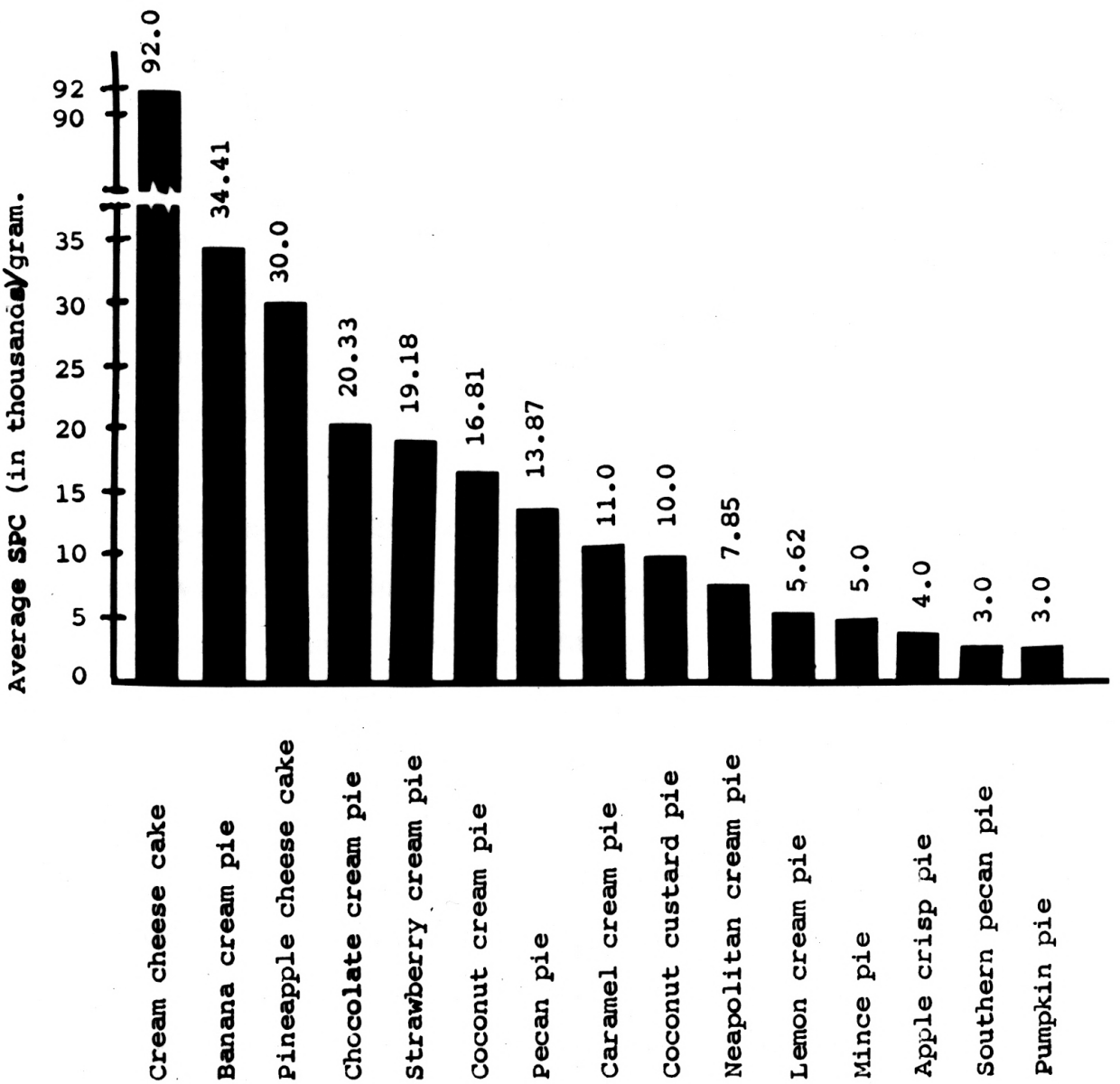
about 5 percent of the samples had plate counts less than 3,000 per gram. In resume, it could be said that these frozen food products had fairly low bacterial counts which confirms the finding of Huber et al (1958) who reported that 86 percent of the meat products and 88 percent of the vegetable products had total counts of less than 50,000 per gram and in both these products the distribution curves were skewed well towards the low count side (below 10,000 per gram). Kereluk and Gunderson (1959a) also found total bacterial counts under 25,000 per gram in 75 percent of most pies and below 50,000 in 83 percent of the samples. However, a few of the samples did show much higher counts. This sort of sudden rise of population in a few of the samples could quite logically be presumed due to transient thawing either during transport or while lying exposed for a longer period at a retailers shop. Similar observations in samples purchased from retail shops were made by Kereluk and Gunderson (1959a).

From data presented in Table 1 it would appear that no significant variation in the bacterial population was observed among the brands of the same variety except that the banana cream pies of brand "B" had considerably higher average counts than the other samples of brand "A" and "C". This could probably be due to the factors mentioned above. It was of interest to note that the products of brand "D", cream cheese cakes and pineapple cakes showed consistently higher counts. None of the samples of these varieties had plate counts between 3,000 to 20,000 per

gram, the range in which about 80 percent of the total samples were found. The containers of these samples were smaller and thicker in size and they contained fresh eggs, cream cheese cakes and baker's cheese cakes. A predominance of Gram positive spore-bearing and non-spore-bearing rods were observed in these varieties which might have been left undamaged during the subsequent process of cooking and freezing. Thus, the higher total counts in these products were probably due to different ingredients having a different microbial flora, different methods of processing and packing. However, no samples of these varieties belonging to other brands were available for brand-wise comparative study of the bacterial population.

Studies of the average bacterial population of different varieties of the samples revealed variations among them. The overall picture of the average bacterial population found in different varieties of the samples has been presented in Figure 1. It would appear that pumpkin pies and southern pecan pies had the minimum average plate count of 3,000 per gram, whereas cream cheese cakes had the maximum counts of 92,000 per gram. The second highest average bacterial population was 35,000 per gram found in banana cream pies. Pineapple cheesecakes had an average of 30,000 per gram and the remainder of the varieties had average counts below 21,000 per gram. Of the remaining 12 different varieties, nine had average counts less than 15,000 per gram and three of the varieties had average bacterial populations varying from 16,000 to 21,000 per gram. These

Figure 1. Average standard plate count (in thousands) per gram of different varieties of precooked frozen desserts and pies.



results coincide with the findings of Proctor and Phillips (1947) who observed considerable variations in bacterial populations of different products of same general characters. Ross and Thatcher (1958) also found average standard plate counts of 12,000 and 1,000 per gram in pumpkin pies and coconut pies, respectively, and stated that these products had comparatively much lower counts than the chicken, turkey and beef pies.

Coliform Count. Samples plated on violet red bile agar showing the appearance of purplish-red colonies about 0.5 mm or more in diameter, surrounded by a zone of precipitated bile after 20 to 24 hrs incubation at 37° C were taken presumptively as positive for the coliform group of organisms (Standard Methods, 1960).

Thirty-nine (38.2%) of the 102 samples gave a positive "presumptive test". Of 39 positive samples, 24 (23.32%) had coliform plate counts varying between 10 to 275 per gram, which is far above the limits generally accepted as satisfactory. This also suggests imperfect cooking and/or post cooking contamination and reflect the sanitary status of these foods. The rest of the 78 (76.5%) specimens showed numbers varying from a minimum of 0 to a maximum of five coliforms per gram. These findings were similar to the report of Kereluk and Gunderson (1959a) who detected the presence of coliform organisms in 30 percent of meat pies with counts varying from 0 to 154 per gram. Several other workers have reported a much higher number of coliform contaminants in precooked frozen meat products

(Buchbinder et al, 1949; Canale-Parola and Ordal, 1957).

Of 15 different varieties of precooked frozen pies and desserts examined, nine varieties were found contaminated with coliforms (Table 2). Among the positive varieties, 100 percent of samples of apple crisp, 63 percent of coconut cream pies and 58 percent of banana cream pies contained coliform organisms. The average number of coliforms per gram of the positive samples of these three varieties, were 10, 22.14 and 59.3, respectively. The positive samples of lemon cream pies and chocolate cream pies had average counts of 45 and 18 organisms per gram, respectively. All other varieties had an average count of less than 10 organisms of the coliform group per gram of positive samples.

Isolation and Identification. In this study, as defined in Standard Methods for the Examination of Dairy Products (APHA, 1960), all of the aerobic and facultative anaerobic, Gram negative, non-spore-forming, rod shaped bacteria which ferment lactose with gas formation within 48 hr incubation were taken as the members of the coliform group of organisms.

A total of 101 coliform organisms was isolated from the different samples which gave a positive presumptive coliform test on violet red bile agar. Their species were differentiated on the basis of lactose fermentation, indole, methyl red, Voges-Proskauer and sodium citrate (IMViC) tests. Of 101 isolates, 47 (46.5%) were indole and methyl red negative (-), Voges-Proskauer positive (+) and sodium citrate positive or

Table 2. Variety and the number of precooked frozen pies and desserts containing coliform organisms.

Variety of Samples	No. of Samples Examined	No. of Samples Containing Coliform No.	%	Coliform Positive Range	Count/gm Samples Average
Strawberry cream pie	16	9	56.25	5-20	9.4
Banana cream pie	12	7	58.3	5-275	59.3
Lemon cream pie	12	1	8.33	45	45
Coconut cream pie	11	7	63.63	10-45	22.14
Coconut custard pie	10	4	40.	5-5	5
Chocolate cream pie	9	5	55.55	5-60	18
Pecan pie	8	0	0	0	0
Neapolitan cream pie	7	3	42.85	5-10	8.33
Caramel cream pie	5	2	40	5-5	5
Cream cheese cake	5	0	0	0	0
Pumpkin pie	2	0	0	--	--
Pineapple cheese cake	2	0	0	--	--
Southern pecan pie	1	0	0	--	--
Mince pie	1	0	0	--	--
Apple crisp	1	1	100	10	10

negative (±) reactions. These were designated as members of species Aerobacter aerogenes variety I. Twenty (19.8%) of the isolates showed IMViC reactions of ±-++ and were identified as members of Aerobacter aerogenes var II. Fourteen (13.8%) gave IMViC reaction of -+-± and 9 (8.9%) were ++-+, and were designated as members of the species Escherichia freundii var I and II (Intermediates) respectively. Ten (9.9%) of the isolates which were differentiated as Escherichia coli Var II had the IMViC pattern of -+-- and one (.98%) of the cultures identified as Escherichia coli var I gave a ++-- reaction. As presented in Table 3, Aerobacter aerogenes var. I was isolated from samples number 1, 2, 4, 8, 24, 33, 42, 49, 50, 57, 74, 75, 77, 81, 82, 83, 86, 88, 89, 92, 98, and 102 and Aerobacter aerogenes var. II from samples 24, 40, 56, 62, 67, 83, 86, 89, 92, 95, 97, and 98. Samples number 2, 24, 30, 31, 43, 46, 56, 62, 63, 88, and 95 yielded Escherichia freundii var. I and Escherichia freundii var. II was isolated from samples 24, 35, 56, 67, 77, and 92. Escherichia coli var. I was isolated from sample number 92 and samples 20, 25, 28, 43, 54, 58, and 67 harbored Escherichia coli var. II. Twelve (11.76%) of these samples also contained staphylococci (Table 3).

Several workers have proved that the organisms of E. coli in general and E. coli var I (IMViC ++--) in particular, are of fecal origin (Thomson, 1955; Buttiaux and Mossel, 1961). Presence of these organisms in food and water has, therefore, been traditionally considered as an indicator of fecal contam-

Table 3. Number and species of coliform bacteria isolated from precooked frozen pies and desserts.

Specimen No.	Variety of Specimen	Species of Coliform isolated						Total No. of Isolates
		E. coli		E. freundii		Aerobacter aerogenes		
		Variety		Variety		Variety		
		I	II	I	II	I	II	
1	Apple crisp					2		2
28	Banana cream pie		2					2
31*	"			1				1
42*	"					1		1
62	"			1		1	3	5
67	"		1		1		1	3
86	"					5	2	7
89*	"					1	2	3
4*	Caramel cream pie					1		1
49	"					1		1
2	Chocolate cream pie			1		1		2
30	"			2				2
58	"		1					1
75	"					1		1
98*	"					5	3	8
25	Coconut cream pie		2					2
56	"			1	1		1	3
57*	"					2		2
77*	"				2	1		3
81	"					7		7
88	"			1		2		3
92*	"	1			2	1	2	6
8*	Coconut custard pie					1		1
33	"					1		1
50	"					1		1
102	"					1		1
24	Lemon cream pie			1	2	4	2	9
20	Neapolitan cream pie		2					2
74	"					2		2
97*	"						1	1
35	Strawberry cream pie				1			1
40	"						1	1
43	"		1	2				3
46*	"			1				1
54	"		1					1

Table 3. Continued.

Specimen No.	Variety of Specimen	Species of Coliform isolated						Total No. of Isolates
		E. coli		E. freundii		Aerobacter aerogenes		
		Variety		Variety		Variety		
		I	II	I	II	I	II	
63	Strawberry cream pie			2				2
82	"					2		2
83	"					3	1	4
95*	"			1			1	2
	Total	1	10	14	9	47	20	101

* Also yielded staphylococci.

ination. A. aerogenes and A. cloacae strains, and intermediate types of coliforms have been found as normal bacterial flora of soil and vegetables (Thomas and Hobson, 1955) and they occur only rarely in the human intestine (Buttiaux and Mossel, 1961).

Of 102 samples examined, eight (7.84%) were found contaminated with E. coli var I and II, (in a small percentage of the total bacterial flora). The remaining 31 (30.32%) of the positive samples showed a predominance of the organisms of probable non-fecal origin (Table 3). Fecal contamination in such a large percentage (7.84%) of precooked frozen food may be considered most undesirable both from an aesthetic as well as a public health hazard point of view. Ross-Doreen and Thatcher (1958) also found evidences of E. coli in pumpkin pies with an average of 738 organisms per gram.

All the subsurface deep red colonies with a zone of precipitated bile, encountered on violet red bile agar were found to be the members of the coliform group. A majority (about 80%) of these formed "typical" (about 1 mm or more) colonies but a small percentage also formed "small" colonies (about 0.5 mm or less). Only on a few occasions were colonies of other Gram negative rods encountered on violet red bile agar but they formed small dark red circular or lenticular colonies without any reddish zone of precipitated bile and were easily differentiated from the coliform colonies. This confirms the findings of Druce et al (1957) who found almost similar results with the use of violet red bile agar while determining the coli-

form content of milk. Hartman (1958) also reported that 71% of the "small" colonies on violet red bile agar of frozen pot pies belonged to the genera of Escherichia and Aerobacter. The colonial characters of Escherichia and Aerobacter group of organisms on EMB agar were very similar to the descriptions given in the Difco Manual (1953) although, certain variations were found. On EMB agar, out of 34 isolates of the E. coli and E. freundii group, 16 (47%) formed small (about 1 to 2 mm), circular, entire, slightly raised to flat, smooth glistening colonies with a metallic sheen, while 13 (38%) were medium (2 to 4 mm), circular, entire, concave, smooth, glistening with little metallic sheen in the center and 5 (15%) formed medium, circular, irregular, concave colonies with a dark center and light periphery having little or no metallic sheen. Three types of colonial characters were observed on EMB agar among the organisms of Aerobacter aerogenes var I and II. The majority of these formed medium (2 to 6 mm), circular, raised, entire, butyrous colonies with dark center and light periphery. Some of them were large, circular, raised, mucoid colonies with a tendency to coalesce and having a dark center with a light periphery. A small percentage of them formed small, raised, circular, with dark center, light periphery, less mucoid with a metallic sheen in the center, being similar to the bigger colonies of Escherichia.

Staphylococcus. Facultatively anaerobic, catalase positive, non-motile, non-spore bearing, Gram positive spherical

cells usually arranged in irregular clusters were identified as members of the genus Staphylococcus (Evans et al, 1955; and Breed et al, 1957).

Of 102 frozen pies examined, 28 (27.45%) contained staphylococci in numbers varying from a minimum of 10 to a maximum of 100 per gram. Out of 15 different varieties of specimens, all except four varieties, viz. cream cheese cakes, pumpkin pies, pineapple cheese cakes and southern pecan pie, harbored staphylococci. The percentage of contamination, among the eleven different varieties harboring staphylococci varied from 11 percent to 100 percent. Average numbers of staphylococci per gram of the positive samples were found to be highest (50/gm) in caramel cream pies, pecan pies and apple crisp. Coconut custard pies, coconut cream pies and mince pies had an average of 46, 42 and 40 staphylococci, respectively, per gram of the positive samples while the remainder had an average of 30 or less per gram of the positive samples as shown in Table 4.

Seventy-six cultures of catalase positive staphylococci showing uniform growth throughout the deep shake tubes of glucose yeast extract agar were isolated from different positive samples. These isolates were studied for the characteristics usually associated with enterotoxigenic staphylococci such as coagulase activity, chromogenesis, gelatinase activity, hemolysin reaction and anaerobic fermentation of mannitol.

Coagulase Activity. Using Bacto-coagulase plasma, all the isolates, along with a known positive and negative strain, were

Table 4. Staphylococcal count of precooked frozen pies and desserts.

Varieties	Total No. of Samples Examined	Samples Containing Staphylococci No.	%	Staphylococci/gram of Positive Samples Range	Average
Strawberry cream pie	16	3	18.75	20-50	30
Banana cream pie	12	5	41.66	10-30	18
Lemon cream pie	12	2	16.66	10-40	25
Coconut cream pie	11	5	45.45	10-80	42
Coconut custard pie	10	3	30	20-100	46.33
Chocolate cream pie	9	1	11.11	10	10
Pecan pie	8	1	12.5	50	50
Neapolitan cream pie	7	3	42.85	20-30	23.33
Caramel cream pie	5	3	60.0	20-80	50
Cream cheese cake	5	0	0	0	0
Pumpkin pie	2	0	0	0	0
Pineapple cheese cake	2	0	0	0	0
Southern pecan pie	1	0	0	0	0
Mince pie	1	1	100	40	40
Apple crisp	1	1	100	50	50

tested for coagulase production but none of these proved to be coagulase positive. This coincides with the findings of Ross-Doreen and Thatcher (1958) who found pumpkin and coconut pies free from coagulase positive staphylococci but the average staphylococcal content in these samples were 38 to 26 per gram, respectively. Efforts were made to find other references to the various other varieties of products studied, but with no avail.

Since most enterotoxigenic staphylococci have been found to be members of the coagulase positive group (Evans and Niven, 1950; Clark et al, 1961), coagulase production has been considered probably the most reliable diagnostic test for detecting the potential food poisoning type of staphylococci (Recommended Methods for the Microbiological Examination of Foods, 1958). It has been indicated that the necessary level of enterotoxin was produced only after the growth of staphylococci to a population level of several millions (Tanner and Tanner, 1953; Frazier, 1958) and food products having upward of half a million coagulase positive staphylococci per gram should be considered a public health hazard (Recommended Methods for the Microbiological Examination of Foods, 1958). The presence of coagulase negative strains of staphylococci in these foods in numbers varying up to 100 per gram may not, therefore, be of much significance so far as food poisoning is concerned. However, this may not preclude the possibility of the presence of enterotoxigenic strains of staphylococci in these foods nor

can it eliminate the possibility of the presence of enterotoxin which might have been formed in these foods prior to cooking and freezing.

These results point out the need for reliable and simple methods for detecting the presence of enterotoxin in foods and also for the isolation and identification of enterotoxigenic strains of staphylococci.

Chromogenesis. Of 76 isolates (coagulase negative staphylococci), 33 (43%) produced yellow pigment, 16 (21%) were orange pigmented and the remaining 27 (36%) were white. This is in agreement with the findings of Hussemann and Tanner (1949) who found 22% of the coagulase negative strains isolated from foods yellow pigmented, 7% orange and 32% white. Mossel (1962) also observed the production of these pigments in coagulase negative strains isolated from food products and other non-clinical sources and concluded that chromogenesis had limited importance as a diagnostic criterion for coagulase positive staphylococci.

Studies of the different physiological characteristics of all these isolates revealed that out of 16 orange pigmented strains, 3 (18.75%) fermented mannitol anaerobically within 48 hrs whereas only 3 percent and 3.7 percent of the yellow and white strains, respectively, showed this reaction in mannitol agar tubes. Aerobic or delayed partial fermentation in mannitol agar tubes was observed in 30.3 percent of the yellow strains and in 14.8 and 25 percent of white and orange strains. These

Table 5. Physiological characteristics of yellow, white and orange pigment producing staphylococci isolated from frozen food.

	33 Yellow Pigmented Strains			27 White Strains			16 Orange Pigmented Strains		
	Pos.	Neg.	% Pos.	Pos.	Neg.	% Pos.	Pos.	Neg.	% Pos.
Fermentation in B.C.P. Mannitol stab agar									
(a) anaerobic	1	32	3	1	26	3.7	3	13	18.75
(b) aerobic or delayed partial anaerobic	10	23	30.3	4	23	14.8	4	12	25
Hemolysis on Sheep blood agar	16	17	48.5	14	13	51.8	13	3	81.25
(a) (Semiclear) Alpha	9	24	27.3	4	23	14.8	8	8	50
(b) (Clear) Beta	6	27	18.2	9	18	33.3	4	12	25
(c) Hot Cold lysis	1	32	3	1	26	3.7	1	15	6.25
Gelatin hydrolysis	18	15	54.5	11	16	40.7	10	6	62.5

characteristics were observed in such a small number of strains that the difference in percentages may not be considered of any significance. The pattern of hemolysis on sheep blood agar produced by the yellow, white and orange pigmented strains varied. Of 16 orange pigmented strains, 50 percent produced Alpha type, 25 percent Beta type and 6.25 percent "hot cold" hemolysis. Clear zone of hemolysis (β) was showed by 33.3 percent of white strains whereas 14.8 percent of these showed Alpha type and 3.7 percent produced hot-cold lysis. Of the yellow strains, 27.3 percent produced Alpha type hemolysis, 18.2 and 3 percent showed Beta type and hot cold lysis. Table 5 summarizes the different physiological characteristics observed of the yellow, white and orange pigmented strains of staphylococci isolated during the course of this study. It would appear from Table 5 that 62.5 percent of the orange pigmented strains and 40.7 and 54.5 percent of the white and yellow strains, respectively, hydrolysed gelatin.

Mannitol Fermentation. Of the 76 isolates, 5 (6.5%) fermented mannitol anaerobically within 48 hr incubation at 37° C and 18 of these (23.6%) fermented this polyol in the upper 2/3 of the tubed medium, when incubation was continued for seven days. The rest of the cultures showed no fermentation in this medium even after continued incubation for 15 days. These findings confirmed the earlier observations made by several workers (Mossel, 1962; Baird-Parker, 1963) that only a very low percentage of the coagulase negative strains show anaerobic

Table 6. Physiological behavior of mannitol positive and negative staphylococci.

	Mannitol fermenter strain				53 Mannitol non-fermenter		76 Mannitol fermenter as well as non-fermenter	
	5 Fermented within 48 hours		18 Delayed (after 48 hr) and partial fermenter					
	No.	%	No.	%	No.	%	No.	%
Pigment production								
(a) White	1	20	4	22.2	22	41.6	27	35.4
(b) Yellow	1	20	10	55.5	22	41.6	33	43.2
(c) Orange	3	60	4	22.2	9	17.	16	21
Hemolysis on Sheep blood agar	5	100	9	50	29	54.5	43	56.3
(Semi clear) Alpha	2	40	5	27.75	14	26.3	21	27.5
(Clear) Beta	3	60	3	16.65	13	24.44	19	25
Cold lysis	0	0	1	5.5	2	3.75	3	4
Gelatin hydrolysis	3	60	11	60.5	25	47.2	39	51

breakdown of this sugar alcohol. However, Mossel, 1962, observed that 39.0% of the coagulase negative strains oxidized mannitol in the upper layer of the medium. Among the mannitol fermenters three were orange pigmented and one each were white and yellow pigmented, respectively (Table 6). All 5 strains (100%) produced hemolysis on sheep blood agar plates, of which 40 percent showed Alpha type lysis and the remainder (60%) produced clear zones of Beta type hemolysis. In contrast to these, only 54.5 percent of the mannitol non-fermenter strains and 50 percent of the delayed mannitol fermenter strains produced lysis on sheep blood agar. Hydrolysis of gelatin on Frazier's gelatin agar was produced by only 60 percent of the mannitol fermenter strains and 60.5 percent by delayed mannitol fermenter strains whereas 47.2 percent of the mannitol non-fermenters also produced this reaction (Table 6). These findings showed a slightly higher frequency of hemolysis and gelatin hydrolysis in mannitol fermenter strains than the non-mannitol fermenters. The number of mannitol fermenters was so small that these differences may not be of much significance. Not much variation in these characteristics was observed between the delayed mannitol fermenters and non-mannitol fermenters.

Hemolysis. After 36 to 48 hrs incubation at 37° C two types of hemolysis were usually observed on sheep blood agar plates around the line of inoculum: (1) a zone of discoloration or a semiclear zone; (2) broad or thin clear zone of complete hemolysis. They have been described here as Alpha and Beta

hemolysis, respectively. For the observation of "hot cold" lysis all the plates were kept overnight in a refrigerator at 4^{o} to 8^{o} C. In some of the plates discoloration was replaced by complete laking or a clear zone and has been termed cold lysis.

From Table 6 it would appear that 56.3 percent of the 76 isolates produced hemolysis on sheep blood agar plates. Out of these, 27.5 percent showed Alpha type, 25 percent Beta type and 4 percent hot-cold lysis. Hussmann and Tanner (1949) also observed that 85% of the coagulase negative strains produced hemolysis on rabbit blood agar.

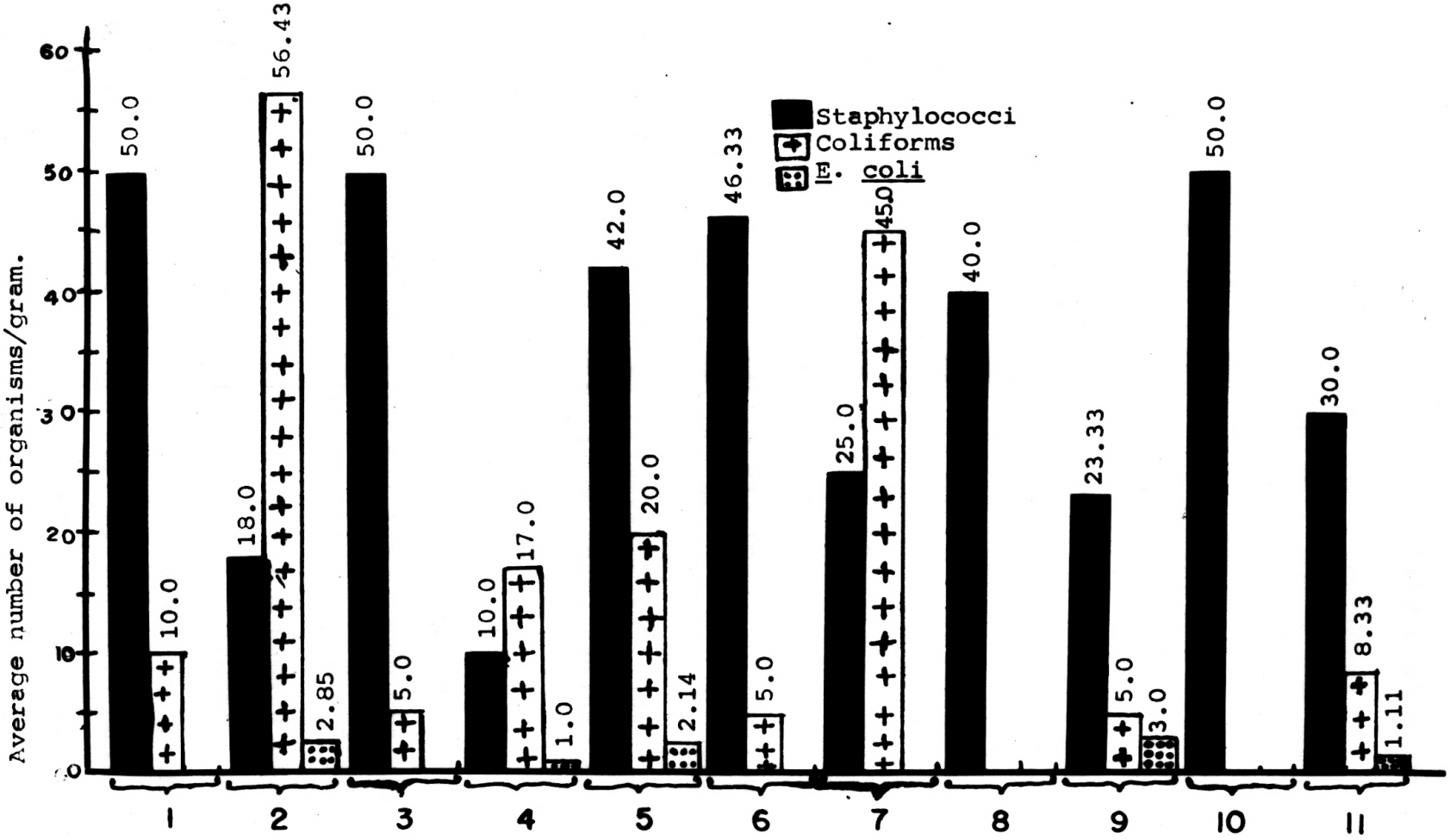
Gelatin Hydrolysis. It would appear from Table 6 that 39 cultures, respresenting 51 percent of the total isolates, were gelatinase positive. All these cultures showed evidence of gelatin liquefication on S-110 medium as well as on Frazier's gelatine agar, except 2 cultures which showed slow gelatin liquefaction only on the latter medium. These figures are qualitatively, in agreement with the results of Mossel (1962) who found that 75.6 of the coagulase negative strains of staphylococci liquefied gelatine.

An overall picture of E. coli, coliforms and staphylococcal content per gram of the positive samples of various varieties has been presented in Figure 2. It shows that the staphylococcal content was higher in all the varieties excepting banana, lemon and chocolate cream pies. All those varieties which were found contaminated with coliform showed evidence of staphylococcal contamination as well. On the contrary, positive

EXPLANATION OF FIGURE 2

Average number of staphylococci, coliforms and *E. coli* per gram of the positive samples of different varieties of precooked frozen dessert-type foods. 1. Apple crisp. 2. Banana cream pie. 3. Caramel cream pie. 4. Chocolate cream pie. 5. Coconut cream pie. 6. Coconut custard pie. 7. Lemon cream pie. 8. Mince pie. 9. Neapolitan cream pie. 10. Pecan pie. 11. Strawberry cream pie.

Figure 2


Average number of organisms/gram.
0
10
20
30
40
50
60
Staphylococci
Coliforms
E. coli
50.0
10.0
18.0
56.43
2.85
50.0
5.0
10.0
17.0
1.0
42.0
20.0
2.14
46.33
5.0
25.0
45.0
40.0
23.33
5.0
3.0
50.0
30.0
8.33
1.11
1
2
3
4
5
6
7
8
9
10
11

samples of pecan pie and mince pie, having fairly high staphylococcal content, showed no evidences of coliforms.

It is a well known fact that the skin and mucous membrane of man and animals are the natural habitat of staphylococci. The existence of large numbers of staphylococci and coliforms has also been found associated with insanitary plant conditions (Huber et al, 1958). Among these two common contaminants, staphylococci have been known to be comparatively resistant to adverse environmental circumstances like cold storage and exposure to higher temperatures. It is, therefore, quite likely that they survived in larger numbers even though the focal point of contamination might have been the same.

E. coli of probable fecal origin was found in small proportions of the coliform population in five of the varieties. The varieties showing evidences of probable fecal contamination, in order of highest to lowest, were neapolitan, banana, chocolate, coconut and strawberry cream pies.

Correlation of the Bacterial Counts. An attempt was made to examine the extent of correlation existing among the three determinations made. From the data compiled in Table 7 it appeared that in a majority of the varieties, standard plate counts were associated with comparable coliform counts. But comparatively lesser correlations existed between the standard plate counts and staphylococcal counts. However, in many of the cases, great variation appeared, viz. cream cheese cakes had the highest average standard plate counts (92,000 per gram) but the

Table 7. Bacteriological content of the frozen pies and desserts.

Type of products	No. of Sample Tested	S.P.C./gram Range (Thousands)	S.P.C./gram Average (Thousands)	Average No. coliform/gram Range	Average No. coliform/gram Average	Average No. of staphylococci/gram Range	Average No. of staphylococci/gram Average
Strawberry cream pie	16	2.0- 94.0	19.18	0- 15	5.30	0- 50	4.4
Banana cream pie	12	4.0-191.0	34.41	0-275	34.60	0- 30	7.5
Lemon cream pie	12	1.5- 23.0	5.62	0- 45	3.75	0- 40	4.17
Coconut cream pie	11	4.0- 59.0	16.81	0- 45	14.09	0- 80	19.0
Coconut custard pie	10	2.0- 19.0	10.0	0- 5	2.0	0-100	14.0
Chocolate cream pie	9	7.0- 55.0	20.33	0- 60	10.0	0- 10	1.1
Pecan pie	8	2.0- 40.0	13.87	0- 0	0.0	0- 50	6.25
Neapolitan cream pie	7	3.0- 16.0	7.85	0- 10	3.56	0- 30	10.0
Caramel cream pie	5	3.0- 20.0	11.00	0- 5	2.0	0- 80	30.0
Cream cheese cake	5	33.0-184.0	92.00	0	0	0 0	0
Pumpkin pie	2	3.0	3.0	0	0	0 0	0
Pineapple cheese cake	2	23.0- 37.0	30.0	0	0	0 0	0
Southern pecan pie	1	3.0	3.0	0	0	0 0	0
Mince pie	1	5.0	5.0	0	0	40	40.0
Apple crisp	1	4.0	4.0	10	10	50	50.0

lowest (0) average coliform and staphylococcal counts. Similar variations were observed in the case of apple cheese cakes which had average standard plate counts of 30,000 per gram but were associated with zero coliform and staphylococci counts. On the contrary caramel cream pies, mince pies and apple crisp had the highest staphylococcal counts (30, 40 and 50 per gram) but their standard plate counts were as low as 11,000, 5,000 and 4,000 per gram. Similar divergences appeared between the staphylococcal counts and coliform counts and vice versa in caramel cream pies and pecan pies.

In resume, it could be said that there was no appreciable correlation between standard plate counts, coliform and staphylococcal counts in precooked frozen dessert-type foods studied. This conforms with the findings of Litsky et al (1957) who reported that there was no apparent correlation between SPC, coliform and enterococci MPN in precooked frozen tuna, chicken and beef pies collected at the retail store level. Ross-Doreen and Thatcher (1958) also found an average SPC count of 12,000 per gram associated with an average of 738 E. coli count and 38 coagulase negative staphylococci per gram in pumpkin pies.

The above-mentioned findings make one wonder whether it would be reasonable to pin point any particular reason for this variation especially when all the possible variable factors that can come into the picture are duly considered. The history of the products obtained from the retailer's shelf and studied was unknown. Each variety had a varied composition and probably a

varied natural microbial flora, even in a single variety the samples were of different brands. Moreover, the number of samples studied was also not sufficient upon which to base a conclusion so far as the correlation between the different determinations are concerned. However, the present study gives an insight into the problem and opens up vistas for future studies of such products from various viewpoints. In order to have a better understanding as far as the different bacteriological content of the various varieties of food products are concerned, perhaps it would be more informative and fruitful to study significantly large number of samples of each variety and brand obtained from larger numbers of retail store levels. Perhaps it would be more interesting to study the bacteriological contents of the three important components of each product separately, e.g., the crust, the fillings, and the topping, to obtain a better insight into the bacteriological status of each of the components and possibly explain the cause of the variations in the bacteriological contents of the different varieties.

Recoveries of E. coli from these foods, though in small numbers, point to the possibility of the presence of other enteric pathogens and demands exploratory efforts for the search of organisms causing potential health hazards like Salmonella, enterococci, clostridia and staphylococci.

In the end, it can be said that the present study which was taken as a screening test for a particular type of most commonly consumed food items, has been very informative and fruitful, and

and opened up new avenues of approaching one of the most common sources of possible public health problems.

SUMMARY

The sanitary status of several varieties of precooked frozen dessert type foods was determined. One hundred two samples were collected from the consumer's level from the local stores (Manhattan, Kansas) for this study. Methods of obtaining and processing were described. Foods belonged to 15 different varieties and 5 different brands. They were studied for their total bacterial population, densities of coliform, E. coli and staphylococci of public health significance.

Employing Standard Methods (APHA, 1960), total bacterial population and coliform plate counts were estimated. The isolation procedure for staphylococci and their follow up has been described and discussed.

All samples, excepting two, showed standard plate counts of less than 100,000 per gram and a majority (90%) showed counts below 50,000 per gram. Thirty-nine (38.2%) of the samples contained coliform and 8 (7.84%) harbored E. coli. Coliform counts of 10 and above were observed in 24 (23.32%) of the samples. Twenty-eight (27.45%) of the specimens showed contamination by staphylococci though none of the isolates proved to be coagulase-positive strains. Altogether 101 identifiable organisms of the coliform group and 76 strains of staphylococci were isolated from these foods. Their physiological and

biochemical characters were studied and described.

The results of the determinations obtained were described and compared with the findings of other workers. Standard plate count data of the various varieties of samples were analysed; and variations in the counts among the different varieties of the samples, as also among the samples of the same variety belonging to different brands, were studied and discussed. Coliform counts and the densities of E. coli and staphylococci were compared. Comparative pictures of coliform and staphylococcal contamination of each variety of the samples and possible reasons for the variations were presented. The correlations were presented between the standard plate counts vs. coliform and staphylococcal counts.

The presence of E. coli in 7.8% of these food samples was pointed out and the possibilities of the presence of other gastrointestinal pathogens as a possible source of public health hazard were discussed.

The need for further investigations was suggested and several approaches were presented.

ACKNOWLEDGEMENT

The author is greatly indebted to his major advisor, Professor V. D. Foltz, for his advice and guidance throughout this work. His suggestions and encouragement had a great deal to do with the successful completion of the work as well as the preparation of this manuscript.

Thanks also are due to Dr. T. H. Lord for his invaluable counseling and advice extended all through this work.

LITERATURE CITED

Abrahamson, A. E. 1960. An official looks at sanitation. J. Milk and Food Tech. 23:72-75.

Baird-Parker, A. C. 1962. An improved diagnostic and selective medium for isolating coagulase positive staphylococci. J. Appl. Bact. 25:12-19.

_____. 1963. A classification of micrococci and staphylococci based on physiological and biochemical test. J. Gen Microbiol. 30:409-427.

Barber, M. A. 1914. Milk poisoning due to a type of Staphylococcus albus occuring in the udder of a healthy cow. Phillipine. J. Sci. 9:515-519. (Cited by Haynes and Hucker, 1946).

Bartley, C. H., and L. W. Slanetz. 1960. Types and sanitary significance of fecal streptococci isolated from feces, sewage and water. Am. J. Publ. Hlth. 50:1545-1552.

Bartram, M. T., and L. S. Black. 1936. Detection and significance of coliform group in milk. I. A comparison of media for use in isolation. Food. Res. 1:551-563.

Berry, J. A. 1934. Cold-tolerant microorganisms and frozen pack. Canner. 78(11). 13-14.

Borgstrom, G. 1955. Microbiological problem of frozen food products. Advances in Food Res. 6:163-230.

Breed, Robert. S., E. G. D. Murrey, and Nathan R. Smith. 1957. Bergey's manual of determinative bacteriology. 7 ed. Baltimore: Williams and Wilkins.

Briggs, C. A. E., J. M. Willingale., R. Braude., and K. G. Mitchell. 1954. The normal intestinal flora of the pig. I. Bacteriological methods for quantitative studies. Vet. Rec. 66:241-242.

Buchbinder, Leon., V. Loughlin., M. Walter., and G. Dangler. 1949. A survey of frozen precooked foods with special reference to a chicken a la king. J. Milk and Food Tech. 12:209-231.

Burton, M. O. 1949. Comparison of coliform and enterococcus organisms as indices of pollution in frozen foods. Food Res. 14:434-438.

Buttiaux, R. 1959. The value of the association Escherichieae-group D, streptococci in the diagnosis of contamination in food. J. Appl. Bact. 22:153-158.

Buttiaux, R., and D. A. A. Mossel. 1961. The significance of various organisms of faecal origin in foods and drinking water. J. Appl. Bact. 24:353-364.

Canale-Parola, E., and Z. H. Ordal. 1957. A survey of the bacterial quality of frozen poultry pies. Food Tech. 11:580-582.

Carter, C. H. 1960. Egg yolk agar for isolation of coagulase positive staphylococci. J. Bact. 79:753-755.

Chapman, G. H., C. Berens., A. Peters., and L. Curcio. 1934. Coagulase and hemolysin tests as measures of the pathogenicity of staphylococci. J. Bact. 28:343-363.

Chapman, G. H., C. W. Lieb., and L. C. Curcio. 1937. Isolation and cultural differentiation of food poisoning staphylococci. Food Res. 2:349-367.

Chapman, G. H. 1945. The significance of sodium chloride in studies of staphylococci. J. Bact. 50:201-203.

_____. 1946. A single culture medium for selective isolation of plasma-coagulating staphylococci and for improved testing of chromogenesis, plasma-coagulation, mannitol fermentation and the Stone reaction. J. Bact. 51:409-410.

Clark, W. S., T. D. Moore,, and F. E. Nelson. 1961. Characterization of coagulase positive staphylococci isolated from milk. Appl. Microbiol. 9:195-199.

Clement, M. T. 1961. Effects of freezing, freeze-drying, and storage in the freeze-dried and frozen state on viability of Escherichia coli cells. Can. J. Microbiol. 7:99-106.

Cruickshank, R. 1937. Staphylocoagulase. J. Path. Bact. 45:295-303.

Dack, G. M., W. E. Cary., O. C. Woolpert., and H. Wiggers. 1930. An outbreak of food poisoning proved to be due to a yellow haemolytic staphylococcus. J. Prev. Med. 4:167-175.

Dack, G. M., E. Wheaton., M. N. Mickelson., and M. N. Schuler. 1960. Public health significance of microorganisms in frozen pot pies. Quick Frozen Foods. 22:44-45, 160, 162.

Daucer, Carl. C. 1961. 1960 Summary of disease out-breaks and a 10 year resume. Publ. Hlth. Rept. 76:915-922.

Difco manual of dehydrated culture media and reagent for microbiological and clinical laboratory procedure, 1953. 9th ed. Difco Laboratories. Detroit 1, Michigan.

Dolman, C. E., R. J. Wilson., and W. H. Cockcroft. 1936. A new method of detecting staphylococcus enterotoxin. Can. Publ. Hlth. J. 27:489-493.

Dolman, C. E., and R. J. Wilson. 1940. The kitten test for staphylococcus enterotoxin. Can. Publ. Hlth. J. 31:68-71.

Druce, R. G., N. B. Bebbington., K. Elson., J. M. Harcombe., and S. B. Thomas. 1957. The determination of the coli-aerogenes content of milk and dairy equipment by plating on violet red bile agar incubated at 30° C. J. Appl. Bact. 20:1-10.

Edwards, P. R., and W. H. Ewing. 1962. Identification of Enterobactericeae. Burgess Publishing Co., Minneapolis.

Evans, T. B., and C. F. Niven (Jr.). 1950. A comparative study of known food-poisoning staphylococci and related varieties. J. Bact. 59:545-550.

Evans, J. B., W. L. Bradford., and C. F. Niven (Jr.). 1955. Comments concerning the taxonomy of the genera Micrococcus and Staphylococcus. Intern. Bull. Bacteriol. Nomen. and Taxon. 5:61-66.

Frazier, W. C. 1926. A method for the detection of changes in gelatine due to bacteria. J. Inf. Disease. 39:302-309.

_____. 1958. Food microbiology. McGraw-Hill Book Company, Inc., New York.

Gillespie, W. A., and V. G. Alder. 1952. Production of opacity in egg-yolk media of coagulase-positive staphylococci. J. Path. and Bact. 64:187-200.

Goresline, H. E. 1959. Obtaining precooked frozen foods of high sanitary quality for the armed forces. Assoc. Food and Drug officials. U. S. Quart. Bull. 23:135-139. (Cited by Elliott, R. P., and H. D. Michener. 1961. Appl. Microbiol. 9:452-468).

Gunderson, M. F., and K. D. Rose. 1948. Survival of bacteria in a precooked fresh-frozen food. Food Res. 13:254-263.

Haines, R. B. 1938. The effect of freezing on bacteria. Proc. Roy. Soc. B-124:451-63. (Original not seen. Cited by Borgstrom, G. 1955).

Hartman, P. A. 1958. The selectivity of autoclave-sterilized violet-red-bile-agar. Food Res. 23:532-535.

_____. 1960. Further studies on the selectivity of violet-red-bile-agar. J. Milk and Food Tech. 23:45-48.

Hartman, P. A., and D. V. Hunterberger. 1961. Influence of subtle differences in plating procedure on bacterial counts of prepared frozen food. Appl. Microbiol. 9:32-38.

Hartsell, S. E. 1951. The longivity and behaviour of pathogenic bacteria in frozen foods: The influence of Plating media. Am. J. Publ. Hlth. 41:1072-1077.

Haynes, W. M. C., and G. H. Hucker. 1946. A review of micrococcus enterotoxin food poisoning. Food Res. 11:281-297.

Heller, C. L. 1951. Towards microbiological standards for manufactured foods. Proc. Soc. Appl. Bact. 14:15-22.

Hopton, J. 1961. A selective medium for the isolation and enumeration of coagulase positive staphylococci from foods. J. Appl. Bact. 24:121-124.

Huber, D. A., H. Zaborowski., and M. M. Raymon. 1958. Studies on the microbiological quality of precooked frozen meals. Food Tech. 12:190-194.

Hucker, G. T., R. F. Brooks., and A. J. Emery. 1952. The source of bacteria in processing and their significance in frozen vegetables. Food Tech. 6:147-155.

Hussemann, Dorothy L., and Fred W. Tanner. 1949. A comparison of strains of staphylococcus isolated from food. Food Res. 14:91-97.

Hussemann, D. L. 1951. Effect of cooking on the bacteriological flora of selected frozen precooked foods. Am. Dietet. Assoc. J. 27:855-858.

Ingram, M. 1951. The effect of cold on microorganisms in relation to food. J. Appl. Bact. Proc. 14:243-260.

_____. 1961. Microbiological standards of foods. Food Tech. 15:4-6, 8-9, 12.

Innes, A. G. 1960. Tellurite-egg agar, a selective and differential medium for the isolation of coagulase positive staphylococci. J. Appl. Bact. 23:108-113.

Jones, A. H., and W. E. Ferguson. 1951. A study of methods of preparing food products for microbiological analysis. Food Res. 16:126-132.

Kachikian, R., C. R. Fellers, and Warren Litsky. 1959. A bacterial survey of commercial frozen breaded shrimp. J. Milk and Food Tech. 22:310-312.

Keith, S. C., (Jr.). 1913. Factors influencing the survival of bacteria at temperatures in the vicinity of the freezing point of waters. Science. 37:877-879.

Kereluk, K., and M. F. Gunderson. 1959a. Studies on the bacteriological quality of frozen meat pies. I. Bacteriological survey of some commercial frozen pies. Appl. Microbiol. 7:320-323.

_____. 1959b. Studies on the bacteriological quality of frozen meat pies. Appl. Microbiol. 7:327-328.

_____. 1959c. Studies on the bacteriological quality of frozen meat pies. II. A comparison of the methods for the examination of coliforms. J. Milk and Food Tech. 22:176-178.

Larkin, E. P., Warren Litsky., and J. E. Fuller. 1955. Fecal streptococci in frozen foods. I. A bacteriological survey of some commercially frozen foods. Appl. Microbiol. 3:98-101.

Litsky, Warren., I. S. Fagerson., and C. R. Fellers. 1957. A bacteriological survey of commercially frozen beef, poultry and tuna pies. J. Milk and Food Tech. 20:216-219.

Logan, P. R., C. H. Harp., and W. F. Dove. 1951. Keeping quality of precooked frozen chicken a la king, a bacteriological evaluation of hot and cold packs. Food Tech. 5:193-198.

Lord, T. H. 1959. Determinative bacteriology, Laboratory Manual. Burgess Publishing Co., Minneapolis.

Ludlam, G. B. 1949. A selective medium for the isolation of Staphylococcus aureus from heavily contaminated material. Mon. Bull. Minist. Hlth. 8:15-20.

Machala, W. E. 1961. A bacteriological investigation of frozen foods in the Oklahoma City area. J. Milk and Food Tech. 24:323-327.

McClesky, C. S., and W. N. Christopher. 1941. Some factors influencing the survival of pathogenic bacteria in cold-pack strawberries. Food Res. 6:327-333.

McFarlane, V. H. 1940. Behavior of microorganisms at sub-freezing temperatures. I. Freezing redistribution studies. Food Res. 5:43-57.

_____. 1942. Behavior of microorganisms in fruit juices and in fruit juice sucrose solutions stored at -17.8° C (0° F). Food Res. 7:509-518.

Miller, N. J., and P. S. Prickett. 1938. Note on violet-red-bile-agar for detection of Escherichia coli. J. Dairy Sci. 21:559-560.

Mossel, D. A. A. 1962. Attempt in classification of catalase-positive staphylococci and micrococci. J. Bact. 84:1140-1147.

Mundt, J. O., A. H. Johnson., and R. Kachikian. 1958. Incidence and nature of enterococci on plant materials. Food Res. 23:1-8.

Ostrolenk, M., and A. C. Hunter. 1946. The distribution of enteric streptococci. J. Bact. 51:735-741.

Phillips (Jr.), A. W., and B. E. Proctor. 1947. Growth and survival of an experimentally inoculated Staphylococcus aureus in frozen precooked foods. J. Bact. 54:49(Abstract).

Proctor, B. E., and A. W. Phillips (Jr.). 1947. Micro-biological aspects of frozen precooked foods. Refrig. Eng. 53:30-33.

_____. 1948. Frozen precooked foods. Am. J. Publ. Hlth. 38:44-49.

Raj, H., and J. Liston. 1961a. Survival of bacteria of public health significance in frozen sea food. Food Tech. 15:429-434.

_____. 1961b. Detection and enumeration of fecal indicator organisms in frozen sea foods. I. Escherichia coli. Appl. Microbiol. 9:171-174.

Recommended methods for the microbiological examination of foods. 1958. Am. Publ. Hlth. Assoc. Inc., 1790 Broadway, New York.

Ross-Doreen, A., and F. S. Thatcher. 1958. Bacteriological content of marketed precooked frozen foods in relation to public health. Food Tech. 12:369-371.

Standard methods for the examination of dairy products. 1960. 11th ed. Am. Publ. Hlth. Assoc. Inc., 1790 Broadway, New York.

Standard methods for the examination of water and waste water. 1960. 11th ed. Am. Publ. Hlth. Assoc. Inc., 1790 Broadway, New York.

Silverman, G. J., J. T. R. Nickerson., D. W. Duncan., N. S. Davis., and M. M. Joselow. 1961a. Microbial analysis of frozen raw and cooked shrimp. I. General results. Food Tech. 15:455-458.

Silverman, G. J., N. S. Davis., J. T. R. Nickerson., D. W. Duncan., I. Tezcan., and M. Johnson. 1961b. Microbial analysis of frozen raw and cooked shrimp. II. Certain characteristics of Staph. isolates. Food Tech. 15:458-464.

Stone, R. V. 1935. A cultural method for classifying staphylococci as of the "food poisoning" type. Proc. Soc. Exper. Biol. and Med. 33:185-187.

Straka, R. P., and J. L. Stokes. 1956. Sound handling practices best antidote for microbial hazards of pre-cooked FF. Quick Frozen Foods. 18:182-186.

Tanner, F. W., and L. P. Tanner. 1953. Food born infections and intoxications. 2nd ed. Garrard Press, Champaign, Ill.

Thatcher, F. S., and W. Simon. 1956. A comparative appraisal of the properties of "Staphylococci" isolated from clinical sites and from dairy products. Can. J. Microbiol. 2:703-714.

Thomson, S. 1955. The numbers of pathogenic bacilli in faeces in intestinal diseases. J. Hyg. Camb. 53:217-224.

Thomas, S. B., and P. M. Hobson. 1955. Coli-aerogenes bacteria isolated from ears and panicles of cereal crops. J. Appl. Bact. 18:1-8.

Tiedeman, W. D., and S. E. Smith. 1945. Practical application of several coliform tests to pasteurized milk. J. Milk Tech. 8:323-330.

Tressler, D. K., and C. F. Evers. 1957. The freezing preservation of foods. II. The AVI Publishing Co., Inc., Westport, Conn.

Weiser, H. H. 1951. Survival of certain microorganisms in selected frozen foods. Quick Frozen Foods. 13(7):50-52.

Woodburn, M. J., and D. H. Strong. 1960. Survival of Salmonella typhimurium, Staphylococcus aureus and Streptococcus faecalis frozen in simplified food substrates. Appl. Microbiol. 8:109-113.

Yale, M. W. 1937. Comparison of solid with liquid media as a means of detecting the presence of lactose fermenting bacteria in pasteurized milk. Am. J. Publ. Hlth. 27:564-569.

Zaborowski, H., D. A. Humber., and M. M. Raymon. 1958. Evaluation of microbiological methods used for the examination of precooked frozen foods. Appl. Microbiol. 6:97-104.

Zebovitz, E., J. B. Evans., and C. F. Nivan (Jr.). 1955. Tellurite-glycine agar: A selective plating medium for the quantitative detection of coagulase-positive staphylococci. J. Bact. 70:686-690.

SANITARY STATUS OF PRECOOKED FROZEN DESSERT-TYPE FOOD

by

NAGENDRA NATH SHARAN VERMA

Graduate Bihar Veterinary College, Patna, 1947
Post-Graduate (Vet. Sci.), Indian
Veterinary Research Institute, U. P., 1953

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements of the degree

MASTER OF SCIENCE

Department of Bacteriology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1964

ABSTRACT

This investigation was undertaken with the objective of assessing the sanitary status of the precooked frozen dessert-type foods available at the consumer level. The study comprised bacteriological analyses for the total bacterial population, densities of coliform, Escherichia coli and staphylococci in various types of these foods as available in the local market (Manhattan, Kansas). Plate count agar and violet red bile agar (Difco) were used for estimating the total bacterial population and coliform counts, respectively. Staphylococcus Medium 110 was used for primary isolation and enumeration of staphylococci. Counting of colonies and computation of plate counts were done according to the procedure described in Standard Methods for the Examination of Dairy Products (APHA, 1960).

Of 102 precooked frozen dessert-type foods examined, only 2 (1.96%) had counts above 100,000 per gram and less than 200,000 per gram. The largest percentage (90.16%) of the samples had counts below 50,000 per gram and 8 (7.84%) of these had counts varying from 51,000 to 100,000 per gram. Not much variation in plate count data was observed among the samples of different brands but variations did exist to a certain extent among the samples of different foods tested.

Thirty-nine (38.2%) of these specimens showed evidences of coliform contamination and 8 (7.84%) of these were found contaminated with E. coli of probable fecal origin. The coliform counts in 24 (23.32%) of the samples varied from 10 to 275 per

gram and the remainder had counts of less than 10 per gram.

The presence of staphylococci was observed in 28 (27.45%) of the samples with varying numbers which went up to 100 per gram.

From the positive samples 101 coliform organisms were isolated and differentiated into different species on the basis of lactose fermentation and IMViC reactions. One of these isolates proved to be E. coli var I and 10 were E. coli var II, whereas the remainder were E. freundii (intermediates) and Aerobacter species.

All 76 strains of staphylococci isolated from these foods were subjected to the usual tests for pathogenity, viz, (a) coagulase production in citrated rabbit plasma, and (b) production of hemolysis on five percent sheep blood agar plates. The strains were also tested for other characteristics which have been of particular interest in enterotoxin-producing strains of staphylococci, such as, anaerobic fermentation of mannitol, gelatinolytic action and pigment production. The relationships between the different physiological characteristics of these isolates were studied and discussed.

Of 76 strains isolated, 5 fermented mannitol anaerobically, 19 produced beta hemolysis and 2 alpha hemolysis on sheep blood agar, 39 hydrolysed gelatin and 16 were orange pigmented, but none of these isolates was found to be coagulase positive. Thus, as such, they were not of much significance so far as food poisoning was concerned; this did not preclude the possibility of the presence of enterotoxigenic strains of staphylococci in

these foods, nor can it eliminate the possibility of the presence of enterotoxin that might have formed in these foods prior to cooking or freezing.

The results of these studies offer evidences suggesting that although these foods had fairly low total bacterial populations, they were not only associated with high coliform contamination but some of these foods indicated evidence of fecal pollution too, which, both from the aesthetic as well as the public health point of view cannot be taken as desirable. Moreover, their presence also suggests the possibility of the contamination of these foods with other enteric pathogens (like Salmonella), enterococci, clostridia and staphylococci and demands an exploratory search for these organisms.

Some of the pertinent problems of analysis and interpretation which have arisen from the present study have been discussed and future approaches to the problems are suggested.