

AN ABSTRACT OF THE THESIS OF

WILLIAM SCOTT TANNER for the M. S. in BACTERIOLOGY
(Degree)

Date thesis is presented May 10, 1950

Title SOME MICROBIOLOGICAL STUDIES OF NON- AND PARTIALLY PRE-
COOKED FROZEN FOODS WITH PARTICULAR REFERENCE TO THE PRESENCE OF
COLIFORMS STAPHYLOCOCCI AND SALMONELLA GROUPS

Abstract approved by [REDACTED]

(Major Professor)

Fifty-three samples of non- and partially precooked frozen foods were examined for the presence of food-poisoning staphylococci (Micrococcus pyogenes aureus), food-infecting Salmonella, Shigella, coliforms, and bacterial plate count.

Food-poisoning staphylococci were found in nine of thirty-one poultry products examined. Of the foods examined, chicken a la king most frequently contained this organism. Plate counts in a majority of the foods examined ranged from several hundred thousand to one or two million bacteria per gram. Coliform counts were higher than generally considered permissible for foods prepared under sanitary conditions. In no instance were enteric pathogens encountered.

Results of this study as a whole indicated the necessity for improved sanitation in preparation and handling of these foods.

SOME MICROBIOLOGICAL STUDIES OF NON- AND PARTIALLY PRECOOKED
FROZEN FOODS WITH PARTICULAR REFERENCE TO THE PRESENCE
OF COLIFORM STAPHYLOCOCCI AND SALMONELLA GROUPS

by

WILLIAM SCOTT TANNER

A THESIS

submitted to


OREGON STATE COLLEGE


in partial fulfillment of
the requirements for the
degree of


MASTER OF SCIENCE

June 1950

APPROVED:


Head of Department and Professor of Bacteriology
In Charge of Major


Chairman of School Graduate Committee


Dean of Graduate School

Date thesis is presented May 10, 1950
Typed by Virginia Tanner

Acknowledgement

The writer wishes to express his thanks to Professor Simmons for his suggestions and direction of this work. Thanks are also extended to Professor Bollen, who aided in the preparation of this thesis.

TABLE OF CONTENTS

	page
I. Introduction	1
II. Object of investigation	6
III. Historical.	7
Food poisoning microorganisms	7
Food infecting microorganisms	9
<u>Shigella</u> organisms	11
Bacteria as indicators of sanitary conditions	12
Resistance of microorganisms to freezing	14
IV. Contemporary Microbiological Studies of Frozen Poultry and Pastry Products.	17
V. Experimental	20
VI. Discussion.	24
VII. Summary.	25
VIII. Conclusions	25
IX. Literature Cited.	26

SOME MICROBIOLOGICAL STUDIES OF NON- AND PARTIALLY PRECOOKED
FROZEN FOODS WITH PARTICULAR REFERENCE TO THE PRESENCE
OF COLIFORMS, STAPHYLOCOCCI AND SALMONELLA GROUPS

INTRODUCTION

The commercial production of frozen foods has increased to large proportions since its beginning in 1861 (9, p. 695). There appears to be no reason why this increase should not continue. Storage facilities for frozen foods are becoming more widely available and more highly perfected as time passes. Obviously this increased production of frozen food reflects consumer demand. The flavor, odor, appearance, and nutritive values of frozen foods place them in a favorable light in relation to consumer preference. To the consumer freezing brings foods nearer to their fresh state than any type of preservation yet employed (16, p. 22). However, as is the case with any type of preservation method for food, there are some undesirable features, and these also should be considered in relation to the desirability of frozen foods. One of these is the relative expense of these foods, and the other is the presence of microorganisms which are harmful, or which indicate that the food was not handled under sanitary conditions. This study is concerned with the latter aspect only. Before reporting the findings of these studies, it may prove of interest to summarize the development of commercial freezing and frozen foods.

Frozen foods are by no means of recent date. Peoples of frigid areas have practiced preserving their food by freezing for thousands of years by means of natural climatic conditions. Frozen foods for people of the tropical and warmer temperate climates, however, are comparatively new (9, p. 695). Their increased availability in these

climates is due to the development of mechanical refrigeration. The importance of this simple fact should not be overlooked. The development and increased availability of frozen foods parallels the development and increased availability of mechanical refrigeration.

Mechanical refrigeration developed slowly until the invention of a sulfuric acid refrigerating apparatus by Dr. William Cullen of Scotland in 1820. His apparatus was used for cooling biologicals. Salt and ice freezing mixtures had been used at this time, but they were found satisfactory only for local uses. Linde in Germany, in the middle 1800's made the first practical contribution to mechanical refrigeration with the introduction of the expanded air machine. The fact that Milwaukee became the center of the beer industry is largely due to Vilter making his ammonia refrigerating machines there. Fish was frozen for the first time by mechanical refrigeration in 1891 at Sandusky, Ohio. Although this was the first commercial venture employing mechanical refrigeration, it doesn't mark the beginning of commercially frozen foods. It was preceded by ice and salt freezing of fish in 1861, poultry in 1865, and meats in 1880. Frozen fruits were introduced in 1905, vegetables in 1930, precooked entrees in 1942, and bakery products about a year later (9, p. 695).

Since 1940 an almost startling array of frozen food preparations have made their appearance on the market. The most recent types are the non- and partially precooked specialty foods. These were unknown before the war, but may now be found in wide variety at many markets. For example, there are frozen chicken a la king, chicken pies, chicken tamales, turkey tamales, turkey pies, frozen cookies, fruit

and non-fruit pies, and biscuits. These foods are composites of several ingredients. In some cases, such as in chicken a la king, the nature of the composite in the thawed state is a creamy, semi-liquid product. The same is true of the other chicken and turkey products, excepting the tamales. These are more of a pasty semi-solid consistency. Foods of this nature, especially since they are only partially cooked prior to distribution, are conducive to a rapid and uninhibited growth of microorganisms if allowed to remain at room temperature. Non-fruit pies, such as pumpkin and custard, are also of interest. Prior to cooking they are in a liquid state, and after cooking they are semi-solid. In either state they may support rapid growth of microorganisms, for example food poisoning Micrococcus pyogenes aureus. Frozen pies are in the category of non-precooked foods, hence contaminating microorganisms are not removed until the product is cooked after purchase. Fruit pies should be considered in a different category than non-fruit pies, because the acid conditions of the pies due to the fruit therein exerts an inhibitory effect upon microorganisms. Frozen cookies and biscuits also have a semi-solid consistency when thawed. In addition, cookies contain large amounts of sugar, another factor which aids microbial growth. Although cookies and biscuits may support a large number of microorganisms, they are not frequently found to do so. The important foods to consider then are the non-fruit pies, and the prepared chicken and turkey products. Their consistency, essentially neutral reaction, and content of sugar and other necessary nutrients are all factors which favor the development of large numbers of microorganisms, both harmless and potentially harmful.

Two other factors may also be considered in relation to the flora of microorganisms found in frozen foods. One pertains to the initial contamination of the food by handlers, dirty equipment, and other sources of bacteria, and the other pertains to the time the food is allowed to remain at a temperature favorable for growth of microorganisms. A study of these factors is not within the scope of this investigation. It would involve inspection of the frozen food plant, and the tracing of the food thru various distribution and retail establishments after it left the plant. It may be stated, however, that frozen foods are handled by numerous persons who have no idea of what microorganisms are, and the factors which influence their development. This factor may influence the bacterial count of a food significantly. For example, a food may be allowed to remain at room temperature for several hours before freezing due to production difficulties, or in other cases remain unfrozen for several hours for no apparent reason. In either case the amount of resulting contamination is due to the ignorance or lack of concern on the part of the handlers.

As stated by Frobisher (10, p. 655), "Freezing must be prompt and rapid to be effective. The frozen foods are virtually the same as fresh foods so long as they remain frozen....It is of especial importance to note that, if frozen foods are once thawed, they may undergo rapid microbial decomposition....Interruptions to electric current supplying frozen food lockers may cause serious damage if prolonged for twenty-four to forty-eight hours or more, since microorganisms, including dangerous pathogens like Clostridium botulinum may grow while the food is thawed, and their activities may not be apparent when the

food is used after refreezing. Indeed, food frozen after being thawed unknown to the consumer, may be a very dangerous product."

Gastro-intestinal disturbances with symptoms of nausea, vomiting, and diarrhea are associated with food-poisoning in the usual sense. Poisoning due to agents other than bacteria are not considered under this popular concept.

The term "food-poisoning", so limited, is still erroneous however because it is not the food per se which poisons, but certain types of bacteria and their products. The use of this term probably arises from the older conception that ptomaines caused the poisoning associated with foods. This is no longer considered true. Ptomaines are organic bases related to amines or substituted ammonias. They are formed during advanced decomposition of proteins by microorganisms. Only a few are violently toxic, and then only in massive doses; therefore a large amount of rotten food would have to be ingested to acquire an active dose of any ptomaine. Under ordinary conditions of diet, ptomaine poisoning is due to decomposed food products, but most of the cases of the so-called ptomaine poisoning are infections or intoxications due to bacteria of the Salmonella and staphylococcus (Micrococcus) groups (10, p. 663).

The contamination which frozen foods may receive prior to freezing, the ideal nature of the food for supporting microbial growth, and the significance of the flora of microorganisms that are present in the food when it reaches the consumers' tables prompted the following investigation.

OBJECT OF INVESTIGATION

It is felt that as new frozen food products appear on the market, that people qualified to examine these products should do so, and establish proof of their wholesomeness. The purpose of this study is two-fold: 1) an attempt to determine the sanitary history of the food as evidenced by standard plate counts, and coliform counts; 2) to isolate any representatives of the Micrococcus, Salmonella, and Shigella groups that may be present.

HISTORICAL

There are few references pertaining to the microbiology of foods which were examined in this investigation. Four reports (4, 12, 13, 14) were found in the literature, but until several similar investigations point out similar results, an accurate and complete picture of these newer frozen foods cannot be obtained. Before discussing the few studies which have been made on these foods, it appears necessary to discuss several topics which have direct bearing upon this problem. These topics are food poisoning and food infecting microorganisms, bacteria as indicators of sanitary conditions, and the resistance of microorganisms, bacteria as indicators of sanitary conditions, and the resistance of microorganisms to freezing.

Food poisoning microorganisms

Staphylococci are the most common cause of food poisoning according to Dack (6, p. 80). Accurate records on the occurrence of this type of food poisoning are lacking, since it is not reportable.

Certain staphylococci elaborate several toxins which contribute in varying degree to their ability to produce infection (8, p. 329). For the purpose of this study, the staphylococci which produce a heat stable, potent enterotoxin which is the cause of acute gastro-intestinal disturbances in man are of direct concern. This enterotoxin is a distinct entity. It is quite resistant to heat, resisting boiling for thirty minutes (8, p. 330).

Parasitic staphylococci are part of the permanent bacterial flora of the skin and nasopharynx. Potentially pathogenic forms are

constantly carried on the skin or in the nasal passage by approximately twenty and fifty percent, respectively, of all individuals. If pathogenic staphylococci are found in food, they may be assumed to have been derived from a human being or animal source (8, p. 326). The most common infections often due to staphylococci are furuncles, boils, acne, sinus, and colds. The nose in particular, even if apparently healthy, harbors strains of enterotoxigenic staphylococci (6, p. 100). Workers with sinus infections, acne, recurrent boils, or colds should be excluded from the handling of food products. The conditions necessary for an outbreak of staphylococcus food poisoning are usually: contamination of food with enterotoxin-producing staphylococci, a suitable food in which the organisms can grow, and the keeping of this food for a sufficient time at a temperature compatible with growth (6, p. 102). The foods most suitable for growth are sugar-containing creamy products, such as cream puffs, custard pies and eclairs.

The symptoms of staphylococcus food poisoning usually appear in three hours, and occasionally from one to six hours, after ingestion of the contaminated foods. The incubation time is dependent upon the susceptibility of the individual. The first symptom observed usually is salivation. This is followed by nausea, vomiting, retching, abdominal cramps, and diarrhea. In severe cases, blood may be discharged with the feces and vomitus. Headache, muscular cramping, and sweating, frequently occur when symptoms are moderately severe. Marked prostration, vomiting, and diarrhea occur in severe cases. Staphylococcus food poisoning in normally healthy individuals is seldom fatal. A few fatal cases have occurred in which inadequate treatment was given.

The symptoms appear rapidly and are of short duration. People suffering from staphylococcus food poisoning usually recover within twenty-four hours (8, pp. 332-335).

Food infecting microorganisms

Salmonella organisms are the cause of gastro-enteritis infections in man. Symptoms of such infections are characterized by nausea, vomiting, abdominal pain, and diarrhea. The gastro-enteritis caused by Salmonella occurs after an incubation period of from eight to forty-eight hours following consumption of contaminated food. The onset is nearly always sudden. Headache, chills, and abdominal pain are common. The infection is more severe in infants and young children. Blood cultures are generally negative, however the organisms may be recovered from the vomitus or feces (8, pp. 387-388). Salmonella typhimurium is the organism most commonly isolated from cases with Salmonella gastro-enteritis infections in this country (6, p. 133). Dack (6, p. 127), states that Salmonella food infections account for only a small number of cases of food poisoning outbreaks, and that only in a small number of such infections do the organisms invade the host's tissues and cause death.

The salmonella food poisoning and the staphylococcus food poisonings differ in that the former is an infection due to the organisms, and the latter is due to potent enterotoxins produced by the organisms. Salmonella have never been shown by American investigators to produce enterotoxic substances. Therefore, salmonella are considered as only pathogenic, and not toxigenic (9, p. 699). They are commonly referred to as food-borne infections, and poisoning due to staphylococci

enterotoxins is referred to as food-borne intoxications.

Salmonella species are also the cause of typhoid and paratyphoid fevers. The organisms involved are Salmonella typhosa, S. paratyphi (paratyphoid A), S. schottmuelleri (paratyphoid B), and S. hirschfeldii (paratyphoid C). Typhoid and paratyphoid fevers differ significantly from the gastro-enteritis infections caused by the other salmonella. The onset of typhoid fever is insidious, often beginning gradually with a loss of appetite and a headache. These symptoms appear from seven to fourteen days after becoming infected. The fever rises in a step-like manner to an average of 104° F. During the first week of the disease the organisms are present in the blood, the patient is usually prostrate, and may have a diarrhea. However, constipation is more common. Rose spots, abdominal tenderness, cough and bronchitis are symptoms of the first and second weeks. In more severe cases, a person becomes delirious and shows the so-called typhoid state. After the third week, the temperature returns to normal. The mortality rate in typhoid fever ranges from fifteen to twenty percent (8, p. 386-387).

Paratyphoid infections are similar to typhoid infections, but are much milder and less typical than typhoid fever. The onset of symptoms is one to ten days after infection occurs. Fever and malaise last from one to three weeks. Rose spots are rare (8, p. 387).

Salmonella species are also a common cause of diarrheal diseases. According to Rubenstein, Feemster, and Smith (15, pp. 841-853), Salmonella are the most important cause of diarrheal diseases. They found that four types of Salmonella were responsible for seventy

percent of eight hundred and eleven cases investigated.

The Salmonella are transmitted by the four "F's" (10, pp. 722-723), feces, foods, fingers, and flies. The source of all Salmonella organisms causing infections is the reservoir of organisms living in the tissues of human beings and animals (8, p. 393).

It is of interest to note here in discussing food poisoning, that the older conception that ptomaines caused food poisoning is of questionable merit. This fact was mentioned earlier, and is stated again to emphasize it. Food poisoning is most often caused by staphylococci, and sometimes is caused by salmonella (10, p. 663).

Shigella organisms

Shigella organisms are the cause of bacillary dysentery. The symptoms are intestinal disturbances ranging from very mild diarrhea to severe dysentery. Intense inflammation and ulceration of the large bowel may occur, often with scar formation and stricture of the bowel after recovery. In some epidemics, especially those due to Shigella dysenteriae, the fatality rate is high (10, p. 726).

The natural habitat of the dysentery bacilli is the intestinal tract of mammals. Man is the species most often affected. The naturally occurring disease appears to be limited to man and perhaps monkeys. Dogs have rarely been found to harbor Shigella. As yet, no animal reservoir of significance has been discovered (8, pp. 397-408).

Dysentery bacilli are transmitted by infected food or water, and gain entrance to the human intestinal tract by means of these media. In direct contrast to typhoid bacilli, they cause no septicemic phase, being limited to the intestinal wall, and bowel (8, p. 402).

The incubation period of bacillary dysentery is variable, but may be as short as twenty-four hours. Abdominal discomforts, and cramps (gripping) are the first symptoms, and usually appear suddenly. They are followed by diarrhea which is accompanied by straining and tenesmus. Stools are liquid from the start. Large amounts of mucus and blood are passed in severe cases. Recovery is usually uncomplicated as the disease is self limiting (8, p. 403). A small number of persons so infected become chronic carriers.

Bacteria as indicators of sanitary conditions

The introduction of liquifiable solid media, nutrient agar, afforded a method by which the numbers of bacteria in various materials might be determined by adding to the liquid medium in plates, a measured portion of the substance, mixing, allowing the plates to solidify and counting the numbers of colonies that developed after incubation. This technique, to which have been added several requirements for standardizing determinations, was presented to the dairy industry in 1910 by the American Public Health Association (11, p. 4). Its use has spread to other types of investigations; for example, foods and water.

The standard plate count as used today indicates the approximate or relative numbers of viable bacteria present in various foods, milk and water. As devised, it is a routine measure, being rapid, inexpensive, and relatively simple to carry out. As Hammer (11, p. 3) states, it is not necessary for a routine count to indicate the actual number of bacteria in a sample, but rather that a representative portion be determined. Limits of numbers of bacteria set by ordinances,

and regulations, recognize that routine methods of counting do not necessarily give as high counts as might be obtained by direct microscopic counts.

Since its beginning the plate count has met with a large amount of adverse criticism (16, pp. 320-325). The primary objections to the plate count are: 1) Pathogenic organisms are not detected; 2) The counts are not representative of the actual numbers of bacteria present; 3) Wide variations in counting are encountered; 4) The period required before results are available is too long. It is not the purpose here to discuss these adverse criticisms in detail, but suffice it to say that in spite of these criticisms the standard plate count is still widely in use today, and has proven quite valuable.

A standard plate counting technique for frozen foods was devised by the Committee on Standard Methods for the Microbiological Examination of Foods (5, pp. 332-334). This standard method was used for the frozen foods examined in this investigation. The method is explained in a following section.

The standard plate count has been of considerable value in quality control work in spite of its short-comings. Plate counts of raw and pasteurized milk are an aid in placing milk in its proper grade. Plate counts are frequently used in bacteriological water analyses, and to estimate numbers of microorganisms in foods at various stages of manufacture.

Escherichia coli, a very common inhabitant of the intestinal tract, has been used for many years in water analyses and similar types

of investigations as an indicator of fecal pollution. It has found use in food and milk analyses as an indicator of the sanitary conditions under which foods are handled. Fecal pollution of food, as evidenced by the presence of E. coli points out the possibility that other enteric organisms might also be there. Some of the enteric organisms are pathogenic.

Resistance of microorganisms to freezing

The ability of microorganisms to resist freezing and continued storage in foods at freezing, and below freezing temperatures, does not appear to be generally understood. Some individuals believe that freezing merely places the microorganisms in a state of suspended animation, and that when normal temperatures are resumed, that all cells will become active again. These conclusions are of poor logic. Since a few cells survived in suspensions which were maintained at temperatures comparable to that of liquid air, it was believed that freezing did not kill microorganisms. These conclusions were based upon work of a qualitative nature. When quantitative determinations were made, which involved the counting of viable cells before and after freezing, a marked decrease in population was evident (16, p. 25). The actual number of surviving organisms would be influenced by the numbers of bacteria in the initial product and its nature.

On the other hand, there are people who believe that frozen foods are sterile foods. As stated by Proctor and Phillips (12, p. 48), "All too often frozen foods are found displayed in retail stores in the thawed condition." Such conditions seem to indicate ignorance

as to the non-sterility of the foods, or else gross carelessness.

Extensive studies have been made to determine the resistance of pathogenic and non-pathogenic microorganisms in frozen fruits and vegetables. A summary of a few of these studies will be presented here.

Magoon reported that the old belief that freezing destroys molds, yeast, and bacteria must be revised, for, while the majority of microbial flora is usually killed by freezing temperatures in food products, a small portion can survive. Some were found that were able to resist temperatures of -252° C. for as long as ten hours with no apparent deleterious affect. Some were not only unharmed by cold but were even capable of increasing in number at temperatures well below 0° C. (16, p. 25).

Haines found that the death of bacteria frozen rapidly at -70° C. was most rapid if they were stored at temperatures ranging from -1° C. to -5° C., and not at temperatures of -20° C. Microorganisms were also found to differ in their susceptibility to freezing. Spores and Micrococcus pyogenes aureus were resistant, while Pseudomonas pyocyaneus was sensitive (16, p. 26).

To determine whether pathogenic organisms could survive in frozen fruits, Wallace and Tanner (16, p. 29) inoculated cherries and cherry juice with cultures of Salmonella typhosa, S. schottmülleri, S. aertrycke, Escherichia coli, and Proteus vulgaris. The inoculated foods were stored at -17.8° C. and -40° C. for twenty weeks in the case of the cherries, and four weeks in the case of the cherry juice. The organisms survived in the cherry juice less than four weeks, and in the

cherries for two to three months.

Mc Gleskey and Cristopher tested the viability of several organisms inoculated into sliced sweetened strawberries which were then frozen and stored at -18° C. They reported the following survivals: Salmonella typhosa, six months; Staphylococcus aureus, five months; Salmonella aertrycke and S. schottmüller, one month; S. paratyphi was not recovered. Salmonella typhosa inoculated into unsliced sweetened berries was present in small numbers after storage at -18° C. for fourteen months (16, p. 29).

Enterotoxin-producing microorganisms are of especial interest in frozen foods. Jones and Lochhead found that staphylococci survived freezing in vegetables. Fifty different strains of Staphylococcus spp. were isolated. Using eighteen strains which produced enterotoxin, eight strains were still able to produce toxin in frozen corn defrosted at room temperature, but were unable to produce enterotoxin in a refrigerator at 4.4° C. to 10° C. where no multiplication of the cells was observed (9, p. 698).

It is evident from consideration of these studies, that many microorganisms are able to resist freezing and below freezing temperatures for considerable lengths of time. This need not make consumers of frozen foods unduly apprehensive. If foods are maintained frozen until ready for prompt consumption, there should not be any danger provided that the food was not heavily contaminated by the manufacturer.

CONTEMPORARY MICROBIOLOGICAL STUDIES OF
FROZEN POULTRY AND PASTRY PRODUCTS

Buchbinder, et al (4, pp. 209-213), studied the possibility of potential health hazards existing in frozen precooked foods. They state that these foods have been on the market for the past six or seven years, and there appears to be no recorded evidence of their implication in outbreaks of food poisoning. Nevertheless, because some of these frozen precooked foods contain ingredients which readily promote bacterial growth, including that of food poisoning pathogens, and because of scanty literature on the subject, they made a study of these foods.

They obtained the following information from the examination of thirty-nine samples of frozen chicken a la king:

- 1) 7.7% of all samples had coliform counts of less than 500,000, but greater than 100,000.
- 2) 10.3% of all samples had coliform counts greater than 10,000.
- 3) 18.0% of all samples had coliform counts greater than 1,000.
- 4) 38.5% of all samples had coliform counts greater than 100.
- 5) Twelve samples of chicken a la king yielded staphylococci of the food poisoning type.
- 6) 82% of the samples had plate counts exceeding 100,000; 64% had plate counts exceeding 500,000; 54% had plate counts exceeding 1,000,000.

Examinations of other chicken-containing frozen foods, and freshly prepared restaurant-made chicken a la king showed that these were of a much lower bacterial count. No enteric pathogens were

found.

A recent study by Proctor and Phillips (13, p. 49) is of interest here. They examined a variety of frozen precooked meat, poultry, and fish products for their ability to support growth of experimentally inoculated cultures of enterotoxic Staphylococcus aureus (Micrococcus pyogenes aureus). The food samples were inoculated with water suspensions of the organisms and stored at -18° C. for several months. Results indicated that creamed food products, such as chicken a la king, ham a la king, creamed tuna, and creamed salmon are good substrates for the development of S. aureus. Samples of creamed food products heavily inoculated with S. aureus were found to contain large numbers of these after storage at -18° C. for nine months, although there were some reductions in S. aureus counts. In a later paper (12, p. 46) they state that creamed fish frequently show direct microscopic counts of 5,000,000 per gram, and coliforms in excess of 100 per gram. Pastry products were found to have the lowest bacterial population.

Rose and Gundeson (14, p. 47) analysed for total coliform bacterial numbers, for hemolytic S. aureus, and for members of the Salmonella and Shigella groups at various stages of boned chicken production, and in the final chicken containing product. They found that cooking of eviscerated chicken prior to boning reduces the bacterial load to as low as seventy-three organisms per gram of meat. After coming into contact with the hands of workers and contaminated equipment, the chicken becomes seeded with a heavy inoculum of bacteria which rapidly increases to as many as nine million bacteria

per gram during the approximately two and a half hours required for the boning operation. Further increases were noted to arise from plant production malpractices, the most important of which was a holding time of from one to three hours at room temperature before freezing. In a chicken-containing frozen food, members of the Salmonella are rapidly killed by storage at -14° C., but from two to twenty percent of the inoculum can survive storage for ninety-two days. The bacterial count of cold-stored chicken-containing food depends primarily on the initial contamination rather than on the storage time, according to these workers. This was their conclusion after finding excessive contamination in material stored from three and one-half to five and one-half months at this low temperature. Hemolytic Staphylococcus aureus, but not members of the Salmonella and Shigella group, have been isolated from frozen foods.

EXPERIMENTAL

Frozen chicken a la king, chicken pie, chicken tamale, turkey pie, turkey tamale, chicken chow mein, pumpkin pie, cherry pie, and cookies were examined for standard plate count, coliform counts, food-poisoning staphylococci, food-infecting Salmonella and Shigella.

The standard plate counts were carried out according to the tentative methods recommended by the Committee on Standard Methods for the Microbiological Examinations of Foods (5, pp. 332-334). The procedures and standard conditions of this method for determining the plate count of bacteria were followed exactly. Duplicate samples were taken of each food examined and mixed with sterile water in blender jars. Dilutions of 1 to 1,000; 1 to 10,000; and 1 to 100,000 were poured with dextrose-tryptone agar for determining the plate count. The results are recorded as the plate count of microorganisms per gram.

One of the duplicates used for dilutions was also used for the enumeration of coliforms by the most probable numbers technique, as employed for determining the probable number of coliforms in water (2, p. 203). Results are recorded as the most probable number of coliforms per gram of food.

The determinations for food poisoning staphylococci were based upon the use of Difco Staphylococcus Medium Number 110. Chapman (7, p. 110) clearly states that on this medium, with few exceptions, food poisoning staphylococci produce colonies that have golden pigmentation, ferment mannitol, and give a gelatinase reaction. The medium contains a relatively high concentration, 7.5% sodium chloride and

therefore is selective for staphylococci. It is well suited for pigment formation, may be used for the determination of the fermentation of mannitol, for the Stone type gelatinase test, and gives a growth satisfactory for the coagulase test that may be used for additional confirmation (7, p. 110). Food-poisoning staphylococci were confirmed by the following characters: 1) gram positive, spherical cells, grouped in one's, two's and grape-like packets; 2) ferment mannitol; 3) produce a golden pigmentation; 4) hydrolyse gelatin; 5) and coagulate human blood plasma.

Salmonella and Shigella isolation and identification was attempted by using selective media such as Salmonella-Shigella agar and bismuth sulfite agar. In the event that typical colonies developed which were representative of these organisms, they were picked from the plate and inoculated into Russel's double sugar agar. If Russel's medium showed reactions characteristic of these organisms, then physiological, morphological, serological and cultural characteristics were determined, and the organisms were identified accordingly (3).

Information obtained from these examinations is summarized in the following tables.

STANDARD PLATE COUNT OF ORGANISMS FOUND IN FROZEN
CHICKEN AND TURKEY PREPERATIONS AND IN
FROZEN PASTRIES

<u>Type of food</u>	<u>No. of samples</u>	<u>Range in plate counts</u>				
		1000	10000	100,000	over	
		<u>1000</u>	<u>9900</u>	<u>99000</u>	<u>990,000</u>	<u>1 M*</u>
Chicken a la king	18	4	0	0	8	8
Chicken pie	9	0	0	0	5	4
Turkey pie	4	0	0	0	3	1
Turkey tamale	6	0	0	4	2	0
Chicken tamale	6	0	0	2	4	0
Chicken Chow Mein	3	3	0	0	0	0
Pumpkin pie	3	0	0	0	3	0
Cookies	4	0	0	4	0	0
Cherry pie	3	0	0	3	0	0

* One million or over

Table II

DISTRIBUTION OF STAPHYLOCOCCI* SALMONELLA AND SHIGELLA
IN SOME FROZEN CHICKEN AND TURKEY PRODUCTS AND IN
SOME FROZEN PASTRIES

<u>Type of food</u>	<u>No. of samples</u>	<u>Occurence of</u>		
		<u>Staphylococci</u>	<u>Salmonella</u>	<u>Shigella</u>
Chicken a la king	18	6	0	0
Chicken pie	9	2	0	0
Turkey pie	4	1	0	0
Turkey tamale	6	0	0	0
Chicken tamale	6	0	0	0
Chicken Chow Mein	3	0	0	0
Pumpkin pie	3	0	0	0
Cookies	4	0	0	0
Cherry pie	3	0	0	0

* Staphylococci of food poisoning type

Table III
AVERAGE COLIFORM COUNTS AND pH OF FROZEN FOODS

<u>Type of food</u>	<u>Number of samples</u>	<u>Average of the most probable no. of col- iforms per gram</u>	<u>Average pH</u>
Chicken a la king	18	240	6.7
Chicken pie	9	160	6.5
Turkey pie	4	70	6.5
Turkey tamale	6	35	5.9
Chicken tamale	6	14	6.2
Chicken Chow Mein	3	0	---
Pumpkin pie	3	1600	6.6
Cookies	4	0	6.6
Cherry pie	3	48	4.0

DISCUSSION

Chicken a la king appears to be a heavily contaminated food in the majority of cases. Bacteriological plate counts of this food often are as high as one or two million per gram. In addition, food poisoning staphylococci are of frequent occurrence. It may be of interest to note that the author and his wife, in the early part of the investigation, ate freshly thawed portions of the chicken a la king which remained after the samples were taken. After heating to serving temperature the food was eaten at 6:30 P.M., and at 10:30 P.M. both became nauseated, vomited, and had diarrhea. Upon completion of the analysis of the chicken a la king two days later, food poisoning staphylococci were found in large numbers. The bacterial plate count of the food was one and a half million. In consideration of the fact that the enterotoxins of food poisoning staphylococci are heat stable, and that they would not be destroyed in the short time the food was heated, it was believed that staphylococci were responsible for the food poisoning.

Chicken pies appeared to contain large numbers of organisms quite frequently. Occasionally they were found to contain food poisoning staphylococci. Chicken tamale, and pumpkin pie were in some instances, found to have high plate counts, but staphylococci were not encountered. Pumpkin pie in addition to having a high plate count, had a high coliform count, and was found to contain Proteus sp., Alcaligenes fecalis, and members of the para-colon group of bacteria (3). Other foods examined gave no evidence of food poisoning

potentialities.

SUMMARY

Fifty-three samples of poultry products and pastries were examined for sanitary quality with special reference to food poisoning bacteria. Most of the products gave high plate counts and showed excessive numbers of coliforms. Food-poisoning staphylococci were found in nine of the thirty-one poultry products examined. Enteric pathogens were not encountered.

CONCLUSIONS

The results of this investigation confirm and extend other investigations of a similar nature. The poor sanitary quality, and in some cases the food poisoning potentialities of the cream style and pastry foods, emphasizes the fact that more care should be exercised in their preparation. Frozen foods generally have been remarkably free from direct implication in transmitting food poisoning and infectious diseases. However, this investigation shows that food poisoning through certain specialty foods may offer a serious problem as soon as these become extensively available. The frozen food industry, which thus far has failed to establish a sanitary code, should require strict sanitation in preparation and handling of cream style poultry products and pastries.

LITERATURE CITED

1. American Public Health Association and Association of Official Agricultural Chemists. Standard methods for the examination of dairy products. Ninth edition. New York. 1948.
2. American Public Health Association. Standard methods for the examination of water and sewage. Ninth edition. New York, 1946. 286 p.
3. Bergey, David H., et al. Manual of determinative bacteriology. Sixth edition. Baltimore, the Williams Wilkins Company, 1948.
4. Buchbinder, Leon, et al. A survey of frozen precooked foods with special reference to chicken a la king. Journal of milk and food technology, 12:209-213, July-August 1949.
5. Committee on Standard Methods for the Microbiological Examination of Foods. Microbiological examination of foods. American Journal of public health, 36:332-335, April 1946.
6. Dack, G. M. Food poisoning. Second edition. Chicago, the University of Chicago Press, 1949. 184 p.
7. Difco Laboratories. Difco manual. Eighth edition. Detroit, 1948. 223 p.
8. Dubos, Rene. Bacterial and mycotic infections of man. Philadelphia, J. B. Lippincott Company, 1948. 785 p.
9. Fitzgerald, Gerald A. Are frozen foods a public health problem? American Journal of public health, 37:695-701, June 1947.
10. Frobisher, Martin. Fundamentals of bacteriology. Fourth edition. Philadelphia, W. B. Saunders and Company, 1949. 936 p.
11. Hammer, Bernard W. Dairy bacteriology. Third edition. New York, John Wiley and Sons Incorporated, 1948. 593 p.
12. Proctor, Bernard E., and Phillips, Arthur W. Frozen precooked foods. American Journal of public health 38:44-49, January 1948.

13. Phillips, Arthur W., and Proctor, Bernard E. Growth and survival of an experimentally inoculated Staphylococcus aureus in frozen precooked foods. Journal of bacteriology 54:49, July 1947.
14. Rose, Kenneth D., and Gunderson, Millard F. Some bacteriological aspects of the commercial production of boned chicken. Journal of bacteriology 54:47, July 1947.
15. Rubenstein, A. D., R. F. Feemster, and H. M. Smith. Salmonellosis as a public health problem in wartime. American Journal of public health 34:841-853, July 1942.
16. Tanner, Fred Wilbur. Microbiology of foods. Second edition. Champaign, Garrard press, 1944. 1196 p.