The Effect of Quick Freezing on the Bacteria in Food Products

by

Lawrence Parker Abare

Submitted in Partial Fulfillment of the Requirements

for the

Degree of

Bachelor of Science

from the

Massachusetts Institute of Technology

1930

Course VII

Signature redacted

Signature of author _____

Certification of Department _____

Professor A. L. Merrill
Secretary of the Faculty
Massachusetts Institute of Technology
Cambridge, Massachusetts

Dear Professor Merrill:

The following thesis is submitted as a partial fulfillment of the requirements for the degree of Bachelor of Science.

Respectfully submitted,

Signature redacted

Acknowledgement

The author wishes to express his thanks and appreciation for the advice given him during the work by Professor S. C. Prescott of the Department of Biology and Public Health.

The Effect of Quick Freezing on the Bacteria in Food Products

Introduction

The recent rise to popularity of the "quick freeze" method of preserving food products - particularly meat and fish - has raised the question as to what is its effect on the bacteria present in such products. In order to answer this question, and because little or no attention has been paid to this phase of quick freezing, the present investigation was undertaken.

Table of Contents

Quick Freezing

It is not within the scope of this thesis to decide on the merits of "quick freezing" versus slow freezing. Neither is it necessary to pass judgment on the various methods of quick freezing. It will, however, be of value to explain briefly what quick freezing is and how it differs from ordinary slow freezing.

"Air frozen", "slow frozen" and "sharp frozen" are synonyms referring to products frozen by placing them in a room, the temperature of which is kept in the vicinity of -10°F.

"Quick frozen" refers to products frozen by direct contact with a liquid refrigerant such as sodium chloride brine or by indirect contact with very cold calcium chloride brine. The temperatures used for quick freezing vary from -20°F. to -50°F. according to the method which is used.

The advantages of quick freezing over slow freezing are best portrayed in a paper by Birdseye (7), the originator of a method of quick freezing, from which the following is abstracted.

Animal tissues are composed largely of a semi-liquid protein gel containing appreciable amounts of sodium, potassium, calcium, magnesium, manganese, iron and zinc

salts in the form of chlorides, phosphates, sulphates, bromides and iodides. Such a solution does not freeze homogeneously - pure water ice first begins to separate out at a temperature of approximately 31° F. and as the temperature is lowered, more pure water ice separates out, leaving a concentrated solution of the salts in the remaining liquid. It has been estimated that the water content of fish is not entirely frozen until a temperature of -70° F. (-56.67° C.) is reached.

The greater part of the water content, however, is frozen by the time the temperature has reached 25° F. During the period when the temperature is between 31° F. and 25° F. the individual ice crystals are constantly increasing in size and when the lower temperature is reached the growth of the individual crystals for the most part ceases. This zone, from 31° F. to 25° F. may then be termed the "zone of maximum crystal formation". Since the size of crystals depends on the time allowed for their formation, it is obvious that if this zone is passed through more quickly, it will result in the formation of smaller ice crystals.

The formation of large ice crystals in and between the cells results in a more or less serious rupture of

the more delicate of the tissues and a compression of the tougher fibers into dehydrated layers or bundles. Cells which are in this condition autolyze more rapidly and are more susceptible to bacterial contamination than are fresh undamaged cells. Quick freezing because of better methods of heat conduction and the use of lower temperatures, passes the product through the zone of maximum crystal formation so very rapidly that the resulting ice crystals are too small to materially injure the tissues.

Methods of Quick Freezing

There are many methods of quick freezing - to do any more than enumerate them would take more time and space than is justifiable.

The principal methods of quick freezing are:-

1. Birdseye - The product is packaged before freezing and placed between two metal belts against which is sprayed calcium chloride brine at a temperature of -45°C. to -50°C. A package two inches thick is frozen in from 1½ to 2 hours.

2. Cooke - The unwrapped food product is placed on a flat metal slab to the bottom of which are attached numerous metal fins. These fins dip into a -20°F. brine solution and aid in the conduction of heat from the product to the brine. Fish fillets are frozen in from 35 to 40 minutes.

3. Kolbe - The unwrapped food products are placed in round metal pans which are floated on calcium chloride brine around a raceway. The brine is kept at a temperature of -20°F. Fish fillets are frozen in from 25 to 30 minutes.

4. Peterson - The unwrapped food product is placed in cans similar to ice cans which are immersed in a brine whose temperature is -25°F. to -30°F. Cakes two inches thick are frozen in 45 minutes.

Effect of Cold and Freezing on Bacteria

The first extensive investigations on this subject were made by Prudden (2) in 1887. He kept pure cultures of B. prodigiosus and B. proteus for fifty-one days at temperatures ranging from -10°C. to 1°C. and found them sterile at the end of this time. Prudden concludes that

> "the greatest reduction occurs during, or shortly after the sudden reduction of temperature to freezing, and, if after this the bacteria remain in ice a comparatively gradual reduction goes on; if bacteria are thawed out and immediately refrozen another large increment is destroyed."

Prudden believed that the greatest destruction occurred when the water did not freeze.

In 1902 Sedgwick and Winslow (4) after a thorough study of the literature and much experimentation came to the conclusion that there is

> "during the first half hour of freezing, a heavy reduction.....amounting to perhaps 50%. After this brief period of sudden but uncertain 'reduction' the destruction of the germs proceeds pretty regularly as a function of the time."

Keith (3) in 1913 tested the resistance of B. coli to freezing when suspended in water, physiological salt solution, various dilutions of fat free milk, various mixtures of glycerine and water, and in solutions of cane sugar and of dextrose. He believes that water-bearing food materials freeze in such a manner that most of the bacteria present are extruded from the water crystals along with other non

aqueous substances (including air) and lie in or among them without being injured. On the other hand more watery substances and water itself would not offer such protection and there would be a greater destruction of the bacteria. Keith offers this as an explanation of the fact that the least watery foods contain the greatest number of bacteria after freezing and the foods having the highest water content the least number of bacteria. From his experiments Keith drew the following conclusions:-

> "Low temperatures alone do not destroy bacteria. On the contrary, they appear to favor bacterial longevity doubtless by diminishing destructive metabolism. Frozen food materials, such as ice cream, milk and egg substance, favor the existance of bacteria at low temperatures, not because they are foods, but apparently because they furnish physical conditions somehow protective of the bacteria."

In 1915 Hilliard, Torossian and Stone (1) published notes on the germicidal effect of freezing and low temperatures. They suggested that

> "bacteria may be killed by the mere fact of low temperature interfering with metabolism; by freezing of the cell contents and rupture of the membrane by internal pressure; by external pressure or grinding developed during crystallization; by expansion of the frozen medium within the receptacle; or by more or less prolonged suspension of metabolic activities, leading to slow death from age or starvation."

Hilliard and Davis (2) in 1918 performed further experiments in order to make comparisons between the effect

of cold and the effect of crystallization. The following are the conclusions drawn by them with the understanding that the work was not extensive enough to render them final:-

> "1. Intermittent freezing of bacteria exerts a more effective germicidal action than continuous freezing.
>
> 2. The reduction is much less in milk and cream than in pure tap water when freezing temperatures are applied, due, no doubt, to physical protection offered to the bacteria by the colloidal and solid matter in suspension.
>
> 3. The degree of cold below freezing is not a very important factor in the destruction of bacteria. There is no critical temperature below freezing where the germicidal effect is greatly accelerated.
>
> 4. The death-rate of B. coli is much higher in media which are frozen solid than it is in the same media not solid and at a slightly lower temperature.
>
> 5. Crystallization, probably resulting in mechanical crushing, is an important germicidal factor in causing the death of bacteria at zero degrees Centigrade and below. The greatest reduction occurs promptly upon freezing and refreezing, but is not caused so much by the sudden change in temperature as by this mechanical factor."

The general concensus of opinion seems to be that there is a double effect - that of the cold itself and that of the crystallization. The former acts chiefly through interferance with normal metabolism and the latter through mechanical injury to the bacterial cell.

It is apparently conceded by most of the investigators that greater protection is offered to the bacteria by foods and colloidal materials than is offered by pure water.

Effect of Quick Freezing on Bacteria

The only work on the bacteriology of quick frozen food products that the writer was able to find was in a paper by Birdseye (8). The bacterial counts given in this paper were performed at the General Seafoods Co., Gloucester, on products frozen by the Birdseye method.

Following is the method that was used in making counts:-

> A comparatively large sample is taken - usually 10 grams - mascerated with knives and forceps and placed in a flask with 100 cc of sterile water, which is later diluted sufficiently to assure ease of counting. One cubic centimeter of the diluted sample is plated on Bacto nutrient agar and incubated 48 hours at 20° C. The count is recorded in bacteria per gram.

The following are some counts made using frozen fillets, the sample being taken while the product was still hard frozen :-

Haddock	29,000	24,300
	46,000	28,000
	29,000	38,600
	15,300	18,000
Mackerel	17,300	
Lemon Sole	58,000	
	35,300	
Dab Sole	34,000	
	35,000	
	35,500	

The following is the latter part of a table showing the progressive Infection and Disinfection of fish during Dressing Operations:-

	Flesh (Bacteria per gram)
7. After leaving brining machine (Sodium chloride and Sodium hypochlorite)	97,600
8. After being packaged, and held at -2.5° C. for 12 hours	77,450
9. After being frozen	31,950

The above table does not give a true picture of the effect of quick freezing since many of the less resistant bacteria have been killed off by repeated washings with chlorinated sea water and by the sodium hypochlorite treatment.

Total Counts

The attempt to obtain total counts of the bacteria present in various food products and to find the effect of quick freezing on these counts did not prove as satisfactory as was hoped for. Two factors were largely responsible for the difficulty encountered - first, the fact that with such food products as meat and fish it is very difficult to obtain a representative sample for a bacteriological count and second, that the quick freezing plant was located at Gloucester.

This latter fact made it impractical to obtain total counts on the same product before and after freezing since such a procedure would necessitate shipment of the product from Cambridge to Gloucester and back again. The time occupied in this shipment and the varying temperatures to which the product would be subjected would materially affect the number of bacteria present and would tend to make worthless any results which might be obtained.

The writer seriously considered the practicability of quick freezing in the laboratory with the aid of dry ice or other refrigerants but due to the difficulty of duplicating the conditions of commercial quick freezing, failure of which would make any results subject to criticism, the idea was abandoned.

The factor of location was remedied towards the latter part of the investigation when a local freezing plant was established. Three satisfactory determinations were made - too few on which to make any general statement regarding the percentage decrease in the number of bacteria due to quick freezing, but sufficient to promise results from further research along this line.

The difficulty of obtaining a representative sample was finally overcome by carefully following a fixed method of procedure, by using a comparatively large sample and by grinding and thoroughly mixing the entire amount before taking samples. The writer is of the opinion, gained through the sad experience of several unsuccessful determinations, that the counts on samples taken from various parts of a fish fillet or cut of meat will vary so greatly as to make them practically worthless.

Run #1

The first attempt to obtain total counts was made on several quick frozen products received February 20, 1930. Due to delays while in transit the products were nearly thawed out when the package was opened. Rough bacterial counts were made at this time by four other students and the products were then stored in a refrigerator at an average temperature of 25° F. Four days later total counts were made by the writer, the dilutions used being based on the rough counts previously obtained, with the belief that the counts would be materially the same. They proved to be so much greater, however, that accurate counts could not be made and although of little value, they are reproduced below. Benefiting by this experience, all later samples were plated in six dilutions.

These products were frozen for the most part a month before they were received. The following are the dates of freezing:-

Haddock Fillets	January 22, 1930
Gray Sole Fillets	September 3, 1929
Lamb Chops	January 30, 1930
Raspberries	June, 1929

A 10 gram sample was used for all the counts. It was removed from the container with the aid of forceps and knives

and weighed in a petri dish. The haddock and sole fillets were ground with sterile sand in a mortar - the lamb chops were ground in a meat grinder and the raspberries were ground in a mortar without the aid of sand. All sampling apparatus was sterilized in the autoclave before use.

Total Counts

(Bacteria per gram)

	20° C	37° C
Haddock Fillets	25,000 (Estimated)	440
Gray Sole Fillets	200,000 (Estimated)	5,100
Lamb Chops	50,000,000 (Estimated)	210,000
Raspberries	1,000	1,000

The counts, inaccurate as they are, serve to show the wide difference which exists between the counts on plates incubated at 20° C and those incubated at 37° C. This result was also evidenced in all later determinations.

Run #2

This run was carried out with the intention of making a comparison between the numbers of bacteria present in the frozen and unfrozen product. Since it was impossible to make counts on the same sample before and after freezing for reasons previously mentioned, it was decided to make comparisons of certain products which could be obtained in the unfrozen and frozen condition and which it was reasonably certain had, up until the time of freezing, undergone similar treatment.

For this purpose several samples were ordered from Gloucester - they arrived at 5:00 p.m. and were stored in the unopened shipping container in a refrigerator at 25 F. until 10:00 a.m. the following morning. The fresh products had been packed without ice or other refrigerant in a separate package - the frozen products had been packed with a small quantity of dry ice and were still frozen hard.

The following method of sampling was employeed on this and all subsequent total count determinations:-

> A ten gram sample is placed in a sterile mortar and ground with a small amount of sterile sand until thoroughly mascerated. It is then transferred to a wide mouth glass stoppered bottle containing 100 cc of sterile water. The stopper is replaced and the bottle is shaken vigorously for two minutes. The contents are allowed to settle one-half minute and the supernatant liquid used for making plates and further dilutions.

The following results were obtained:-

	Bacteria per gram	
	20°C	37°C
Unfrozen Oysters	7,600	400
Frozen Oysters	14,800	270
Unfrozen Fillet of Sole	117,000	21,800
Frozen Fillet of Sole	16,800	1,260
Unfrozen Haddock Fillets	24,300	3,020
Frozen Haddock Fillets	13,600	7,700

The results of this run evidenced the impossibility of making any legitimate comparison between the frozen and unfrozen condition in separate samples.

Runs #2, 3 and 4

The purpose of these runs was to determine the effect of storage at below freezing temperatures on the number of bacteria in quick frozen foods. For this purpose the frozen products used in Run #2 were stored in the icing unit of a "Frigidaire" and counts were made on them at weekly intervals for two weeks. The temperature of the icing unit varies from about - 3°F to 12°F. The following are readings of a thermometer placed in this unit:-

9:30 a.m.	4°F
10:30 a.m.	0°F
12 noon	-1°F
1:30 p.m.	3°F
4:20 p.m. (Motor running)	10°F

The following counts were obtained:-

	Bacteria per gram		
	Feb. 28	Mar. 7	Mar. 15
Frozen Oysters			
20°C	14,800	10,800	8,500
37°C	270	280	120
Frozen Haddock			
20°C	13,600	11,200	11,600
37°C	7,700	3,300	4,800

	Feb. 28	Mar. 7	Mar. 15
Frozen Sole			
20°C	16,800	18,800	23,100
37°C	1,300	2,800	2,100

Per Cent Reduction

	2/28 to 3/7	3/7 to 3/15	2/28 to 3/15
Frozen Oysters			
20°C.	26.6%	21.3%	42.6%
37°C.	3.7% *	57.2%	55.6%
Frozen Haddock			
20°C.	17.7%	3.6% *	14.7%
37°C.	57.1%	34.1% *	37.7%
Frozen Sole			
20°C.	11.9% *	22.9% *	37.5% *
37°C.	115.0% *	25.0%	61.5% *

* Increase

These results are far from satisfactory - there is an increase in the number of bacteria in one-half of the cases and a decrease in the other half. The increases are probably

due to the fact that the samples were taken from a different part of the same fillet and that the fillets were not ground up as they should be. It will be noticed that the only increase occurring in the case of the oysters is a negligible one. It is to be expected that the oysters would be more homogeneous in their bacterial content due to their contact with each other after being shucked and to their high water content.

The possibility of there being an actual increase in the number of organisms in those cases in which it apparently occurred is very slight. The temperature was sufficiently low at all times to prevent bacterial growth and every means was taken to prevent contamination.

Run #5

In this run it was possible to eliminate most of the factors which had made the other determinations unsuccessful. The products used for the test were purchased in a local market, taken to the laboratory and coarsely ground in a sterile meat grinder. Before taking samples for the counts the ground material was thoroughly mixed to insure an even distribution of the bacteria. The products were frozen at a local quick freezing plant and immediately brought back to the laboratory where samples were again taken for total counts.

The foods used for this test were hamburg steak, lamb's liver and cod steaks.

Total Counts

Bacteria per Gram

	20°C.	37°C.
Unfrozen Liver	860,000	150,000
Frozen Liver	128,000	18,600
Unfrozen Hamburg	51,000,000	116,000
Frozen Hamburg	15,000,000	67,000
Unfrozen Cod	2,000,000	42,000
Frozen Cod	930,000	50,000

Per Cent Reduction
in Number of Bacteria
due to Quick Freezing

	20° C.	37° C.
Liver	85.1%	87.6%
Hamburg	70.6%	42.2%
Cod	53.5%	19.1% *

* Increase

These counts indicate that a sizeable reduction in the number of bacteria is effected by quick freezing. One determination is far too few on which to base any generalizations regarding this effect, however, although it is sufficient to suggest paths for future investigation. There is evidently a difference in the amount of protection offered to bacteria by different food products - this degree of protection is probably a function of several factors - cell structure, water content, and chemical composition.

The per cent reduction as well as the actual reduction due to freezing is probably influenced by the initial number of bacteria present in the food though the evidence offered by the above counts is not conclusive.

Percentage Ratio of Number of Bacteria on Plates Incubated at 37°C. to Those Incubated at 20°C.
(on the basis of a one gram sample)

Unfrozen Sole	18.6%
Unfrozen Haddock	12.4%
Unfrozen Oysters	5.3%
Unfrozen Cod	2.1%
Unfrozen Hamburg	0.23%
Unfrozen Liver	17.5%
Frozen Sole	10.6% *
Frozen Haddock	42.2% *
Frozen Oysters	1.9% *
Frozen Cod	5.38%
Frozen Hamburg	0.45%
Frozen Liver	14.5%

* Average of Three Determinations.

It would be expected, barring outside contamination, that the ratio 37°C./20°C. organisms would be greater in the hamburg steak and liver since these products are derived from warm blooded animals. This is not the case for the hamburg steak has the lowest ratio of any of the products which is probably due to it having been kept at a temperature

more favorable for the growth of these organisms.

In a like manner it would be expected that the ratio would be smaller in the case of seafoods whose natural habitat is comparatively cold sea water. While this proved to be the case in some instances, it was not true in others making it impossible to draw any legitimate conclusions.

It was hoped that these ratios would show whether or not quick freezing was more fatal to organisms whose optimum temperature was 37°C. than to organisms whose optimum was 20°C. If such was the case, the ratios would be smaller in the frozen products. Unfortunately one-half of the ratios are larger in the frozen than they are in the unfrozen products so that it is again impossible to draw any conclusions.

Types of Organisms

For the purpose of determining the types of organisms which survive or do not survive quick freezing, sixty-two organisms were isolated from frozen and unfrozen products. The source of these organisms was as follows:

16 from frozen sole
12 from frozen haddock
8 from frozen lamb chops
5 from frozen raspberries
7 from frozen oysters
6 from unfrozen oysters
4 from unfrozen sole
4 from unfrozen haddock

These sixty-two organisms were, in order to determine their cultural reactions, inoculated into most of the common bacteriological media. These cultural reactions will be found in the appendix.

For convenience, these organisms as isolated were assigned numbers. The organisms numbered from 1 to 15 and 46 to 63 inclusive were isolated from plates incubated at 20°C. and those numbered from 16 to 45 inclusive were isolated from plates incubated at 37°C. The numbers assigned and the source of the organisms are as follows:

Frozen Sole - Nos. 1, 2, 3, 4, 16, 17, 18, 19, 20, 21, 38, 39, 40, 56, 57, 58

Frozen Haddock - Nos. 5, 6, 7, 8, 22, 23, 43, 44, 45, 61, 62, 63

Frozen Lamb Chops - Nos. 9, 10, 11, 12, 24, 25, 26, 27

Frozen Raspberries - Nos. 13, 14, 15, 28, 29

Frozen Oysters - Nos. 33, 34, 35, 46, 47, 48, 49

Unfrozen Oysters - Nos. 30, 31, 32, 50, 51, 52

Unfrozen Sole - Nos. 36, 37, 54, 55

Unfrozen Haddock - Nos. 41, 42, 59, 60

The morphological and cultural characteristics revealed that the following pairs of organisms apparently are duplicates:-

#49 and #51 - the former was isolated from frozen oysters and the latter from unfrozen oysters.

#60 and #62 - #60 was isolated from unfrozen haddock and #62 from frozen haddock.

#18 and #19 - Both were isolated from frozen sole.

Other organisms showed close similarity but due to slight differences in cultural reactions, confirmation of which time did not permit of, could not be included in this list.

Psychrophiles

In order to ascertain which of the sixty-two organisms were psychrophiles or at least those which were able to grow at low temperatures they were inoculated into nutrient broth and placed in a "Frigidaire", which was kept at 25°F. The tubes were observed for growth one week, two weeks and one month after inoculation - the final comparison being made at this last inspection. A slight growth was not considered positive. The following are the results obtained and the amount of growth as estimated from the degree of cloudiness, amount of sediment or presence of pellicle:-

	No.	Isolated from
Very Abundant Growth	2	Frozen Sole
	8	Frozen Haddock
	10	Frozen Lamb
	11	Frozen Lamb
	12	Frozen Lamb
	46	Frozen Oyster
	47	Frozen Oyster
Abundant Growth	20	Frozen Sole
	48	Frozen Oysters
	55	Unfrozen Sole
	60	Unfrozen Haddock
	62	Frozen Haddock
Moderate	3	Frozen Sole
	5	Frozen Haddock
	7	Frozen Haddock
	15	Frozen Raspberries

No.	Isolated from
24	Frozen Lamb
25	Frozen Lamb
26	Frozen Lamb
28	Frozen Raspberries
34	Frozen Oysters
36	Unfrozen Sole
37	Unfrozen Sole
38	Frozen Sole
42	Unfrozen Haddock
49	Frozen Oyster
50	Unfrozen Oyster
59	Unfrozen Haddock

Isolated from	Total number	Number showing growth	Per Cent showing appreciable growth at 25° F.
Unfrozen Sole	4	3	75%
Frozen Sole	16	4	25%
Total	20		
Unfrozen Haddock	4	3	75%
Frozen Haddock	12	4	33.3%
Total	16		
Unfrozen Oysters	6	1	16.7%
Frozen Oysters	7	5	71.4%
Total	13		
Frozen Lamb Chops	8	6	75%
Frozen Raspberries	5	2	40%
	Total 30		
Organisms from Unfrozen Products	14	7	49.6%
Organisms from Frozen Products	48	21	43.7%

These results show no greater percentage of psychrophiles in the quick frozen than in the unfrozen products. There is no reason to believe that psychrophiles are better able to withstand the effect of quick freezing than are other bacteria.

Bacilli, Cocci and Spore Formers

	Forms	Per Cent of Total
#1 to #29	17 Bacilli	58.6%
Frozen Products	15 Spore Formers	88.2% *
(29 organisms)	12 Cocci	41.4%
#30 to #63	7 Bacilli	36.8%
Frozen Products	1 Spore Former	14.3% *
(19 organisms)	12 Cocci	63.1%
Unfrozen products	4 Bacilli	28.6%
(14 organisms)	0 Spore Formers	0.0% *
	10 Cocci	78.5%

*Per Cent of total bacilli

The organisms numbered from 1 to 29 inclusive were isolated from food products which had been stored at least one month at -20° F. after being frozen at a temperature of -45° F. (See page 11) It is interesting to note the high percentage of bacilli present in this group and the even higher percentage of spore formers. Whether this is due to the fact that these products were originally infected with a larger number of spore formers or whether the percentage has been increased by freezing is problematical.

The organisms numbered from 30 to 63 inclusive consist of 19 organisms from frozen products and 14 from unfrozen products. The frozen products show a greater percentage of bacilli and a smaller percentage of cocci than the unfrozen products.

Spoilage Organisms

The ability of the organisms to spoil the product from which they were isolated was investigated by growing each of them in an infusion made for this product. After growth had taken place the infusion was submitted to an olfactory test and in some cases to tests for ammonia or indol or for both.

Following are the results of these tests:-

Haddock Infusion	Olfactory Test
#5	No spoilage
#6	No spoilage
#7	Spoilage
#8	No spoilage
#59	No spoilage
#60	No spoilage
#61	Spoilage
#62	Spoilage
#63	Spoilage
#22	Spoilage
#23	No spoilage
#41	Spoilage - sour
#42	No spoilage
#43	Extreme spoilage, indol
#44	No spoilage
#45	Spoilage

Sole Infusion	Olfactory Test	Test for NH_3
#1	Spoilage	
#2	Extreme spoilage	Ammonia(and indol)
#3	Spoilage	(Slight indol)
#4	Slight spoilage	
#54	Extreme spoilage	Ammonia
#55	Slight spoilage	
#56	Slight spoilage	
#57	Extreme spoilage	
#58	Unspoiled	
#16	Spoilage	Ammonia
#17	Spoilage	Ammonia (indol)
#18	Spoilage	Ammonia
#19	Spoilage	Ammonia
#20	Slight spoilage	Ammonia
#21	Extreme spoilage	Ammonia
#36	Spoilage	
#37	Slight spoilage	
#38	Extreme spoilage	Ammonia
#39	Slight spoilage	
#40	Slight spoilage	

Oyster Infusion

	Olfactory Test	Test for NH_3
#46	Spoilage	Positive
#47	Spoilage	Positive
#48	Spoilage, sour	
#49	Not inoculated	
#50	Spoilage	Positive
#51	Not inoculated	
#52	Spoilage	Positive
#30	Spoilage	Positive
#31	Unspoiled	
#32	Spoilage	Positive
#33 -	Spoilage	Positive (indol)
#34	Spoilage	Positive
#35	Not inoculated	

Lamb Infusion

	Olfactory Test	Test for NH_3
#24	Spoilage	Positive
#25	Spoilage	Positive
#26	Extreme spoilage	Positive
#27	Extreme spoilage	
# 9	Slight spoilage	Positive
#10	Spoilage, sour	Positive
#11	Spoilage	Positive
#12	Extreme Spoilage	Positive

Conclusions

From the foregoing discussions and data the writer ventures to draw certain conclusions, appreciating, however, that the work is not extensive enough to render any of these statements final.

1. Quick freezing effects a sizeable reduction in the number of bacteria present in a food product.

2. The percentage reduction in the number of bacteria due to quick freezing varies with the food product and with the initial number of bacteria present in the product.

3. Sterilization of the food product is not effective by quick freezing at a temperature of -45° F. or by later storage at -20° F. for periods as long as six months.

4. No special type or class of bacteria are outstanding among those surviving quick freezing.

Appendix

#1 - Isolated from frozen sole.

Rods - long chains, 0.8 x 3.0 microns
Spores - 0.8 x 1.6 microns, ellipsoidal
Gram stain - negative
Gelatin - slight napiform liquefaction in eight days
Nitrate broth - nitrite
Agar slant - filiform, moderate, flat, glistening, smooth, opaque
Optimum temperature - 20° or 37° C. Doubtful growth at 25° F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#2 - Isolated from frozen sole.

Rods - single, 0.6 x 1.0 microns
Gram stain - negative
Gelatin - stratiform liquefaction
Nitrate broth - no nitrite
Agar slant - moderate, filiform, flat, glistening, smooth, translucent
Optimum temperature - 20° C. No growth at 37° C. Abundant growth at 25° F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - peptonization (entire in five days)
Lead acetate agar - H_2S

#3 - Isolated from frozen sole.

Cocci - single, pairs, fours, clusters - 0.8 micron
Gram stain - negative
Gelatin - no liquefaction
Nitrate broth - no nitrite
Agar slant - moderate, filiform, raised, glistening, contoured, opaque, iridescent
Optimum temperature - 20° C. No growth at 37° C. Moderate growth at 25° F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#4 - Isolated from frozen haddock.

Cocci - single, pairs, clusters, 0.8 microns
Gram stain - negative
Gelatin - no liquefaction
Nitrate broth - no nitrite
Agar slant - scanty, filiform (effuse), raised, glistening, contoured, opaque
Optimum temperature - 20° C.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#5 - Isolated from frozen haddock

Cocci - single, pairs, fours, clusters, 0.8 microns
Gram stain - positive
Gelatin - no liquefaction
Nitrate broth - nitrite
Agar slant - scanty, beaded, flat, glistening, contoured (slight), opaque
Optimum temperature - 20°C. No growth at 37°C. Moderate growth at 25°F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#6 - Isolated from frozen haddock.

Cocci - single, pairs, fours, clusters, 0.9 microns
Gram stain - positive
Gelatin - no liquefaction
Nitrate broth - no nitrite
Agar slant - scanty, filiform, raised, glistening, contoured, (slight), opaque
Optimum temperature - 20°C. No growth at 37°C.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#7 - Isolated from frozen haddock.

Cocci - single, pairs, fours, clusters, 0.8 microns
Gram stain - positive
Gelatin - no liquefaction
Nitrate broth - no nitrite
Agar slant - scanty, beaded, flat, glistening, contoured, (slight), opaque
Chromogenesis - pale yellow brown (brown orange in old cultures)
Optimum temperature - 20°C. No growth at 37°C. Moderate growth at 25°F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#8 - Isolated from frozen haddock.

Cocci - single, pairs, clusters, 1.0 micron
Gram stain - negative
Gelatin - no liquefaction
Nitrate broth - nitrite
Agar slant - scanty, beaded, raised, glistening (slight), rugose, opaque
Optimum temperature - 20°C. No growth at 37°C. Abundant growth at 25°F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - slight H_2S

#9 - Isolated from frozen lamb chop.

Rods - single, pairs, 1.5 x 3.0 microns
Spores - ellipsoidal, 1.0 x 1.8 microns
Gram stain - positive
Gelatin - infundibuliform liquefaction
Nitrate broth - no nitrite
Agar slant - abundant, spreading, flat, dull, rugose, (slight), opaque
Optimum temperature - 20°C. or 37°C.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - acid curd, peptonization (slight), reduction of litmus
Lead acetate agar - no H_2S

#10 - Isolated from frozen lamb chops.

Rods - 0.5 x 0.9 microns
Gram stain - negative
Gelatin - crateriform liquefaction
Nitrate broth - nitrite
Agar slant - abundant, rhizoid, flat, glistening, smooth, opaque
Optimum temperature - 20°C. or 37°C. Abundant growth at 25°F.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - acid, no gas
Litmus milk - acid curd, peptonization
Lead acetate agar - no H_2S

#11 - Isolated from frozen lamb chops.

Cocci - single, clusters, 0.5 microns
Gram stain - negative
Nitrate broth - no nitrite
Gelatin - slight napiform liquefaction in eight days
Agar slant - moderate, filiform, raised, glistening, smooth, opaque, iridescent
Optimum temperature - 20°C. No growth at 37°C. Abundant growth at 25°F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#12 - Isolated from frozen lamb chops.

Cocci - single, clusters, 0.4 microns
Gram stain - negative
Gelatin - napiform liquefaction
Nitrate broth - no nitrite
Agar slant - moderate, filiform, raised, glistening, opaque, iridescent
Optimum temperature - 20°C. Abundant growth at 25°F.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - acid, no gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#13 - Isolated from frozen raspberries.

Rods - single, 1.0 x 3.0 microns
Spores - 0.8 x 1.8 microns
Gram stain - negative
Gelatin - saccate liquefaction
Nitrate broth - nitrite
Agar slant - abundant, spreading, flat, dull, rugose, opaque
Optimum temperature - 20°C. or 37°C.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - acid, no gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#14 - Isolated from frozen raspberries.

Rods - single, chains, 1.6 x 3.5 microns
Spores - ellipsoidal, 1.3 x 1.8 microns
Gram stain - positive
Gelatin - infundibuliform liquefaction
Nitrate broth - no nitrite
Agar slant - moderate, echinulate, flat, dull, smooth, opaque - at 37°C. filiform, raised, glistening,
Chromogenesis - dirty brown, pale brown tint at 37°C.
Optimum temperature - 20°C. or 37°C.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#15 - Isolated from frozen raspberries.

Cocci - single, 0.5 microns
Gram stain - negative
Gelatin - crateriform liquefaction
Nitrate broth - nitrite
Agar slant - scanty, beaded, flat, glistening, contoured (slight), opaque, iridescent
Chromogenesis - dirty brown yellow
Optimum temperature - 20° C. or 37° C. Moderate growth at 25° F.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - acid, no gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#16 - Isolated from frozen sole.

Rods - chains, 1.0 x 3.2 microns
Spores - cylindrical, 0.9 x 1.6 microns
Gram stain - positive
Gelatin - saccate liquefaction
Nitrate broth - nitrite
Agar slant - abundant, spreading, flat, glistening, smooth, opaque, iridescent
Optimum temperature - 20° C. or 27° C.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - peptonization
Lead acetate agar - no H_2S

#17 - Isolated from frozen sole.

Rods - chains, 1.5 x 3.0 microns
Spores - ellipsoidal, 0.8 x 2.0 microns
Gram stain - positive
Gelatin - saccate liquefaction
Nitrate broth - nitrite and ammonia
Agar slant - abundant, rhizoid, flat, dull, smooth, opaque
Optimum temperature - 20°C. or 37°C.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - peptonization
Lead acetate agar - no H_2S

#18 - Isolated from frozen sole.

Rods - single, 0.8 x 3.0 microns
Spores - ellipsoidal, 1.0 x 1.6 microns
Gram stain - negative
Gelatin - stratiform liquefaction
Nitrate broth - nitrite and ammonia
Agar slant - moderate, filiform, flat, dull, rugose, opaque
Optimum temperature - 20°C. or 37°C.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - peptonization
Lead acetate agar - no H_2S

#19 - Isolated from frozen sole.

Rods - single, 0.8 x 3.0 microns
Spores - ellipsoidal, 0.8 x 1.5 microns
Gram stain - negative
Gelatin - infundibuliform liquefaction
Nitrate broth - nitrite and ammonia
Agar slant - moderate, filiform, flat, dull, rugose, opaque
Optimum temperature - 20°C or 37°C
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - peptonization
Lead acetate agar - no H_2S

#20 - Isolated from frozen sole.

Cocci - single, pairs, fours, 1.6 microns
Gram stain - negative
Gelatin - no liquefaction
Nitrate broth - nitrite
Agar slant - scanty, beaded, raised, glistening, contoured, opaque
Optimum temperature - 20°C. or 37°C. Abundant growth at 25°F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#21 - Isolated from frozen sole.

Cocci - single, clusters, 1.0 microns
Gram stain - negative
Gelatin - infundibuliform liquefaction
Nitrate broth - nitrite
Agar slant - moderate, beaded, raised, dull, smooth, opaque
Optimum temperature - 20°C. or 37°C.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#22 - Isolated from frozen haddock.

Rods - single, 0.9 x 2.5 microns
Spores - 0.8 x 1.4 microns
Gram stain - positive
Gelatin - infundibuliform liquefaction
Nitrate broth - nitrite and ammonia
Agar slant - moderate, filiform, flat, dull, rugose, opaque
Optimum temperature - 20°C. or 37°C.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - peptonization
Lead acetate agar - no H_2S

#23 - Isolated from frozen haddock.

Cocci - single, clusters, 0.4 microns
Gram stain - negative
Gelatin - no liquefaction
Nitrate broth - nitrite
Agar slant - filiform, scanty, flat, glistening, smooth, opaque
Optimum temperature - 37°C. No growth at 20°C.
Dextrose broth - acid, no gas
Lactose broth - acid, no gas
Sucrose broth - acid, no gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#24 - Isolated from frozen lamb.

Rods - single, short chains, 0.7 x 2.5 microns
Spores - 1.0 x 1.6 microns
Gram stain - positive
Gelatin - infundibuliform liquefaction
Nitrate broth - nitrite and ammonia
Agar slant - moderate, filiform, flat, dull, rugose, opaque
Optimum temperature - 20°C. or 37°C. Moderate growth at 25°F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - peptonization
Lead acetate agar - H_2S

#25 - Isolated from frozen lamb.

Rods - single, pairs, 0.8 x 3.0 microns
Spores - ellipsoidal, 0.9 x 1.5 microns
Gram stain - negative
Gelatin - infundibuliform liquefaction
Nitrate broth - nitrite and ammonia
Agar slant - moderate, filiform, flat, dull, rugose, opaque
Optimum temperature - 20°C. or 37°C. Moderate growth at 25°F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - peptonization
Lead acetate agar - no H_2S

#26 - Isolated from frozen lamb.

Rods - single, 0.4 x 1.6 microns
Spores - 0.8 x 1.5 microns
Gram stain - negative
Gelatin - infundibuliform liquefaction
Nitrate broth - no nitrite
Agar slant - abundant, spreading, flat, glistening, smooth, opaque
Optimum temperature - 37°C. Moderate growth at 25°F.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - acid, no gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#27 - Isolated from frozen lamb.

Rods - single, 0.6 x 2.5 microns
Spores - 0.6 x 1.6 microns
Gram stain - negative
Gelatin - infundibuliform liquefaction
Nitrate broth - nitrite and ammonia.
Agar slant - moderate, filiform, flat, dull, rugose, opaque
Optimum temperature - 20° C. or 37° C.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - peptonization
Lead acetate agar - no H_2S

#28 - Isolated from frozen raspberries.

Rods - single, pairs, 0.5 x 1.8 microns
Spores - ellipsoidal, 0.8 x 1.5 microns
Gram stain - negative
Gelatin - infundibuliform liquefaction
Nitrate broth - nitrite and ammonia
Agar slant - moderate, filiform, flat, dull, rugose, opaque
Optimum temperature - 20° C. or 37° C. Moderate growth at 25° F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - peptonization
Lead acetate agar - no H_2S

#29 - Isolated from frozen raspberries.

Rods - long chains, 1.2 x 3.8 microns
Spores - ellipsoidal, central, 1.3 x 1.8 microns
Gram stain - positive
Gelatin - stratiform liquefaction
Nitrate broth - no nitrite
Agar slant - moderate, filiform, flat, dull, smooth, opaque
Optimum temperature - 20°C. or 37°C.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#30 - Isolated from unfrozen oysters.

Cocci - clusters, 0.7 microns
Gram stain - positive
Gelatin - infundibuliform liquefaction
Nitrate broth - no nitrite
Agar slant - scanty, beaded, flat, glistening, smooth, opaque
Chromogenesis - pale yellow, yellow orange in old cultures
Optimum temperature - 37°C.
Dextrose broth - acid, no gas
Lactose broth - acid, no gas
Sucrose broth - acid, no gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#31 - Isolated from unfrozen oysters.

Rods - single, 0.3 x 0.8 microns
Gram stain - negative
Gelatin - moderate crateriform liquefaction
Nitrate broth - nitrite and ammonia
Agar slant - moderate, filiform, convex, glistening, smooth, translucent
Optimum temperature - 20° or 37° C.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - acid, no gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#32 - Isolated from unfrozen oysters.

Cocci - single, clusters, 0.8 microns
Gram stain - negative
Gelatin - no liquefaction
Nitrate broth - no nitrite
Agar slant - scanty, beaded, flat, glistening, smooth, opaque, (very white)
Optimum temperature - 37° C. No growth at 20° C.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#33 - Isolated from frozen oysters.

Rods - chains, 1.6 x 2.5 microns
Spores - ellipsoidal, 0.8 x 2.0 microns
Gram stain - negative
Gelatin - infundibuliform liquefaction
Nitrate broth - nitrite and ammonia
Agar slant - abundant, spreading, flat, glistening, smooth, opaque
Optimum temperature - 37°C.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - acid, no gas
Litmus milk - peptonization
Lead acetate agar - no H_2S

#34 - Isolated from frozen oysters.

Cocci - clusters, 0.9 microns
Gram stain - positive
Gelatin - no liquefaction
Nitrate broth - nitrite and ammonia
Agar slant - filiform, flat, glistening, smooth, opaque
Chromogenesis - pale orange
Optimum temperature - 37°C. Moderate growth at 25°F.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#35 - Isolated from frozen oysters.

Cocci - clusters, 0.4 microns (0.3 - 0.6 microns)
Gram stain - negative
Gelatin - infundibuliform liquefaction
Nitrate broth - no nitrite
Agar slant - scanty, beaded, glistening, smooth, translucent
Optimum temperature - 37° C.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#36 - Isolated from unfrozen sole.

Cocci - single, pairs, fours, 1.0 microns (0.8 - 1.2 microns)
Gram stain - negative
Gelatin - no liquefaction
Nitrate broth - nitrite
Agar slant - scanty, beaded, flat, glistening, smooth, translucent
Optimum temperature - 20° C. or 37° C. Moderate growth at 25° F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - slight H_2S

#37 - Isolated from unfrozen sole.

Cocci - 0.6 microns
Gram stain - positive
Gelatin - no liquefaction
Nitrate broth - nitrite
Agar slant - moderate, filiform, raised, glistening, smooth, opaque
Chromogenesis - brilliant yellow
Optimum temperature - 20°C. or 37°C. Moderate growth at 25°F.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - slight H_2S

#38 - Isolated from frozen sole.

Rods - single, 0.7 x 1.8 microns
Gram stain - negative
Gelatin - crateriform liquefaction (moderate)
Nitrate broth - nitrite
Agar slant - moderate, filiform, flat, glistening, smooth, opaque
Chromogenesis - light brown orange (not like #7)
Optimum temperature - 37°C. Moderate growth at 25°F.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - acid, no gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#39 - Isolated from frozen sole.

Cocci - sarcinae, 0.8 microns
Gram stain - positive
Gelatin - no liquefaction
Nitrate broth - nitrite
Agar slant - moderate, filiform, slightly raised, glistening, contoured, opaque
Chromogenesis - cream color (yellow tint at 20° C.)
Optimum temperature - 20° C. or 37° C.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#40 - Isolated from frozen sole.

Cocci - 0.7 microns
Gram stain - negative
Gelatin - slight napiform liquefaction
Nitrate broth - no nitrite
Agar slant - scanty, beaded, flat, glistening, smooth, opaque
Optimum temperature - 37° C.
Dextrose broth - acid, no gas
Lactose broth - acid, no gas
Sucrose broth - acid, no gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#41 - Isolated from unfrozen haddock.

Cocci - clusters, (chains) 0.8 microns
Gram stain - positive
Gelatin - crateriform liquefaction (moderate)
Nitrate broth - no nitrite
Agar slant - moderate, filiform, flat, glistening, rugose, opaque
Chromogenesis - orange
Optimum temperature - 37°C.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#42 - Isolated from unfrozen haddock.

Cocci - clusters, 1.0 microns
Gram stain - positive
Gelatin - moderate crateriform liquefaction
Nitrate broth - no nitrite
Agar slant - moderate, filiform, flat, glistening, smooth, opaque (very white)
Optimum temperature - 20°C. or 37°C. Moderate growth at 25°F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#43 - Isolated from frozen haddock.

Rods - single, 0.8 x 1.8 microns
Gram stain - negative
Gelatin - no liquefaction
Nitrate broth - no nitrite
Agar slant - moderate, filiform, slightly raised, glistening, smooth, opaque
Chromogenesis - pale brown orange
Optimum temperature - 37°C.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - acid, no gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#44 - Isolated from frozen haddock.

Cocci - single to clusters, 0.8 microns
Gram stain - negative
Gelatin - slight crateriform liquefaction
Nitrate broth - nitrite
Agar slant - moderate, beaded, flat, dull, contoured, opaque
Optimum temperature - 37°C.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#45 - Isolated from frozen haddock.

Cocci - single, pairs, sarcinae, 0.7 microns
Gram stain - positive
Gelatin - moderate crateriform liquefaction
Nitrate broth - no nitrite
Agar slant - scanty, effuse, raised, glistening, rugose, opaque
Chromogenesis - brilliant yellow
Optimum temperature - 20°C. or 37°C.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - acid, no gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#46 - Isolated from frozen oysters.

Rods - 0.4 x 0.9 microns
Gram stain - negative
Gelatin - infundibuliform liquefaction
Nitrate broth - no nitrite
Agar slant - moderate, filiform, raised, glistening, slightly contoured, opaque
Chromogenesis - pale brown yellow
Optimum temperature - 20°C. No growth at 37°C. Abundant growth at 25°F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - peptonization
Lead acetate agar - H_2S

#47 - Isolated from frozen oysters.

Rods - 0.4 x 0.5 microns
Gram stain - negative
Gelatin - stratiform liquefaction
Nitrate broth - no nitrite
Agar slant - moderate, filiform, convex, glistening, smooth, translucent
Chromogenesis - greenish tinge
Optimum temperature - 20° C. Abundant growth at 25° F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - slight peptonization
Lead acetate agar - H_2S

#48 - Isolated from frozen oysters.

Rods - 0.4 x 1.2 microns
Gram stain - negative
Gelatin - no liquefaction
Nitrate broth - no nitrite
Agar slant - moderate, filiform, slightly raised, glistening, contoured, fluorescent
Optimum temperature - 20° C. No growth at 37° C. Abundant growth at 25° F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#49 - Isolated from frozen oysters.

Rods - single, 0.3 x 1.5 microns
Gram stain - negative
Gelatin - moderate napiform liquefaction.
Nitrate broth - no nitrite
Agar slant - abundant, filiform, flat, glistening, smooth, opaque
Chromogenesis - yellow brown
Optimum temperature - 20°C. or 37° C.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#50 - Isolated from unfrozen oysters.

Rods - single, 0.6 x 1.0 microns
Gram stain - negative
Gelatin - infundibuliform liquefaction
Nitrate broth - no nitrite
Agar slant - moderate, filiform, slightly raised, glistening, smooth, opaque, viscid
Chromogenesis - deep orange (brown orange in old cultures)
Optimum temperature - 20°C. no growth at 37°C. Moderate growth at 25°F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#51 - Isolated from unfrozen oysters.

Rods - single, 0.3 x 1.4 microns
Gram stain - negative
Gelatin - moderate napiform liquefaction
Nitrate broth - no nitrite
Agar slant - abundant, filiform, flat, glistening, smooth, opaque
Chromogenesis - brown yellow
Optimum temperature - 20°C. or 37°C.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#52 - Isolated from unfrozen oysters.

Cocci - clusters, o.8 microns
Gram stain - negative
Gelatin - no liquefaction
Nitrate broth - no nitrite
Agar slant - moderate, filiform, raised, glistening, slightly contoured, opaque, iridescent
Optimum temperature - 20°C. or 37°C.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#53 - Isolated from unfrozen oysters.

No growth.

#54 - Isolated from unfrozen sole.

Rods - 0.4 x 0.5 microns
Gram stain - negative
Gelatin - moderate, beaded, liquefaction
Nitrate broth - nitrite and ammonia
Agar slant - moderate, filiform, slightly raised, slightly glistening, contoured, translucent, iridescent
Optimum temperature - 20°C. or 37°C.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - peptonization
Lead acetate agar - no H_2S

#55 - Isolated from unfrozen sole.

Cocci - single, pairs, clusters, 0.8 microns
Gram stain - positive
Gelatin - no liquefaction
Nitrate broth - nitrite
Agar slant - moderate, filiform, slightly raised, glistening, smooth, opaque, viscid
Chromogenesis - cream color
Optimum temperature - 20°C. No growth at 37°C. Abundant growth at 25°F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#56 - Isolated from frozen sole.

Cocci - pairs, 0.5 microns
Gram stain - positive
Gelatin - moderate mapiform liquefaction
Nitrate broth - nitrite
Agar slant - abundant, filiform, raised, glistening, smooth, opaque
Chromogenesis - yellow green in old cultures
Optimum temperature - 20°C.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#57 - Isolated from frozen sole.

Cocci - single, clusters, 1.0 micron
Gram stain - positive
Gelatin - slight napiform liquefaction
Nitrate broth - no nitrite
Agar slant - moderate, filiform, raised, slightly glistening, slightly rugose, opaque
Chromogenesis - deep brown orange
Optimum temperature - 20°C.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#58 - Isolated from frozen sole.

Cocci - o.8 microns
Gram stain - negative
Gelatin - no liquefaction
Nitrate broth - nitrite
Agar slant - filiform, abundant, raised, glistening, smooth, opaque
Chromogenesis - cream color (yellow green in old cultures)
Optimum temperature - 20°C. Moderate growth at 25°F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#59 - Isolated from unfrozen haddock.

Cocci - clusters, 0.8 microns
Gram stain - positive
Gelatin - moderate napiform liquefaction
Nitrate broth - no nitrite
Agar slant - moderate, filiform, raised, glistening, slightly rugose, opaque
Chromogenesis - pale orange
Optimum temperature - 20°C. or 37°C. Moderate growth at 25°F.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#60 - Isolated from unfrozen haddock.

Cocci - single, clusters, 1.0 microns
Gram stain - Negative
Gelatin - no liquefaction
Nitrate broth - no nitrite
Agar slant - moderate, filiform, convex, glistening, smooth, opaque, viscid.
Chromogenesis - cream color
Optimum temperature - 20°C. Moderate growth at 25°F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#61 - Isolated from frozen haddock.

Cocci - single, pairs, clusters, 1.0 microns
Gram stain - positive
Gelatin - no liquefaction
Nitrate broth - nitrite
Agar slant - scanty, filiform, slightly raised, glistening, slightly contoured, opaque
Chromogenesis - pale orange
Optimum temperature - 20°C. or 37°C.
Dextrose broth - acid, no gas
Lactose broth - no acid or gas
Sucrose broth - acid, no gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

#62 - Isolated from frozen haddock.

Cocci - single, pairs, clusters, 0.8 microns
Gram stain - negative
Gelatin - no liquefaction
Nitrate broth - no nitrite
Agar slant - moderate, filiform, convex, glistening, smooth, opaque
Chromogenesis - cream color
Optimum temperature - 20°C. Abundant growth at 25°F.
Dextrose broth - no acid or gas
Lactose broth - no acid or gas
Sucrose broth - no acid or gas
Litmus milk - no reaction
Lead acetate agar - H_2S

#63 - Isolated from frozen haddock.

Cocci - single, pairs, 1.0 microns
Gram stain - positive
Gelatin - moderate napiform liquefaction
Nitrate broth - nitrite and ammonia
Agar slant - moderate, beaded, raised, glistening, rugose, opaque
Optimum temperature - 20°C. or 37°C.
Dextrose broth - acid, no gas
Lactose broth - acid, no gas
Sucrose broth - acid, no gas
Litmus milk - no reaction
Lead acetate agar - no H_2S

Methods

Media - All culture media used was manufactured by the Digestive Ferments Co.

Nutrient Agar	pH 6.7 and pH 7.3
Nutrient Gelatin	pH 6.6
Nutrient Broth	pH 6.8
Dextrose Broth	pH 6.76
Sucrose Broth	pH 6.8
Lactose Broth	pH 6.8

Tests -

Ammonia - tested for with Nessler's reagent.

Indol - Ehrlich - Böhme

Solution #1

Para-dimethyl-amino-benzaldehyde	1 gr.
Ethyl alcohol (95%)	95 cc.
Hydrochloric acid, concentrated	20 cc.

Solution #2

Saturated aqueous solution of potassium persulfate.

Procedure

The organism is grown for 48 hours in a peptone solution containing tryptophane. To about 10 cc. of the culture medium add 5 cc. of solution #1 and 5 cc. of solution #2. Shake. The appearance of a red color in five minutes is evidence of the presence of indol.

Nitrite - The organism is grown in nitrate broth. The nitrite test is performed by adding 1 cc. of sulphanilic acid and 1 cc. of alpha naphthyl-amine acetate. The presence of a pink or red color is indicative of nitrites.

Acid in Sugar Broths - Tested for with Brom cresol purple. When the acid is present the indicator turns yellow.

Bacterial Stains

Gram Stain - Hucker modification

Solution A

Crystal violet (85% dye content)	4 g.
Ethyl alcohol (95%)	20 cc.

Solution B

Ammonium oxalate	0.8 g.
Water	80 cc.

Mix solutions A and B

Lugol's Iodin Solution

Iodin	1 g.
Potassium iodide	2 g.
Water	300 cc.

Counterstrain

Safranin (saturated solution in 95% alcohol)	10 cc.
Water	100 cc.

Technic

Stain 1 minute with the gentian violet solution; wash in water; immerse in iodin solution for 1 minute; wash in water and blot dry; decolorize in 95% alcohol for 30 seconds with gentle agitation; cover with counterstain for 10 seconds. Then wash, dry, and examine with the oil immersion lens.

Gram plus organisms will retain the color of the crystal violet; Gram negative organisms will lose this color and take that of the safranin.

Bibliography

Effect of Freezing and Cold on Bacteria

1. Hilliard, C.M., Torossian, C. and Stone, R.
1915. Notes on the factors involved in the germicidal effect of freezing and low temperatures.
Science 42: Nov. 26

2. Hilliard, C.M. and Davis, Mildred A.
1918. Germicidal Action of Freezing Temperatures upon Bacteria.
Jour. of Bact. 3: 423-431

3. Keith, S. C.
1913. Factors influencing the survival of bacteria at temperatures in the vacinity of the freezing point of water.
Science 37: 877-879 June 6

4. Sedgwick, W. T. and Winslow, C. E. A.
1902. Experiments on the effect of freezing and other low temperatures on vitality of bacillus of typhoid fever.
Mem. Acad. Arts and Sc. 12.(5)

5. .....
1918. Bibliography of articles on the effect of cold temperatures on bacteria.
Jour. of Bact. 3: 423

Quick Freezing

6. Birdseye, Clarence
1929. Some scientific aspects of packaging and quick freezing perishable flesh products.
Jours of Ind. and Eng. Chem. 21: 414

7. 1929. " " " " " " 21: 573

8. 1929. " " " " " " 21: 854

9. 1930. Packaging Quick Frozen Foods
Food Industries 2: 156-158

10. Burton, Laurence V.
1930. How Fillets are Frozen by the Cooke Method
Food Industries 2: 146-151

11. Carroll, Thomas J.
1930. A Kolbe Installation at the Gorton Pew Plant
Food Industries2:169-172

12. Kolbe, Carl F.
1930. Why Quick Freeze?
Food Industries 2: 165-168

13. Pattison, E. Scott
1930. Freezing Fish by the Birdseye Method
Food Industries 2: 159-161

14. Taylor, Harden F.
1929. The Growth and Future of the Frozen Fish Industry.
Fishing Gazette - Review Number 1929

15. 1930. Solving the Problems of Quick Freezing
Food Industries 2: 146-151