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TECHNICAL REPORT
66-35-FD

A STUDY OF THE MICROBIOLOGY OF SELECTED DEHYDRATED FOOD PRODUCTS

by

F. E. Wells

Midwest Research Institute
Kansas City, Missouri

Contract No. DA-19-129-AMC-206 (N)

May 1966

UNITED STATES ARMY
NATICK LABORATORIES
Natick, Massachusetts 01760

Food Division
FD-49

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FOREWORD

This investigation was undertaken relative to studies on microbial contamination of dehydrated foods. The area of microbial examinations of dehydrated food items, especially freeze-dehydrated products, has seemingly received less attention than other segments of the processed food industry. It was therefore deemed necessary that a study of the microflora of dehydrated food products used in special military rations be initiated. Subject investigation included studies to determine if subject microorganisms undergoing the freeze-drying process in food items are changed chemically and/or physiologically.

The work covered in this report, performed by Midwest Research Institute, Kansas City, Missouri under Contract No. DA19-129-AMC-206(N) was designed to obtain specific technical information relative to selected dehydrated food products. Dr. Frank E. Wells was the Official Investigator.

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ABSTRACT

The effect of freeze-drying on the viability of six species of bacteria on seven kinds of food is presented. Freeze-drying influences are presented and discussed in relation to the effects of freezing, drying and storage conditions. The influence of food type and physiological age of bacteria is shown to influence both immediate losses in viability as well as the rate at which such losses occur during storage. The rate of decline in bacterial viability during storage is discussed as a function of the degree of metabolic injury sustained by the bacteria during the freeze-drying process.

SUMMARY

Fruits, meats and vegetables were inoculated with strains of Salmonella oranienburg, Staphylococcus aureus, Aerobacter aerogenes, Escherichia coli, Alcaligenes faecalis and spores of Clostridium botulinum (A). The reduction in viability of these bacteria during the freeze-drying process was determined. Calculations were also made of the proportion of the total microbial reduction which was brought about by the freezing and by the drying phases of the freeze-drying process. The rate of reduction in bacterial numbers during storage was also determined.

The freeze-drying process was found to reduce viability by 90 per cent or more in the vegetative cells of five species of bacteria and 50 per cent or more for spores of Cl. botulinum.

The greatest reduction in bacterial numbers occurred during the freezing phase of the process. However, percentagewise, reduction in the numbers of cells through either the influence of freezing or dehydration was found to be of nearly equal value.

The type of food on which the cells were dried influenced survival; foods high in organic acids appeared to support lower populations.

The loss of viability in bacteria was more rapid during early storage periods than during later periods. This early, rapid loss is believed to be related to the degree of metabolic injury to which the cells were subjected during the freeze-drying process.

The age of the cells for two bacterial species (S. oranienburg and S. aureus) at the time of freeze-drying did not greatly affect the percentage of cells which survived when counts were made on "rich" medium. Metabolic damage was greater, however, in "young" cells than in "old" cells and fewer of the younger cells could be recovered after freeze-drying when a "minimal" medium was used for plating.

Storage temperature had a great influence on the rate at which the bacteria on the dry foods declined in viability. Bacterial numbers declined more rapidly at 100°F and 70°F than at 40°F. Packaging the dry foods in nitrogen resulted in higher bacterial survival rates than packaging in air. However, bacteria on the foods stored at 40°F and packaged in air had higher survival rates than those on similar foods packaged in nitrogen and stored at 100°F or 70°F.

The effect of diluent and diluent temperature was found to vary and results were inconclusive. Variations in optimum diluent temperature and types of diluting fluid appear to be related to the type of food sampled and presumably to the different bacterial types found on these foods.

I. INTRODUCTION

Lyophilization is an often-used technique for maintaining cultures of microorganisms. Because of this, questions have arisen about the persistence of viable bacteria on freeze-dried foods.

In order to adequately evaluate the significance of numbers of bacteria on freeze-dried foods or to give meaning to the presence of certain bacterial types, it is necessary to determine the effects of the freeze-drying process on the reactions of bacteria to their environment. To do this, an evaluation of both the intrinsic and extrinsic factors which are associated with the freezing and drying phases of the process (for specific foods) has to be made. We must determine the immediate and long term influences of these factors on loss of viability during the storage period.

Information on the bacteriocidal aspects of the freeze-drying process for individual species and for mixed populations is not available. We need such information on the lethality of the process for certain bacterial "indicator" species, and we need to know how these processing effects will influence the persistence of bacteria on the dried foods during storage.

This study was undertaken for the U. S. Army Natick Laboratories to gather information concerning the persistence of bacteria on freeze-dried foods following dehydration and the proportional influence of the freezing and dehydration phases on the maintenance of viability during the processing of the foods.

We have attempted to relate the results obtained from the freeze-drying studies to the results obtained from a census of bacteria found on certain dehydrated, military rations.

II. METHODS AND MATERIALS

A. Microbial Survey of Dehydrated Military Rations

The census of total aerobic populations on dehydrated Quartermaster rations was made using plate count agar (PCA), Difco, for the

plating medium. Dilution medium was phosphate buffer at pH 7.0. Agar plates were prepared in duplicate and were incubated at 30°C for 48 hr. prior to counting.

Coliform bacteria were enumerated on violet red bile agar, Difco, after 24 hr. incubation.

Anaerobic spore counts were made after the serial dilutions had been heated to 80°C and held for 10 min. in order to initiate the spore germination processes. Platings of aliquots of these dilutions were made on anaerobic agar (BBL). The plates were incubated anaerobically at 35°C.

Examination of the foods for members of the genus Salmonella was done essentially by the method of Angelotti (1963). The recommended method called for use of brilliant green sulfadiazine agar; in our examinations we used brilliant green agar without the addition of sulfadiazine. Numbers of coliforms were so low that overgrowth in the enrichment medium by these organisms was not a problem.

The solid plating medium of Kenner et al.(1961) was used for determining the incidence of fecal streptococci. The plates were incubated at 35°C for 48 hr. Fecal streptococci appeared as pink surface colonies or as pink to red subsurface colonies.

The military foods included in the survey were supplied by the U. S. Army Natick Laboratory, Natick, Massachusetts. All items were a minimum of one year old.

B. Freeze-Dried Foods

The vegetables and fruits used for survival studies were prepared from canned items. The cans were opened aseptically; the solids were drained of their liquids; they were sampled to obtain background counts, and were then inoculated with a buffered suspension of bacteria.

Chicken meat was prepared by cooking cubed, deboned meat in Mylar casings. After the product had been cooked and cooled, it was frozen to -20°F and held until used. For use, the meat was thawed and transferred aseptically to a sterile container. After it had been sampled to obtain a background count, the meat was inoculated with a single species of bacteria.

A similar technique was used for inoculating fish (carp) and for beef (hamburger). The cooking, freezing, and frozen storage reduced residual bacterial counts on these meats to less than 100/g.

Throughout these studies, unless some specific factor was being considered, the following conditions prevailed during the freeze drying process: chamber pressure, less than 500 microns of mercury; condenser temperature, -80°F; freezing temperature, -50°F; platen temperature for drying, 120°F. All processing was done in a Model 11-42 RePP freeze-dryer.

C. Bacterial Test Species

Six species of bacteria were used in these tests: (1) *Salmonella oranienburg*; (2) *Staphylococcus aureus*; (3) *Aerobacter aerogenes*; (4) *Escherichia coli*; (5) *Alcaligenes faecalis*; and (6) *Clostridium botulinum* (A).

Clostridium botulinum spores were used for all tests with this organism. With the other bacteria, 24 hr. cultures were used. All foods were sampled, to determine initial numbers, at the time the trays were placed in the freezer.

D. Counts on Inoculated Foods

For determining the total aerobic population on the military rations, we used plate count agar, Difco, as the plating medium. Phosphate buffer was used as the diluting medium. However, for the inoculated, freeze-dried foods, we used trypticase soy agar (plus dextrose) as a plating medium and 0.1 per cent peptone solution for a diluent. This change was made after tests indicated that phosphate buffer was not satisfactory as a diluent when high dilution of a food was necessary. With the inoculated foods, very high dilutions were often necessary. Trypticase soy agar appeared to give slightly higher counts with frozen and dried foods than did PCA.

The "minimal" agar used for determining metabolically injured cells was prepared according to Davis (1950).

In all cases where comparisons of viable cells were made between a dried food and the original hydrated product, the value for the dehydrated product was adjusted to the number which would have been present at the original moisture level. For the storage studies this adjustment for hydration was not necessary because the initial counts for zero storage time were based on the dry weight.

E. Metabolic Injury

The definition and calculation of metabolic injury used in this study were those of Straka and Stokes (1959).

F. Storage Tests

Reductions in viable bacteria were determined for each food held at three different storage temperatures and under two packaging conditions. Storage temperatures were 100°F, 70°F and 40°F. Each food was sealed in metal containers with nitrogen (to a residual oxygen concentration of 2 per cent or less) and with air.

G. Effect of Diluent on the Recovery of Bacteria from Dry Foods

To study the effect of diluents and diluent temperature on recovery of bacteria from dry foods, four kinds of diluting media were prepared: distilled water, phosphate buffer, 0.1 per cent peptone solution and 0.85 per cent sodium chloride. These diluents were tested at three temperatures, 40°F, 70°F and 110°F. At all test temperatures the dilutions were kept at the specific temperature until the samples were plated. Sample preparation was done as rapidly as possible. No more than 15 min. was allowed to elapse between initial dilution and final plating.

To prevent changes in the diluent temperatures during initial sample preparation, the dry foods were pulverized prior to sampling. This pulverization insured more uniform distribution of contamination among samples.

Two general classes of foods were tested against the different diluents: freeze-dried beef and cabbage.

III. RESULTS

A. Reduction in Bacterial Numbers During Freeze-Dehydration

Two major killing influences act during the process of freeze-dehydration: freezing, and the subsequent effects of drying. In order to more adequately determine at which level greatest lethal influence could be brought to bear, we sampled foods at three stages: i.e., (1) before freezing; (2) after freezing and before lyophilization; and (3) after the products were dried. The effect of freezing was evaluated by determining the number of viable bacteria on the foods after thawing. The lethality of dehydration (plus the concomitant frozen storage influence) was based upon changes in the number of cells which survived freezing.

1. Salmonella oranienburg: S. oranienburg was reduced in numbers during freeze-drying from 95 - 99.9 per cent. The degree of reduction depended somewhat upon the kind of food on which it was dried (see Table I). The higher rate of decline occurred on pears and the lower rate on peas. However, the differences in response among the various foods were generally small. With responses on the other foods falling between the two extremes of pears and peas, the average reduction in S. oranienburg on all test foods was 98.3 per cent.

Because the degree of reduction in viable cells was small among the individual foods, the differences in effect based on food class were also small. For example, the numbers of S. oranienburg were reduced 96.5 per cent by freeze-drying on vegetables, by 99.9 per cent on fruits and 99.4 per cent on meats (see Table II).

2. Staphylococcus aureus: S. aureus was found to possess about the same degree of resistance to total killing effect by freeze-drying on foods as was exhibited by S. oranienburg. The range of values for S. aureus, however, was a little greater than it was for S. oranienburg; reduction extended from 78 - 99.9 per cent. For the most part, the reductions on individual foods were close to the values obtained with S. oranienburg except in the case of fish and corn, Table I. S. aureus appeared to die off on fish at a slower rate (some 20 per cent) than did S. oranienburg. However, the average reduction on all foods in the test amounted to 94.3 per cent for S. aureus while the comparable figure for S. oranienburg was 98.3 per cent.

TABLE I

PER CENT REDUCTION IN VIABLE COUNTS OF SIX SPECIES OF BACTERIA DURING THE FREEZE-DRYING OF SEVEN KINDS OF FOOD

Bacterial Species	Kind of Food							
	Spinach	Corn	Peas	Pears	Chicken	Beef	Fish	Average
Salmonella oranienburg	99.4	95.0	95.0	99.9	99.1	98.4	99.8	98.3
Staphylococcus aureus	99.1	88.0	95.0	99.9	99.9	99.4	78.0	94.3
Aerobacter aerogenes	99.1	98.0	95.0	99.9	97.7	99.4	97.6	98.2
Escherichia coli	99.1	95.0	87.0	99.9	99.9	99.9	98.9	97.2
Alcaligenes faecalis	99.1	95.0	86.0	99.9	99.8	89.0	94.0	94.8
Clostridium botulinum spores	85.3	57.0	42.0	72.7	49.0	58.0	42.0	58.0

TABLE II

EFFECT OF FOOD CLASS ON THE PER CENT OF REDUCTION OF VIABILITY IN SIX SPECIES OF BACTERIA DURING THE FREEZE-DRYING OF FRUITS, VEGETABLES, AND MEATS

Bacterial Species	Food Class		
	Vegetables	Fruits	Meats
Salmonella oranienburg	96.5	99.9	99.4
Staphylococcus aureus	94.3	99.9	92.4
Aerobacter aerogenes	97.6	99.9	98.2
Escherichia coli	94.0	99.9	99.6
Alcaligenes faecalis	93.6	99.9	94.3
Clostridium botulinum spores	64.0	73.0	39.0

When comparisons were made of the loss of viability due to freeze-drying on specific food classes, a greater lethal effect for freeze-drying of fruits was seen, Table II.

3. *Aerobacter aerogenes*: The reduction in the number of viable cells of *A. aerogenes* resulting from the accumulative effects of freeze-drying ranged from 95 - 99.9 per cent. This range was nearly the same as that found for *S. oranienburg*. The average decline in population, based on all foods tested, was identical to that of *S. oranienburg*.

When the survival of *A. aerogenes* on freeze-dried foods was considered from the standpoint of food class, i.e., whether the organism was dried on vegetables, fruits, or meats, little difference in survival pattern was noted amont the three groups. There was essentially a 98 per cent reduction in viable cells on freeze-dried vegetables and meats and a 99 per cent reduction on fruits.

4. *Escherichia coli*: With the exception of drying on peas, *E. coli* did not appear to survive the freeze-drying process any better than *S. oranienburg* or *A. aerogenes*. For the individual foods, the

range of reduction was from 87 - 99.9 per cent. Peas were the only food on which the reduction was less than 95 per cent. The average reduction, based on all foods tested, was 97 per cent, Table I.

Although peas appeared to enhance survivability of E. coli, vegetables, as a class, did not appear to differ greatly from meats or fruits. A 94 per cent reduction in numbers occurred on vegetables; a 99 per cent reduction occurred on meats and on fruits, Table II.

5. Alcaligenes faecalis: A. faecalis, like all other test strains of bacteria was reduced to values less than 10 per cent of the initial numbers by freeze-dehydration on most foods in the study. The average loss of viability in cells of this organism was 95 per cent. The range in values for loss of viability in individual foods extended from 86 per cent on peas to 99.9 per cent on pears. A. faecalis freeze-dried on pears, spinach, and chicken sustained losses in excess of 99 per cent. When losses were compared by food group, a greater destruction of A. faecalis dried on fruit was seen, Table II. Losses on freeze-dried vegetables and freeze-dried meats were about the same, 94 per cent.

6. Clostridium botulinum: Spores of Cl. botulinum were killed by freeze-dehydration, but not to the extreme degree as were the vegetative cells of the other test strains, Table I. The loss of viability on different foods ranged from 42 per cent on fish and peas to 85 per cent on spinach. The average reduction, based on all foods in the test, was 58 per cent.

Spores of Cl. botulinum were reduced in numbers to a lower level on fruits than on vegetables or meats (see Table II). Highest spore survival was obtained on meats.

B. Freezing Effects

1. S. oranienburg: Reductions in the number of S. oranienburg during the freezing phase of the freeze-drying process ranged from 71 - 99 per cent depending upon the kind of food on which the bacteria were frozen (see Table III).

Based on the values for reduction of total counts, it was clear that most of the reduction in the numbers of bacteria during freeze-drying occurred during the freezing cycle.

TABLE III

PER CENT REDUCTION IN VIABLE COUNTS OF SIX SPECIES OF BACTERIA DURING THE FREEZING OF SEVEN KINDS OF FOOD IN PREPARATION FOR FREEZE-DRYING

Bacterial Species	Kind of Food							
	Spinach	Corn	Peas	Pears	Chicken	Beef	Fish	Average
Salmonella oranienburg	97.0	71.0	86.0	95.0	98.0	90.0	99.0	90.8
Staphylococcus aureus	98.0	64.0	67.0	92.0	98.0	68.0	63.0	78.6
Aerobacter aerogenes	97.0	89.0	86.0	76.0	89.0	91.0	36.0	80.6
Escherichia coli	97.0	70.0	51.0	97.0	90.0	91.0	16.0	73.1
Alcaligenes faecalis	98.0	66.0	81.0	90.0	84.0	80.0	77.0	82.3
Clostridium botulinum spores	63.6	50.0	35.0	52.8	40.0	50.0	35.0	46.6

The type of food on which S. oranienburg was frozen appeared to influence the number of cells which survived. On spinach, 97 per cent of the cells were killed by freezing, on corn only 71 per cent. The average reduction for all foods was 90 per cent; the average reduction for S. oranienburg by the total freeze-drying process was 98 per cent. While, roughly only 10 per cent of the initial population was killed by the drying phase, the lethal effect of drying on the survivors of freezing was of a much greater magnitude. Actually, the percentage of viable frozen cells killed by dehydration almost matched the value for destruction through freezing.

2. S. aureus: The reduction in the viable cell count for S. aureus during the freezing phase was generally of a lesser degree than the loss of viability in S. oranienburg. The average reduction in cell count for S. aureus was 10 per cent below the figure for S. oranienburg.

For certain foods, the survival rates for S. aureus were considerably higher than comparable rates for S. oranienburg. While 99 per cent of the cells of S. oranienburg were destroyed by freezing on fish, only 63 per cent of S. aureus cells were killed. Eighty-six per cent of S. oranienburg were destroyed by freezing on peas while only 67 per cent of the cells of S. aureus were killed by freezing on this food.

From the results of this study, S. aureus appears to withstand the lethal influence of freezing and freeze-drying better than some of the other food pathogens (spore forms excepted) on the foods used in these tests.

When the reduction in numbers of S. oranienburg was viewed from the effect of food class, it was seen that the freezing of vegetables and meats resulted in nearly identical losses. Freezing S. oranienburg on fruit appeared to be a somewhat more lethal proposition (see Table IV).

3. A. aerogenes: Survival of A. aerogenes was greater when the bacteria were frozen in fish flesh than on any of the other foods in the tests. Only 36 per cent of the cells were killed on fish while the next lowest rate (on pears) was 76 per cent. The greatest reduction occurred on spinach, 97 per cent. The average reduction for all foods of the test was 80 per cent, and was close to the values found for S. oranienburg and S. aureus.

TABLE IV

EFFECT OF FOOD CLASS ON THE PER CENT OF REDUCTION OF VIABILITY IN SIX SPECIES OF BACTERIA DURING THE FREEZING OF FRUITS, VEGETABLES, AND MEATS IN PREPARATION FOR FREEZE-DRYING

Bacterial Species	Food Class		
	Vegetables	Fruits	Meats
Salmonella oranienburg	85.3	95.0	85.7
Staphylococcus aureus	75.3	92.0	76.3
Aerobacter aerogenes	90.7	76.0	71.0
Escherichia coli	72.7	97.0	65.7
Alcaligenes faecalis	81.7	90.0	80.3
Clostridium botulinum spores	49.5	52.1	30.0

Greater loss of viability was found in vegetables (90 per cent) than in either fruit (76 per cent) or meats (71 per cent). However, frozen meats appeared to allow a somewhat higher level of survival because of the low lethal influence that freezing had for *A. aerogenes* on fish and the influence of this value on the average.

4. *E. coli*: For the most part, *E. coli* was reduced in numbers by freezing in a manner similar to the other bacteria tested. One exception was very noticeable; the reduction of *E. coli* on fish was considerably less than reduction on either of the other meats, Table III. Eighty-four per cent of the initial number of cells survived the freezing process on fish; on chicken and beef, 10 and 9 per cent survived. When the results of individual foods within a food class were averaged, the effects of freezing on fruits, vegetables and meats showed differences. Numbers of *E. coli* on vegetables were reduced 72 per cent by freezing, on meats the numbers were reduced by 65 per cent and on fruits by 97 per cent.

5. A. faecalis: Freezing A. faecalis on the seven foods in this study resulted in an average reduction in numbers of 82 per cent. On single foods, greatest reduction occurred during freezing on spinach. Least loss of viability was noted when the organisms were frozen on corn. Food class had little influence on the survival of A. faecalis on frozen vegetables or meats. In each of these classes, survival was about 20 per cent of the initial population. On fruits, survival was near 10 per cent.

6. Cl. botulinum: Freezing reduced the initial number of spores on all foods (combined average) to 46 per cent of the initial number, Table III. On individual items, loss of viability by spores was greater on spinach. Peas and fish were found to allow the greatest survival of spores for all the foods tested (65 per cent). When averages of the different foods were grouped to show food class effects, meats were found to have more protective influence on the spores than either fruits or vegetables.

Of the total reduction in the initial numbers of spores on spinach, 75 per cent could be attributed to the freezing phase and the remainder to the frozen storage-dehydration phase. On corn, freezing accounted for 88 per cent of the total reduction; for peas the proportion was 47 per cent, for pears 73 per cent and for chicken 53 per cent.

C. Dehydration Effects

1. S. oranienburg: The dehydration effects on all test strains used in this study are shown in Table V. These figures represent the killing effect of dehydration on the cells which survived the freezing process. These figures should not be confused with the percentage of the initial population whose death can be attributed to the drying phase. Further, it must be recognized that not all the reduction in viability during the drying phase can be directly related to dehydration since there is also a factor of continued frozen storage. However, because the two effects cannot be separated, both will be referred to by the term "dehydration effect."

The reduction in viability of frozen cells of S. oranienburg during the drying cycle of the freeze-drying process was found to be nearly the same as the percentage of cells dying during freezing.

TABLE V

PER CENT REDUCTION IN VIABLE COUNTS OF SIX SPECIES OF BACTERIA DURING THE DEHYDRATION CYCLE IN THE FREEZE-DRYING OF SEVEN KINDS OF FOOD

Bacterial Species	Kind of Food							
	Spinach	Corn	Peas	Pears	Chicken	Beef	Fish	Average
Salmonella oranienburg	98.0	82.0	63.0	99.9	94.0	84.0	77.0	85.4
Staphylococcus aureus	98.0	82.0	86.0	99.8	92.0	98.0	40.0	84.1
Aerobacter aerogenes	96.0	83.0	66.0	99.9	88.0	93.0	96.0	97.4
Escherichia coli	95.0	95.0	74.0	99.2	99.9	95.0	98.0	93.7
Alcaligenes faecalis	96.0	85.0	27.0	99.9	75.0	14.0	61.0	65.4
Clostridium botulinum spores	59.0	14.0	11.0	42.0	15.0	14.0	11.0	23.7

Drying appeared to be less detrimental to loss of viability when the cells were dried on peas than on any of the other foods. Vegetables and meats as food groups appeared to have similar influences. Greater loss of viability from drying occurred on fruits (see Table VI).

TABLE VI

EFFECT OF FOOD CLASS ON THE PER CENT OF REDUCTION OF VIABILITY IN SIX SPECIES OF BACTERIA DURING THE DEHYDRATION CYCLE IN THE FREEZE-DRYING OF FRUITS, VEGETABLES, AND MEATS

Bacterial Species	Food Class		
	Vegetables	Fruits	Meats
Salmonella oranienburg	81.0	99.9	85.0
Staphylococcus aureus	88.7	99.8	76.7
Aerobacter aerogenes	81.7	99.9	89.0
Escherichia coli	88.0	99.2	97.6
Alcaligenes faecalis	69.3	99.9	50.0
Clostridium botulinum spores	28.0	42.0	13.3

2. *S. aureus*: Forty per cent of the cells which remained viable on frozen fish were killed by subsequent dehydration. Chicken and beef did not offer this degree of protection; 92 per cent of *S. aureus* dried on chicken and 98 per cent dried on beef were killed. Greatest reduction occurred when this bacterial species was dried on pears. Loss of viability on fruit amounted to 99 plus per cent. As an average, counts of *S. aureus* were reduced to 16 per cent of the cells which survived the freezing phase by drying. Better survival, by food class, was obtained on meats, on vegetables and on fruits in that order.

3. *A. aerogenes*: The average reduction of *A. aerogenes* from drying, on all foods in the test, was 97 per cent. Greatest reduction by drying was observed when *A. aerogenes* was dried on pears and least when on peas. When the effect of dehydration on reduction of viable cell counts

was examined by food class, drying of A. aerogenes on fruits appeared to cause a greater decrease in number than drying on vegetables or meats. Reduction in count on meats was slightly greater than reduction on vegetables, Table VI.

4. E. coli: While the initial freezing effects appeared to be less detrimental to survival of E. coli than to the other bacteria in our studies, dehydration losses on individual foods were generally as great or greater. Reduction in numbers from drying on chicken or fish was higher for E. coli than for any other test species, Table V. It also was found that the average reduction on meat, as a class, was greater for E. coli than for any of the other microorganisms. Loss of viability on fruits was of equal magnitude to the other test strains as was the effect on vegetables.

It may be well to restate that dehydration losses as presented in this study are based on the reduction in the number of cells which appeared to survive the initial freezing. These values cannot be compared to values derived by subtracting freeze-killed from total-killed and assigning the difference to killing during the dehydration phase. In the latter instance both values are based on initial unfrozen numbers.

5. A. faecalis: Of all the bacterial species tested (with the exception of Cl. botulinum spores), A. faecalis appeared to withstand best the killing effects of dehydration. Only on three of the dried foods was the killing of this species as great as that of the other bacteria. These were spinach, pears and corn. The average reduction in viable count on all foods tested was 65 per cent.

Fruit as a class caused a greater loss in numbers of A. faecalis than either meats or vegetables. Meats supported a higher survival population than did vegetables.

6. Cl. botulinum: Spores of Cl. botulinum appeared to be more resistant to dehydration influences than they were to freezing effects. The average reduction in spore count, for all foods, from drying was 24 per cent; the average reduction due to freezing was 46 per cent. Five of the seven test foods were found to support spore populations following drying at levels of 85 - 89 per cent of the viable spores present at the end of the freezing cycle. Reduction of spores on spinach was found to be greater than on any other food, see Table V. Reduction of viability on vegetables and fruits was greater than on meats, see Table VI.

D. Effects of Storage Conditions on Survival of Bacteria on Freeze-Dried Foods

1. S. oranienburg: On all seven test foods, S. oranienburg declined in numbers more rapidly at 100°F storage temperature than at 70°F or 40°F. This was true whether the foods were stored in air-packed cans or in nitrogen. The temperature at which the freeze-dried materials were held had a greater influence on survival than did atmosphere. Regardless of the type of food examined, survival in air-packed containers held at 40°F was better than survival in nitrogen-packed containers held at a higher temperature. At a given temperature, however, survival was better in the food packed in nitrogen, see Figs. 1 - 4.

The percentages of S. oranienburg which persisted at 40°F for different storage periods are given in Table VII. Generally, survival was better on meats than on vegetables or fruit after one month's storage at 40°F. At three months, meats were still more protective, but the difference between the meats and the vegetables at this time was less than the difference at one month. The reduction in numbers of S. oranienburg on the meats at the nine months storage period was to the levels reached on fruits and vegetables.

Because storage at 40°F under nitrogen was found to allow higher rates of bacterial survival, the effectiveness of the other storage conditions was evaluated using this storage environment as base.

S. oranienburg maintained a higher degree of viability at 70°F than it did at 100°F. It also exhibited a wide range of response on the different foods. Thus on peas, over 98 per cent of the cells viable under 40°F storage died under storage at 70°F, more than 99 per cent died at 100°F storage. Of corn, only 53 per cent of the cells viable under 40°F storage died under storage at 70°F and 85 per cent died at 100°F (see Table VIII). The survivors on pears at 70°F appeared to be high, but these figures are probably misleading in that the number of survivors at 40°F were so low that even a few cells surviving at 70°F yielded a high percentage figure.

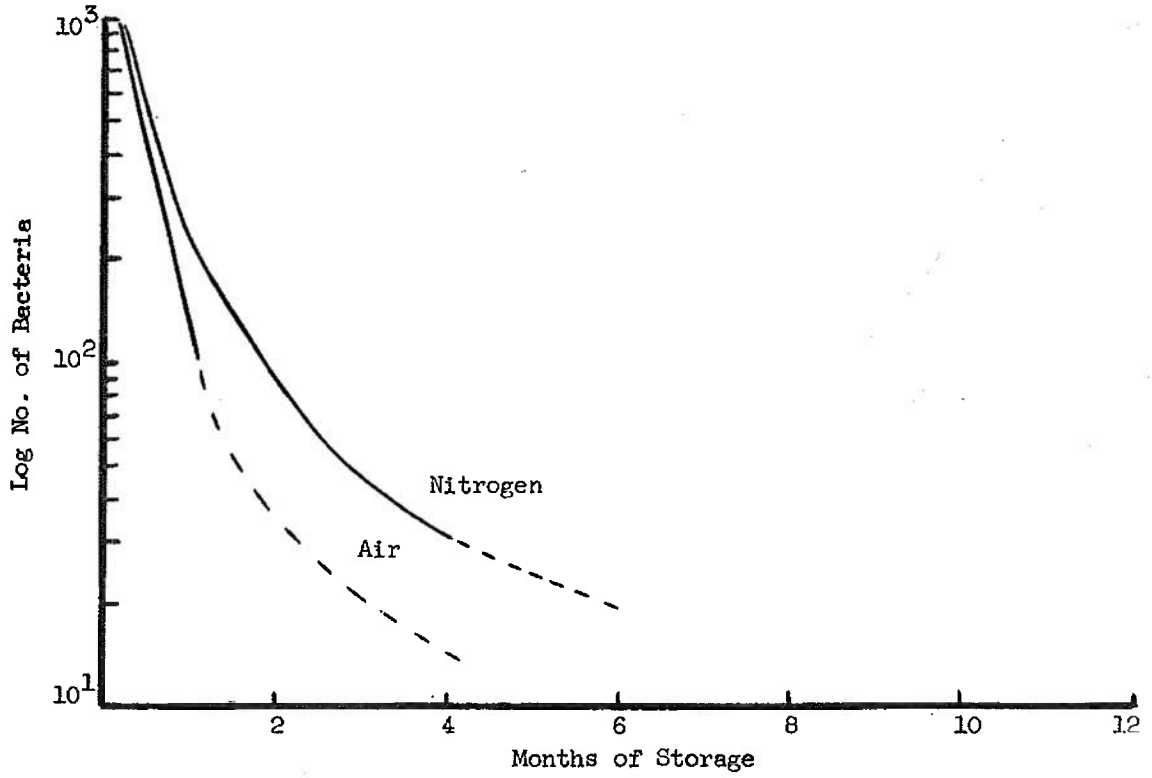

Fig. 1 - Reduotion in the Numbers of Salmonella oranienburg on Freeze-Dried Pears During Storage at 40°F

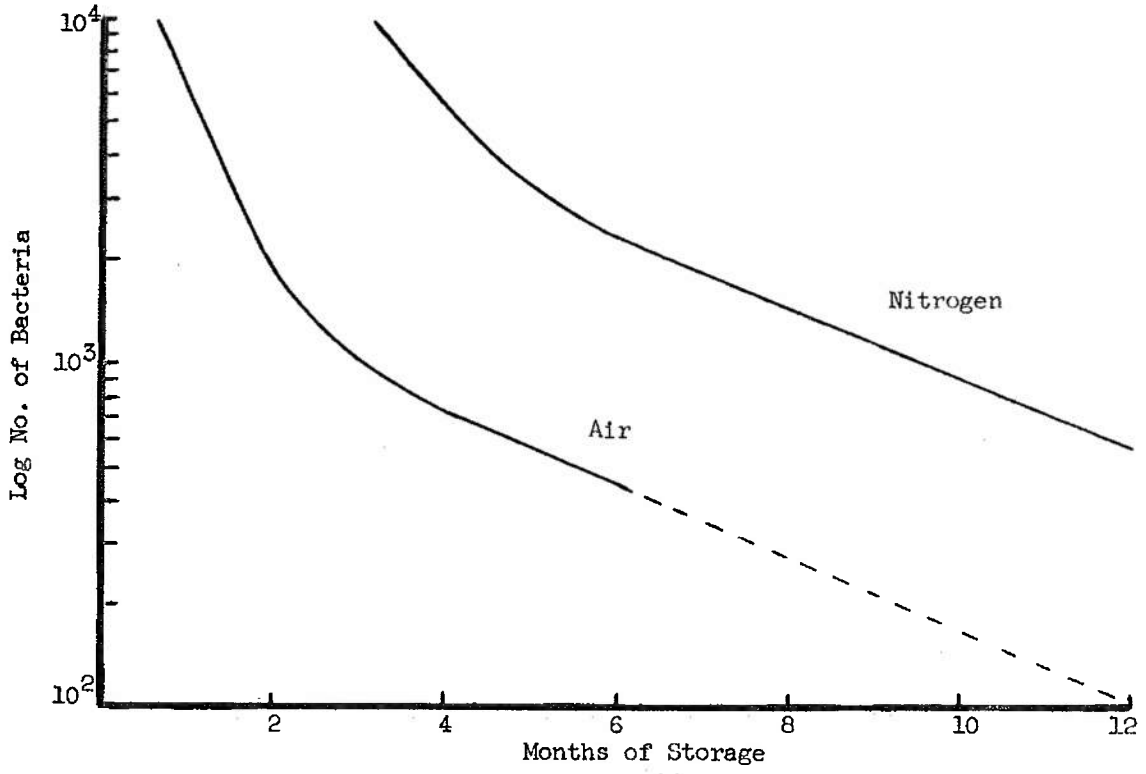

Fig. 2 - Reduction in the Numbers of Salmonella oranienburg on Freeze-Dried Peas During Storage at 40°F

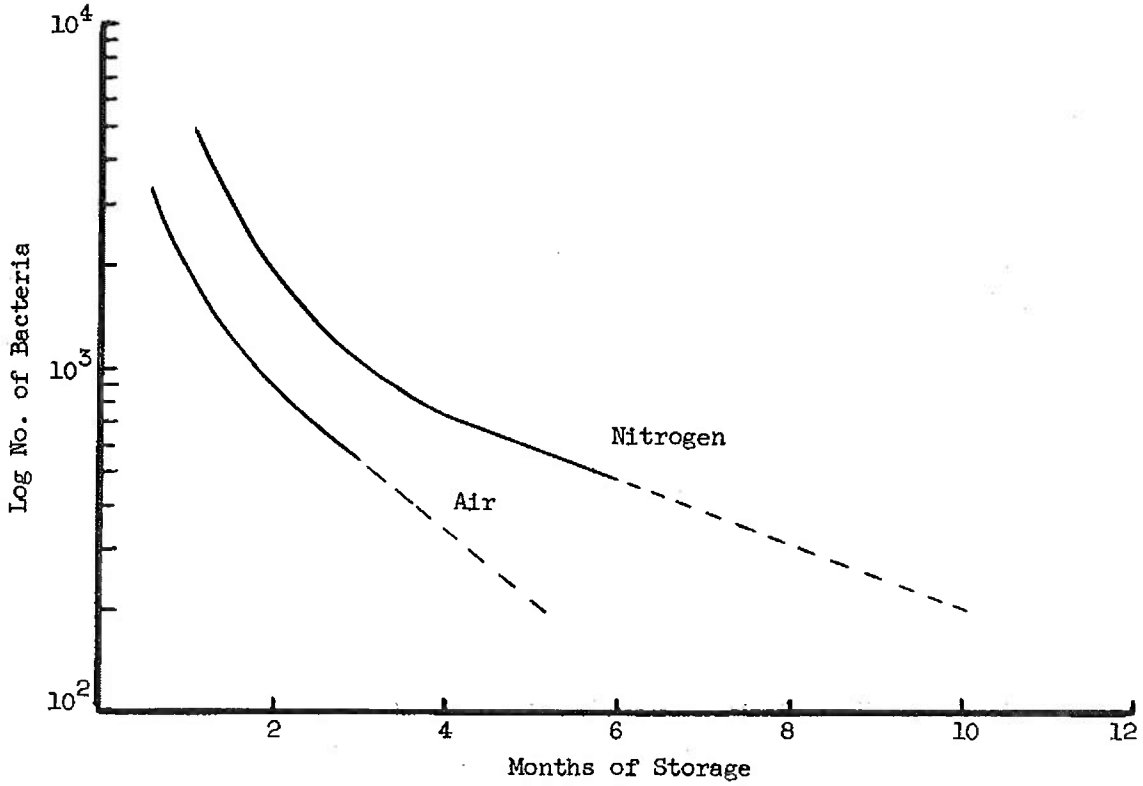

Fig. 3 - Reduction in the Numbers of Salmonella oranienburg on Freeze-Dried Chicken During Storage at 40°F

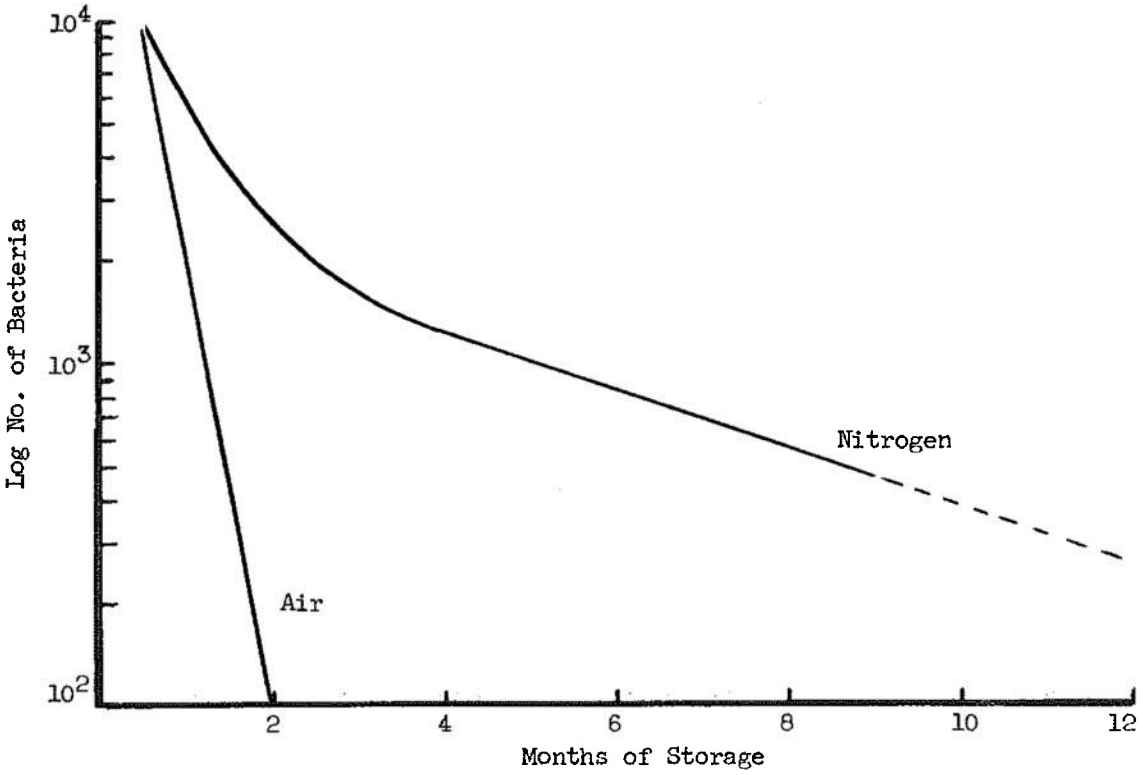

Fig. 4 - Reduction in the Numbers of Salmonella oranienburg on Freeze-Dried Spinach During Storage at 40°F

TABLE VII

PER CENT OF SALMONELLA ORANIENBURG SURVIVING ON FREEZE-DRIED FOODS AFTER STORAGE AT 40°F IN A NITROGEN ATMOSPHERE

Kind of Food	Months of Storage 1	3	9
Spinach	6.2	3.2	0.1
Corn	13.0	5.0	0.2
Peas	13.0	1.5	0.1
Pears	10.0	0.1	0.1
Chicken	41.0	8.0	0.1
Beef	14.2	6.3	0.1
Fish	83.0	36.0	0.1

TABLE VIII

EFFECT OF STORAGE TEMPERATURE ON THE PERSISTENCE OF SALMONELLA ORANIENBURG ON SEVEN FREEZE-DRIED FOODS STORED SIX MONTHS

Dried Food*	Per Cent of Survivors at 40°F Storage Viable Under: 70°F Storage	100°F Storage
Peas	2.5	< 1.0
Corn	46.1	15.3
Spinach	5.9	< 1.0
Pears	75.0	< 5.0
Beef	14.7	2.0
Chicken	11.2	< 1.0
Fish	43.3	26.6

* Sealed in nitrogen.

2. S. aureus: S. aureus responded to storage in a manner similar to S. oranienburg; reduction in numbers was more rapid at 100°F storage than at the lower temperatures. Even under nitrogen, decline at 100°F storage was rapid. The effect of nitrogen packaging on the survival of S. aureus on a number of freeze-dried foods at 40°F is shown in Figs. 5 - 8. The more rapid reduction in bacterial numbers during the early months of storage is indicated by the greater steepness of the curves for these periods of time. This effect is also shown by the percentage of survivors given in Table IX. When survival during storage was compared on a food class basis, meat appeared to have a more protective effect than vegetables or fruits did.

Storage at 70°F also resulted in a more rapid decline of S. aureus than storage at 40°F. Seventy-four per cent of the cells which were viable on freeze-dried corn under 40°F storage were killed by storage under 70°F; 91 per cent of these cells were killed by storage at 100°F. When compared to the survival rates at 40°F, viability of S. aureus appeared most severely reduced when stored at 70°F on freeze-dried peas, spinach and chicken (see Table X).

3. A. aerogenes: A. aerogenes followed the pattern of S. oranienburg and S. aureus in that the initial decline (zero to one-month storage) was more rapid than was the percentage of decline from one to three months. Again, loss of viability was more retarded by storage at 40°F in nitrogen than under any of the other storage conditions. At 100°F, decline in numbers was extremely rapid, whether storage was in air or nitrogen.

Reductions early in storage were more rapid on fruits and vegetables than on meats, a situation which may be related to the degree of metabolic injury to which the cells were subjected on the different foods during freeze-dehydration.

The variability among the different bacteria to storage conditions and kind of food can be seen by a comparison of the results of A. aerogenes (Table XI) to those for S. oranienburg (Table VIII) and S. aureus (Table X). The survival of A. aerogenes at 70°F was higher on peas than was the survival of S. aureus or S. oranienburg. The two pathogens survived at a level less than 10 per cent of the 40°F rate, while A. aerogenes survived at a level near 35 per cent of the 40°F figure. Survival of S. aureus and S. oranienburg was above 25 per cent on corn; it was less than 10 per cent for A. aerogenes.

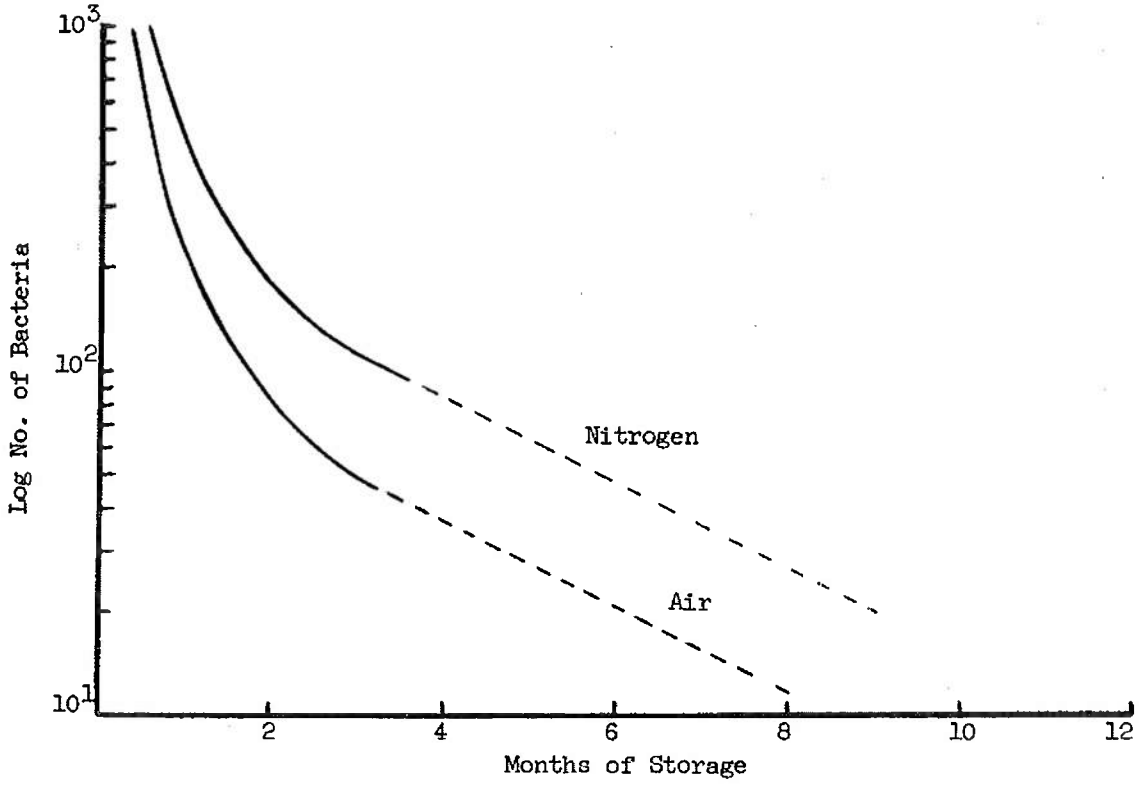

Fig. 5 - Reduction in the Numbers of Staphylococcus aureus on Freeze-Dried Pears During Storage at 40°F

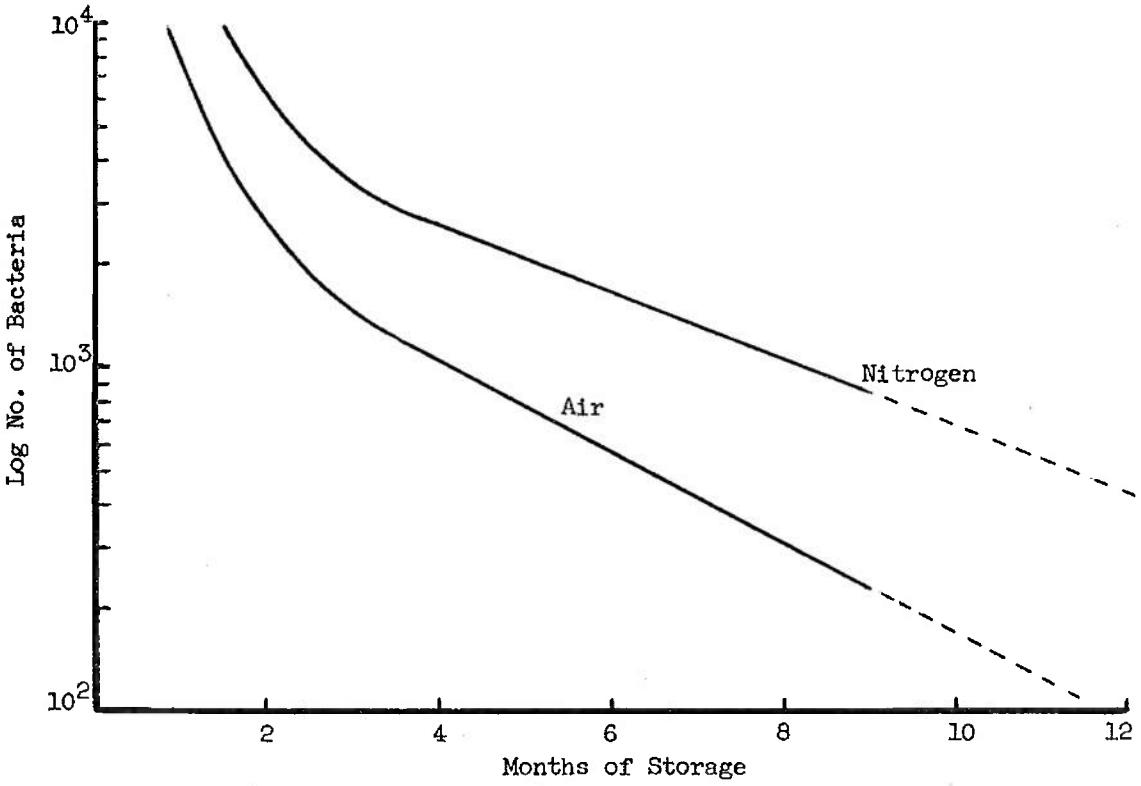

Fig. 6 - Reduction in the Numbers of Staphylococcus aureus on Freeze-Dried Peas During Storage at 40°F

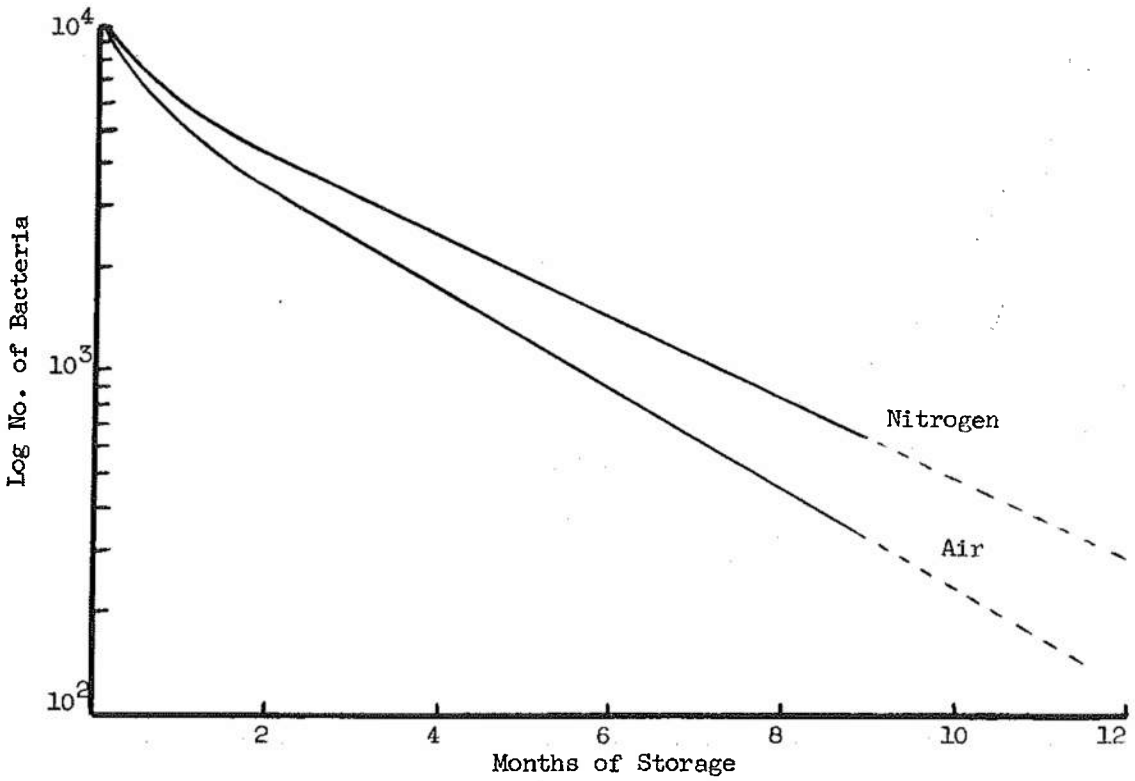

Fig. 7 - Reduction in the Numbers of Staphylococcus aureus on Freeze-Dried Chicken During Storage at 40°F

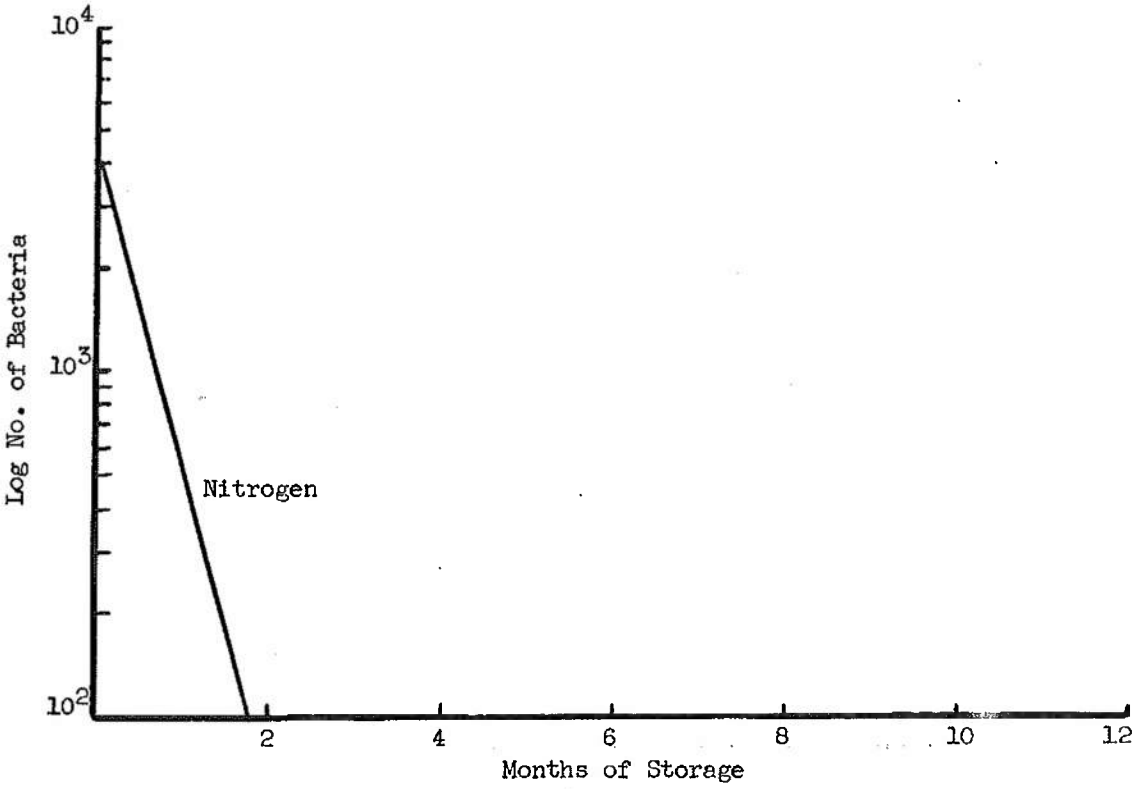

Fig. 8 - Reduction in the Numbers of Staphylococcus aureus on Freeze-Dried Spinach During Storage at 40°F

TABLE IX

PER CENT OF STAPHYLOCOCCUS AUREUS SURVIVING ON FREEZE-DRIED FOODS AFTER STORAGE AT 40°F IN A NITROGEN ATMOSPHERE

Kind of Food	Months of Storage		
	1	3	9
Spinach	0.5	0.1	0.1
Corn	6.4	3.9	0.6
Peas	15.6	3.9	0.6
Pears	33.0	0.1	0.1
Chicken	76.0	6.2	0.1
Beef	17.0	4.0	0.1
Fish	25.0	5.3	0.1

TABLE X

EFFECT OF STORAGE TEMPERATURE ON THE PERSISTENCE OF STAPHYLOCOCCUS AUREUS ON SEVEN FREEZE-DRIED FOODS STORED SIX MONTHS

Dried Food*	Per Cent of Survivors at 40°F Storage Viable at:	
	70°F Storage	100°F Storage
Peas	6.2	< 1.0
Corn	26.0	8.6
Spinach	7.0	< 1.0
Pears	50.0	< 1.0
Beef	16.6	< 1.0
Chicken	4.2	< 1.0
Fish	29.0	< 1.0

* Sealed in nitrogen.

TABLE XI

EFFECT OF STORAGE TEMPERATURE ON THE PERSISTENCE OF *AEROBACTER AEROGENES* ON SEVEN FREEZE-DRIED FOODS STORED SIX MONTHS

Dried Food*	Per Cent of Survivors at 40°F Storage Viable at: 70°F Storage	100°F Storage
Peas	34.4	18.9
Corn	7.1	< 1.0
Spinach	5.4	< 1.0
Pears	< 1.0	< 1.0
Beef	4.0	< 1.0
Chicken	3.4	< 1.0
Fish	26.6	< 1.0

* Sealed in nitrogen.

4. *E. coli*: Decline during storage, for *E. coli*, was similar, in all respects, to the other bacteria which were studied. The reduction in numbers followed the same pattern of decline as shown in Figs. 1 - 4 for *S. oranienburg*. Reductions in numbers were more rapid at 70°F than at 40°F and comparatively were very rapid at 100°F (Table XII). Generally, the cells for *E. coli* survived better on meat items than they did on vegetables at 100°F and 70°F. This response was also noted for storage at 40°F.

5. *A. faecalis*: The general response of *A. faecalis* to the storage conditions was similar to that of the other test organism except it did not exhibit the extreme degree of reduction. The survival of *A. faecalis* on the different test foods was better at both 100°F and 70°F than the other test species (see Table XIII).

TABLE XII

EFFECT OF STORAGE TEMPERATURE ON THE PERSISTENCE OF *ESCHERICHIA COLI* ON SEVEN FREEZE-DRIED FOODS STORED SIX MONTHS

Dried Food*	Per Cent of Survivors at 40°F Storage Viable at: 70°F Storage	100°F Storage
Peas	8.8	< 1.0
Corn	29.0	13.6
Spinach	20.0	< 1.0
Pears	57.0	< 1.0
Beef	11.8	< 1.0
Chicken	15.4	< 1.0
Fish	17.1	< 1.0

* Sealed in nitrogen.

TABLE XIII

EFFECT OF STORAGE TEMPERATURE ON THE PERSISTENCE OF *ALCALIGENES FAECALIS* ON SEVEN FREEZE-DRIED FOODS STORED SIX MONTHS

Dried Food*	Per Cent of Survivors at 40°F Storage Viable at: 70°F Storage	100°F Storage
Peas	10.8	< 1.0
Corn	59.5	52.3
Spinach	8.5	< 1.0
Pears	50.0	< 1.0
Beef	50.0	17.5
Chicken	50.0	5.0
Fish	27.7	< 1.0

* Sealed in nitrogen.

When one compares the persistence of A. faecalis and E. coli, the greater persistence potential for the former can be seen. Because of the better showing of the type of fecal bacteria as represented by A. faecalis, one is tempted to question the utility of E. coli as an index for freeze-dried foods. On the other hand, the decline of E. coli during storage is similar to the degree of reduction for S. oranienburg, and on this basis it (E. coli) may still have significance as an indicator species.

6. Cl. botulinum: Cl. botulinum spores, like the vegetative cells of the other five species, were more rapidly inactivated at 100°F than at lower temperatures. However, these reductions were not as severe or to the degree as that found with the other test strains, Figs. 9 - 12. The values for persistence of Cl. botulinum spores were high at 40°F and remained comparatively high at 70°F. Even at 100°F storage, viability of the spores remained at much higher levels over longer periods of time than did the viability of any of the vegetative test species stored on similar foods (see Table XIV).

The early, rapid storage declines noted with the cells of the other test bacteria were absent, for the most part. Reductions appeared to be more proportional between sampling periods. There is little doubt that the spores will survive freeze-drying in high numbers and will persist on the freeze-dried foods for long periods of time. It also appears that the microbiology of freeze-dried foods stored in excess of nine months to a year will be based on the biology of spore-forming bacteria.

E. Metabolic Damage

For the studies on survival during freeze-drying and storage, we inoculated each food with a bacterial culture which was 24 hr. old. The physiological ages of bacteria on commercially processed foods will undoubtedly vary; therefore, in order to evaluate the effects of cell age on survival potential, we inoculated ground beef with S. oranienburg and S. aureus cultures of different ages and determined the response of these bacteria to freeze-drying variables.

The effect of cell age on viability of S. aureus on freeze-dried beef is given in Table XV. Surprisingly, little difference in survivability appeared to exist among the different aged cells. When, however, one examines Table XVI, it can be seen that cell age had a pronounced effect upon the degree of "metabolic injury" which the cells received. The younger the cells, the greater was the injury.

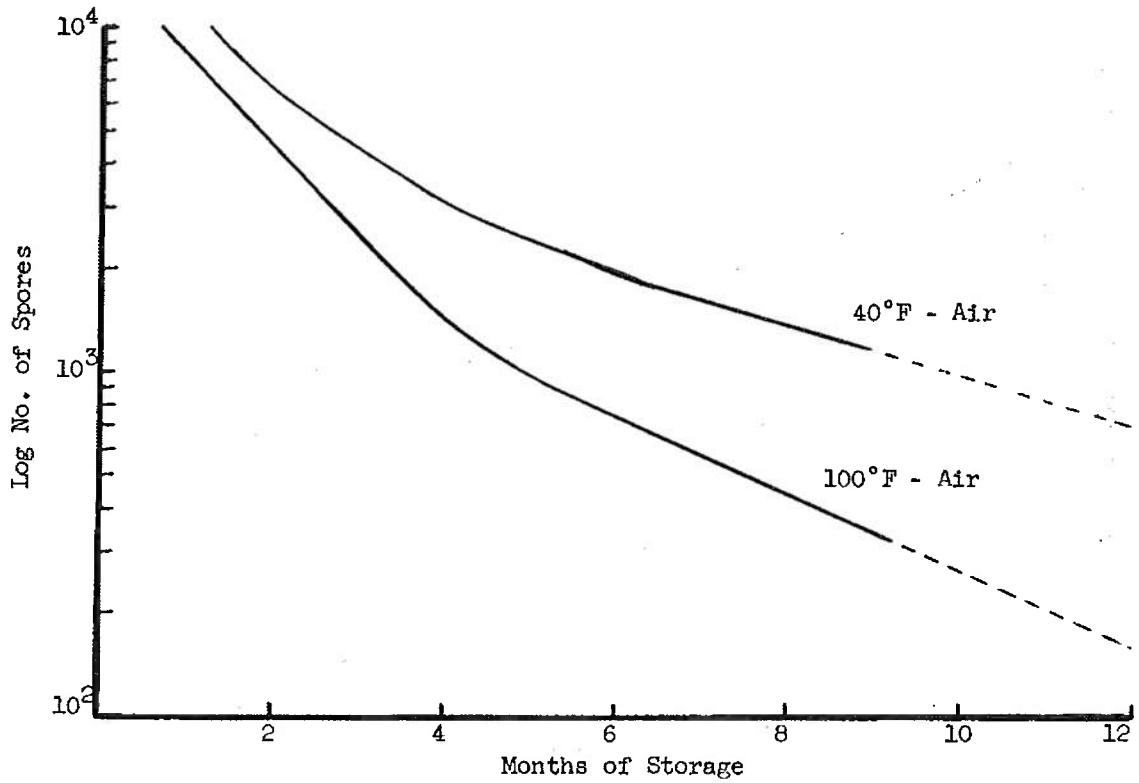

Fig. 9 - Reduction in the Numbers of Clostridium botulinum (spores) on Freeze-Dried Pears During Storage at 100° and 40°F

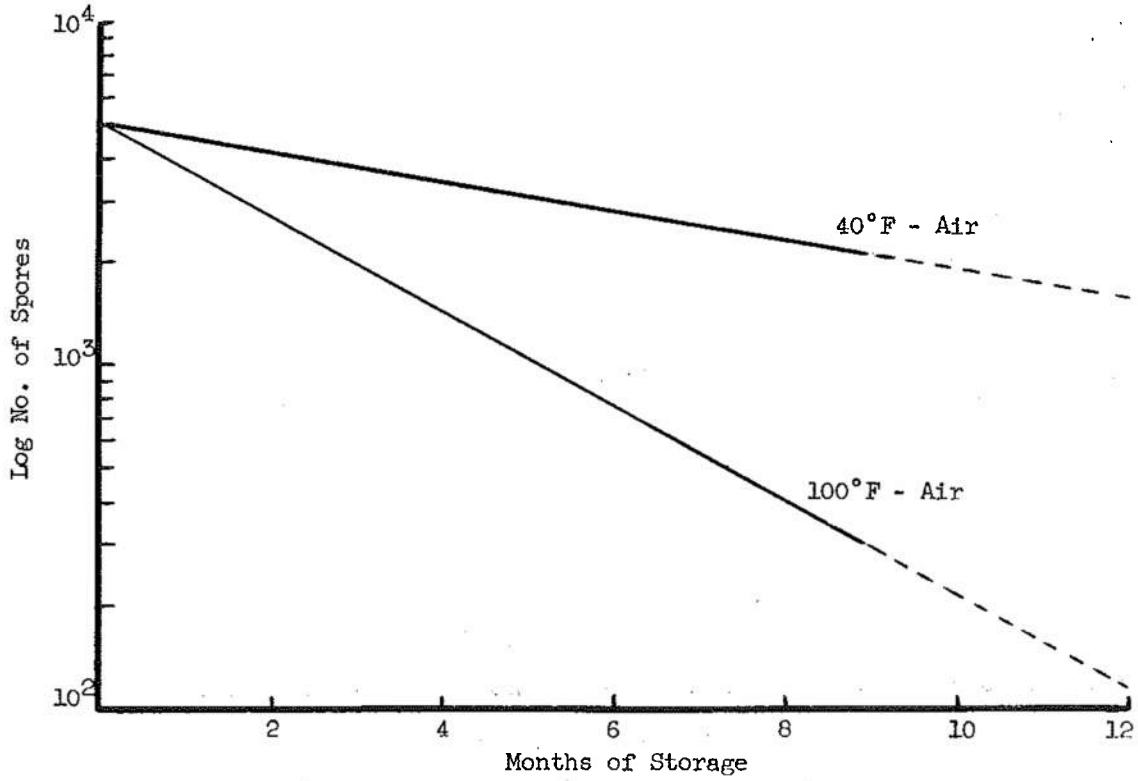

Fig. 10 - Reduction in the Numbers of Clostridium botulinum (spores) on Freeze-Dried Chicken During Storage at 100° and 40°F

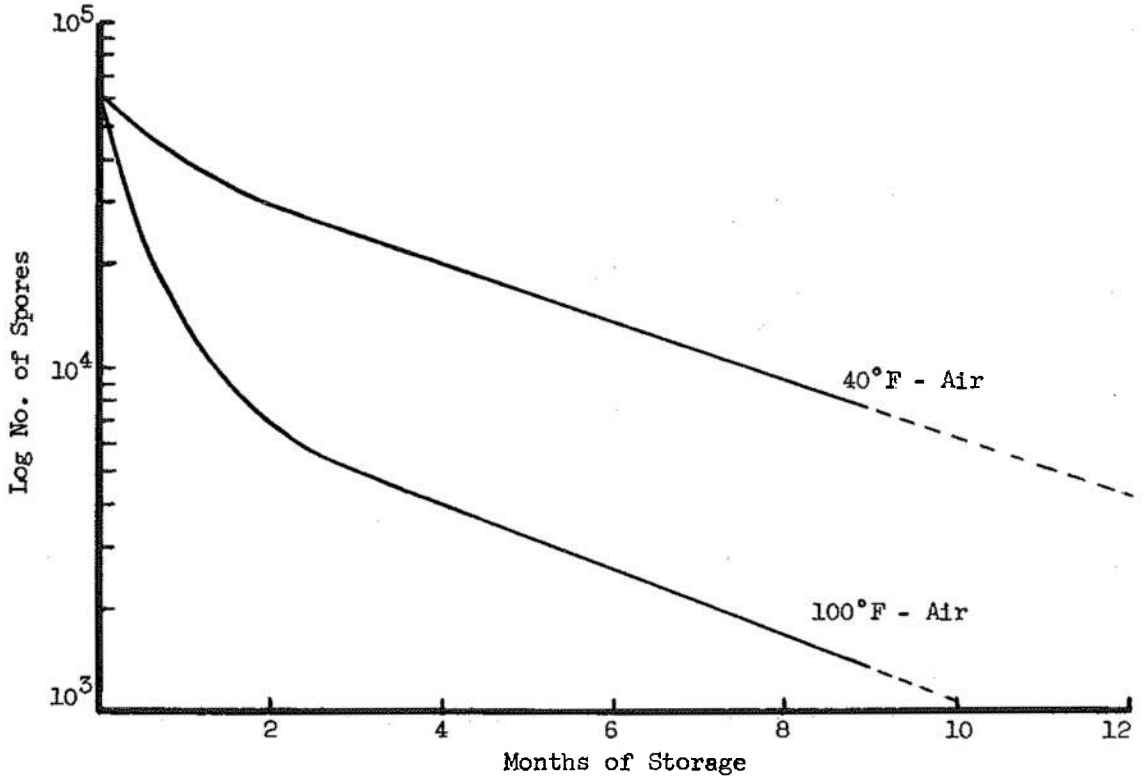

Fig. 11 - Reduction in the Numbers of Clostridium botulinum (spores) on Freeze-Dried Peas During Storage at 100° and 40°F

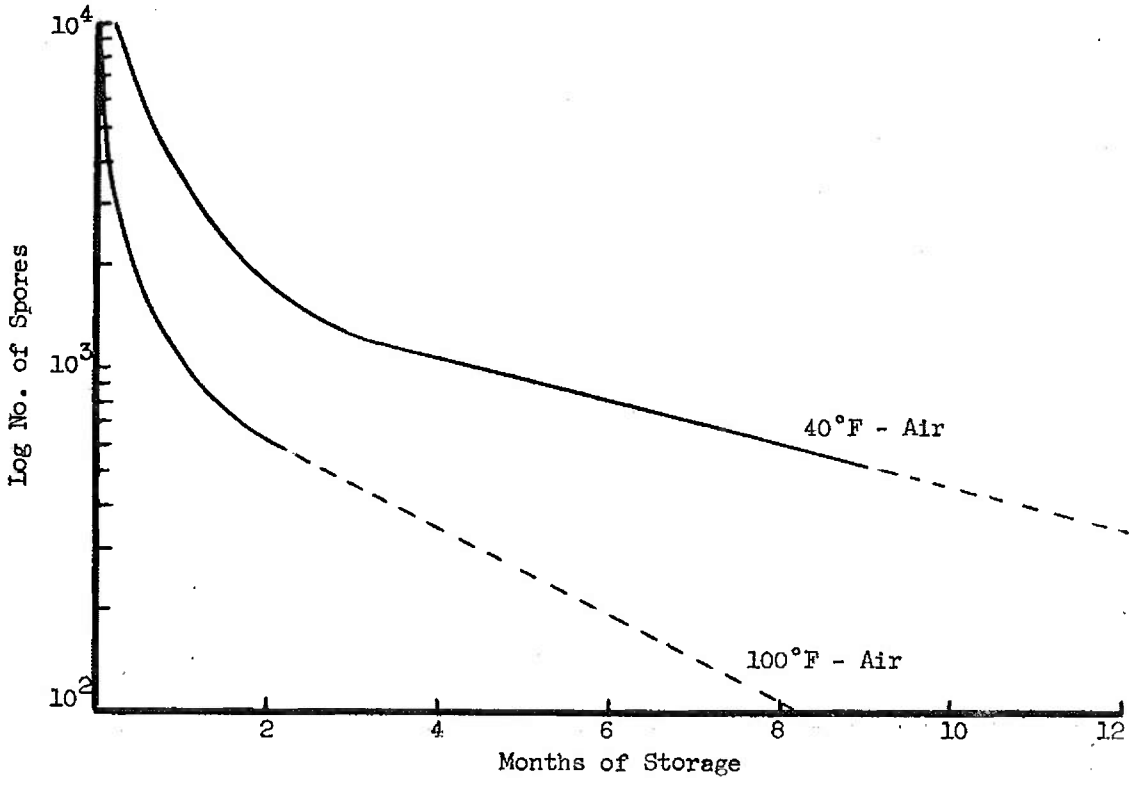

Fig. 12 - Reduction in the Numbers of Clostridium botulinum (spores) on Freeze-Dried Spinach During Storage at 100° and 40°F

TABLE XIV

EFFECT OF STORAGE TEMPERATURE ON THE PERSISTENCE OF *CLOSTRIDIUM BOTULINUM* (SPORES) ON SEVEN FREEZE-DRIED FOODS STORED FOR SIX MONTHS

Dried Food	Per Cent of Survivors at 40°F Storage Viable at: 70°F Storage	100°F Storage
Peas	57.1	8.5
Corn	66.6	41.6
Spinach	26.6	10.0
Pears	54.5	31.8
Beef	82.0	43.2
Chicken	80.0	58.3
Fish	78.0	50.0

TABLE XV

EFFECT OF CULTURE AGE ON THE PERCENTAGE OF REDUCTION IN NUMBERS OF VIABLE BACTERIA ON FREEZE-DRIED BEEF

Age of Culture Used for Inoculum (hr.)	Per Cent Reduction in Viable Count After Drying
Staphylococcus aureus	
6	97.9
18	97.5
24	98.2
48	98.5
Salmonella oranienburg	
6	99.5
18	99.6
24	94.6
48	93.4

TABLE XVI

EFFECT OF CULTURE AGE ON THE PERCENTAGE OF BACTERIA SHOWING METABOLIC DAMAGE CAUSED BY FREEZE-DRYING ON BEEF

Age of Culture Used for Inoculum (hr.)	Per Cent of Cells Showing "Metabolic Injury"
Staphylococcus aureus	
6	58.3
18	33.0
24	18.8
48	15.0
Salmonella oranienburg	
6	55.6
18	42.0
24	29.0
48	27.6

The same general pattern was obtained with *S. oranienburg*, Tables XV and XVI. Age of the cultures used to inoculate the beef prior to freeze-drying apparently had little effect upon the percentage of cells which survived the freeze-drying process. Although there appeared to be a consistent increase in survivors with increase in culture age (99.5 per cent were killed from the 6-hr. culture and 93.4 per cent were killed from the 48-hr. culture), the differences were small. On the other hand, metabolic injury due to freeze-dehydration decreased with increasing culture age. With 6 hr. old cells, nearly 56 per cent showed injury. By the time the culture reached 48 hr. of age prior to inoculation, metabolic injury during drying dropped almost 50 per cent.

Age appeared to have a pronounced effect upon the number of cells of *S. aureus* and *S. oranienburg* which showed metabolic injury. We assume the same is true of other species. Although the injury may not be noticeable in total counts made on "rich" medium, it undoubtedly

is partly responsible for the more rapid decline in bacterial numbers during the early periods of storage. It is the metabolically crippled cells which die early in storage.

Since we wished to relate our findings to commercially produced freeze-dried foods, we examined several U. S. Army Natick Laboratories dehydrated rations, see Table XVII.

For the most part, the counts on these dehydrated foods could be considered low. Based on the findings of Fanelli et al., 1964, and Karlson and Gunderson, 1964, counts on the multicomponent rations were in line with the acceptable levels described for dry soup mixes. Unfortunately, the results of examining the rations cannot be compared too precisely to expected populations, based on our studies of freeze-dried foods, for two reasons. First, all the components of the military rations were not freeze-dried; second, the rations had considerably more handling than our freeze-dried reference materials in that repackaging was necessary, in most cases, for the ration items.

One finding of interest does not appear in Table XVII; examination of colonies which grew on the plate count medium showed that well over half the colonies were spore-forming bacilli; the microbiology of dried foods which have a history of long term storage will likely be related to the presence of spore-forming bacteria.

F. Effect of Diluent and Diluent Temperature on Recovery of Bacteria

The effect of diluent and diluent temperature on the recovery of bacteria from freeze-dried meats and vegetables was examined. Distilled water, 0.85 per cent sodium chloride, phosphate buffer at pH 6.9, and 0.1 per cent peptone solution were tested as diluting media. Each diluent was used at temperatures of 40°F, 75°F, and 113°F. During the transfer of sample dilutions, the temperature of the diluting fluid was kept at the temperature under study.

The effect of diluent and incubation temperatures on recovery of bacteria from meats is presented in the figures of Table XVIII. There appeared to be a small but definite advantage to using 98°F as the temperature for incubation. However, the differences in counts obtained at 75°F and at 98°F were close enough to each other to indicate that an evaluation of the bacteriological quality of meat products could be made at either temperature.

TABLE XVII

BACTERIOLOGICAL SURVEY OF DEHYDRATED ARMY QUARTERMASTER ITEMS

Item	Aerobic Plate Counts (No./g)	Coliform (No./g)	Anaerobic Spore Counts (No./g)	Salmonella (No./g)	Fecal Streptococci (No./g)
Cocoa beverage	950	10	10	Neg.	10
Orange juice	132	10	10	Neg.	0
Fruit cocktail	333	10	10	Neg.	0
Green pea soup	467	10	10	Neg.	0
Apple sauce	37	10	10	Neg.	0
Rice	1,850	10	10	Neg.	0
Spinach	3,450	10	10	Neg.	25
Milk, dry	867	10	10	Neg.	0
Peaches	109	10	10	Neg.	0
Cabbage slaw	450,000	250	50	Neg.	2,100
Cabbage	26,400	10	10	Neg.	1,100
Sweet potato	38	10	10	Neg.	0
Butterscotch pudding	273	10	10	Neg.	0
Grape juice	2,200	10	10	Neg.	0
Orange-grapefruit juice	53	10	10	Neg.	0
Six months peas	533	10	10	Neg.	60
Green beans	13,400	10	10	Neg.	4,000
Peas	7,800	10	10	Neg.	25
Apricots	860	10	10	Neg.	0
Stewed prunes	600	10	10	Neg.	0
Sliced beef and gravy	14,200	10	10	Neg.	5
Macaroni and cheese	1,250	10	10	Neg.	0
Meat balls and gravy	91,000	10	800	Neg.	30
Corn and lima beans	23,000	10	120	Neg.	50
Chicken stew	22,000	10	700	Neg.	350
Beef stew	25,800	10	530	Neg.	17
Chicken and rice	2,800	10	30	Neg.	0
Beef hash	990	10	170	Neg.	90
Mashed potatoes	193	10	10	Neg.	0
Peas; vacuum canned	800	10	10	Neg.	0
Cooked beef; vacuum canned	80	10	10	Neg.	0
Onions; sliced, canned	6,000	10	10	Neg.	-
Carrots; vacuum canned	44,000	5,000	10	Neg.	-
Potatoes; diced	1,300	10	10	Neg.	-

TABLE XVIII

EFFECT OF DILUENT AND DILUENT TEMPERATURE ON RECOVERY OF BACTERIA FROM FREEZE-DRIED MEATS

	Temperature of Diluent					
	40°F		75°F		113°F	
	Plates Incubated at:		Plates Incubated at:		Plates Incubated at:	
Kind of Diluent	75°F	98°F	75°F	98°F	75°F	98°F
Distilled Water	7,500	13,700	9,500	13,200	16,000	22,900
0.85% NaCl	9,200	13,200	10,000	11,700	27,700	22,700
Phosphate Buffer (pH 6.9)	8,700	16,700	15,600	14,300	12,100	15,800
0.1% Peptone	14,200	17,400	10,400	15,300	10,900	13,900

By comparing the counts obtained at 98°F, variation in responses to diluents and diluent temperatures were noted. At a temperature of 40°F, phosphate buffer and peptone solution appeared to be slightly more effective than distilled water or sodium chloride. At a dilution temperature of 75°F, the kind of diluent did not appear to have any great effect on the numbers of bacteria which could be recovered. As diluent temperature was increased to 113°F, distilled water and sodium chloride appeared to be slightly better than peptone solution and phosphate buffer. This is a reversal of the effect at 40°F. The reasons for these shifts are not known, but there is the possibility that they may be related to changes in cell permeability.

The figures presented in Table XIX indicate the responses of bacteria on cabbage to diluent and incubation temperatures. Unlike the meat, higher counts were obtained under incubation at 75°F than were obtained by incubation at 98°F.

With the diluent temperature held at 40°F, better recovery was obtained using peptone solution than was obtained with phosphate buffer, sodium chloride or distilled water. At a diluent temperature of 75°F, the type of diluting fluid did not appear to materially affect the results. When the dilution temperature was raised to 113°F, phosphate buffer was found to be superior to the other types. This may be related to greater stability of temperature-sensitive types in phosphate or it may represent a higher rate of bacterial spore activation in the buffer.

TABLE XIX

EFFECT OF DILUENT AND DILUENT TEMPERATURE ON RECOVERY OF BACTERIA FROM FREEZE-DRIED VEGETABLES

	Temperature of Diluent					
	40°F Plates Incubated at:		75°F Plates Incubated at:		113°F Plates Incubated at:	
Kind of Diluent	75°F	98°F	75°F	98°F	75°F	98°F
Distilled Water	6,000	1,500	20,000	4,000	11,900	3,500
0.85% NaCl	5,900	1,300	15,300	6,700	5,700	1,400
Phosphate Buffer (pH 6.9)	17,600	3,100	17,600	7,100	21,700	3,500
0.1% Peptone	12,400	3,600	17,800	5,200	13,800	3,600

IV. DISCUSSION

It is clear that in the freeze-drying of foods the process results in reduction of cell viability in the bacteria carried on the food. The degree of the reduction is influenced by several factors. Among these variables are the substrates on which the cells are frozen, the species of bacteria, rate of thaw after freezing, characteristics of different mixed populations, the physiological state attained by the bacteria prior to freezing as well as the medium used for culturing after freezing (Ahn et al., 1964; Arpai, 1964; Doebbler and Rinfret, 1963; Kraft et al., 1963; Moss and Speck, 1963; Postgate and Hunter, 1963; Hartsell, 1961; Packer et al., 1965).

We have observed, during this study, slight differences among the six bacterial species in survival on certain types of food. For example, the survival of all test bacteria was low on such items as spinach and pears. This is probably related to the presence of relatively high concentration of organic acids in these foods.

Generally, survival is better on meat items than on vegetables, and these statements can also be applied to the reduction in numbers of bacteria on the freeze-dried products during storage.

As would be expected, differences in storage conditions are reflected in the rates at which bacterial numbers decline. Oxygen has been reported to be primarily responsible for death of lyophilized *Serratia marcescens* (Benedict et al., 1961). The effect of oxygen on death of bacteria during storage of the dried foods in this study could have been slowed by storage of the foods at lower temperatures and, conversely, the effect of increasing storage temperature could have been partly overcome by packaging in nitrogen. However, of the two factors, temperature appeared to be the more important for microbial persistence. Unfortunately, the conditions which result in a maximum rate of decline for bacterial cells also results in maximum decline in the quality of the food.

If we consider only total numbers, freeze-drying can be expected to reduce the populations of bacterial contaminants to reasonable levels. But, if we consider small numbers of food-borne pathogens such as *Salmonella*, freeze-dehydration cannot be expected to immediately reduce these types to acceptable levels if the foods are moderately contaminated prior to freezing and drying.

For most of the bacteria in our tests, the killing effects of freezing and dehydration (with the additional frozen storage factor) were of about equal magnitude. In commercial practice, however, the length of storage of the frozen food prior to dehydration may have a more profound influence upon the killing effect of freezing than we indicate.

Because of the high reduction in vegetative cell numbers during freeze-dehydration and because there is a continued decline in numbers during storage, the long term persistence of bacteria on freeze-dried foods may be related to the possession of a spore; viability in spores of Cl. botulinum was high during freezing and dehydration and persisted at relatively high levels during storage. Despaul (1964) observed that viable spore counts of Clostridium perfringens were reduced one-third by freezing. Values for reduction of Cl. botulinum on foods used in this study were found to be in close agreement with that figure.

Moss and Speck (1963) found that the greater part of injury to frozen cells of Streptococcus lactis occurred during the early stages of storage. Postgate and Hunter (1963) also indicated that A. aerogenes which survived freezing and thawing did not die linearly.

Our studies show that death of cells, as judged by counts on rich medium, does not always indicate the full injury done to a bacterial species by the freeze-dehydration process. Neither do total counts indicate the rate of decline which can be expected during storage.

We had expected that the age of a culture, for instance, would have a decided influence upon the percentage of cells which would survive the freeze-dehydration process. With both S. oranienburg and S. aureus this was not found to be true. However, when metabolic injury (Straka and Stokes, 1959; Postgate and Hunter, 1962) was compared to cell age, a definite relationship was observed: the younger the cells, the greater the injury. While this injury may not be obvious from a total numbers count immediately after freeze-drying, it will undoubtedly be reflected in the death rate of the bacterial cells during storage. Metabolic injury is considered to be one of the reasons for the more rapid decline of bacteria on freeze-dehydrated foods during the early periods of storage. Spores of Cl. botulinum did not decline during storage at the rapid rate exhibited by vegetative cells of S. oranienburg, S. aureus, A. aerogenes, E. coli or A. faecalis; but they did decline in viability more rapidly during the early storage period than in the late.

This study appears to indicate that with good sanitation practices freeze-dehydrated foods can be produced with low bacterial numbers. Although lyophilization, under certain conditions, is used as a means of preserving bacteria, the freeze-drying process as used commercially for food appears to be lethal to 95 per cent or more of vegetative cells of the bacteria which cause food-borne infections and intoxications. The process is not as lethal to spores; 30 per cent may survive.

During storage there is a continued decline in numbers of viable cells (and spores), but some cells will persist on the products for long periods of time when the foods are packed and stored under conditions which will give optimum protection to the quality characteristics of the foods.

V. CONCLUSIONS

1. Freeze-drying of foods brought about a reduction in the number of bacteria on the foods.

2. Loss of viability in the bacterial contaminants during the freeze-drying of foods is related to a number of variable factors: (a) kind of food; (b) type of predominant bacterial species; (c) stage of growth the bacterial cells are in at time freeze-drying is begun; (d) rate of freeze and length of frozen storage; and (e) rate of the dehydration phase.

3. The greatest reduction in numbers of bacteria occurs during the freezing process.

4. Based on the cells which survive the lethal influences of freezing, dehydration causes a percentage reduction in viable cells nearly equal to the reduction caused by freezing.

5. There is a continual decline in the numbers of bacteria on freeze-dried food during storage. This decline is influenced by storage temperature, by storage atmosphere, by type of food, by the number of metabolically injured cells present among the storage population, and by the numbers of spores present in the population.

6. Decline in numbers of cells on storage is more rapid at 100°F than at 70°F or 40°F.

7. Temperature effects can be modified somewhat by the presence of an inert atmosphere during storage. However, temperature appears to be the more important factor, i.e., bacteria on the foods stored at 40°F and in an atmosphere of air had higher survival rates than those on similar foods packaged in nitrogen and stored at 70°F or 100°F.

8. The age of the bacterial population influences the dry-storage potential to a greater degree than it does the reductions of numbers during the freeze-drying processing.

9. Spores are more resistant to the lethal influences of the freeze-drying factors than are vegetative cells, and they (spores) will persist longer during storage.

10. Because the number of cells which survive is low, a selection of variants among certain species may occur.

VI. RECOMMENDATIONS

1. This study has shown that food type, cell age, and storage condition have an influence upon maintenance of viability in bacteria on freeze-dried foods. These factors need to be studied in greater detail to determine the effects of growth of bacteria on a particular food prior to freeze-drying. In addition, the effects of mixed culture growth need to be examined.

2. The long term survival of microbial species on freeze-dried foods may be related to spore forms. Spores of certain species of bacteria, especially *Cl. botulinum*, *Cl. perfringens*, and *B. cereus*, should be studied for effects of freezing on spore viability, spore survivability, and rapidity of spore germination and outgrowth when the foods are rehydrated.

3. The percentage of survivors is low. Therefore, a critical examination for selection of variants by the freeze-drying process should be made. Such selection might alter the classical determinative procedures for species related to disease.

4. The study we have reported was somewhat general in its scope. Much useful information could come by employing a probit method to interpret the inactivation of bacterial spores and vegetative cells from thermal (low temperature) influences. This type of information is available for high temperature inactivation processes but analysis of low temperature effects on spores is meager.

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The effect of freeze-drying on the viability of six species of bacteria on seven kinds of food is presented. Freeze-drying influences are presented and discussed in relation to the effects of freezing, drying and storage conditions. The influence of food type and physiological age of bacteria is shown to influence both immediate losses in viability as well as the rate at which such losses occur during storage. The rate of decline in bacterial viability during storage is discussed as a function of the degree of metabolic injury sustained by the bacteria during the freeze-drying process.

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