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STUDIES IN THE HEAT RESISTANT ORGANISMS OF COLD PACKED CANNED PEAS

THESIS

Submitted to the Faculty of the Michigan Agricultural College in partial fulfillment of the requirements for the degree of Master of Science.

By

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May 1919.

THESIS

The writer wishes to acknowledge her indebtedness to Miss Lae Northrup and Dr. Ward Siltner for the helpful suggestions tendered to her during the course of these investigations.

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Introduction.

During the past few years much has been said about food conservation, the home garden and home canning. Especially has the demonstrators attempted to help the housewife save garden vegetables which would otherwise be wasted.

These efforts have been largely successful. In many instances, however, canned vegetables have spoiled. The reason for this has been given as due to the use of vegetables not fresh or failure to follow the directions as given in the United States Department of Agriculture bulletin number 839 on home canning. From this arose the question as to whether the spoilage was due to either of these causes or both, or if there might be some other cause for this trouble.

It has been the writer's intention during the course of this investigation to solve this problem if possible and determine the cause for the very much larger proportion of spoilage from home canning in comparison with commercial canning.

Review of Literature.

Probably the first bacteriological examination of spoiled canned goods was reported by Russel (17) in 1895 in connection with the occurrence of "swells" in canneries of Wisconsin. In this paper "Gaseous Fermentation in the Canning Industry" he cites the isolation from swelled canned peas, two bacilli which

he did not identify. He recommended the increase of temperature to 242 degrees F. and the pressure to fifteen pounds for twenty-eight minutes.

In 1904, Harding and Nicholson(13) examined an outbreak of spoilage in canned peas. They found spoilage in which there was simply souring of the product and also spoilage in which there was gas present and malodor. A small rod or coccus caused the former and a plump rod with terminal spores which grew vigorously in the presence of sugars at 37 degrees C. caused the latter. Limits for successful processing were determined.

In 1905, Duckwall (12) wrote a book on "Canning and Preserving". One chapter is devoted to canned peas in which he discusses the history of peas, their parasites, their composition and food value, methods of canning, and bacteria associated with spoilage. The organisms discussed were those found by himself and others. He mentions lactic acid bacteria, B. butyricus, B. mesentericus vulgatus, "A butyric acid bacillus which was a strict anaerobe with terminal spores similar to B. tetani", B. megatherium, B. prodigioses, B. subtilis, B. mesentericus ruber, B. mycoides and some organisms not named but described. He recommended a longer sterilization period at a higher temperature, for canned goods.

Savalla (21) published a book in 1916 on "Canning of Fruits and Vegetables". In Part III, he discusses spoilage, giving a report on two hundred and eighty five bacteriological examinations of canned foods from six canneries. Nine spore formers were described but not named. Gas was found in cans of peas but these organisms did not form gas in sugar fermentation tubes.

Dickson (11) in 1917 investigated an occurrence of botu-

lism on the Pacific Coast. He found B. botulinus in three cases. He then inoculated canned peas, beans, and corn with B. botulinus, heating them at a boiling temperature in accordance with Government bulletin directions. These developed gas in three weeks. B. botulinus and B. subtilis were recovered from the cans. He concluded that the time given in the directions for sterilization in the Government bulletins is insufficient.

The Department of Agriculture (19) answered this paper by saying that the spores of B. botulinus are killed by one hour of heating at 175 degrees F. and that there is, therefore, no danger from B. botulinus in canned food. It was also stated that the toxin from B. botulinus is destroyed by boiling a few minutes. However, a warning was given against eating foods showing any signs of spoilage.

A. W. Bitting and A. G. Bitting (4) published their bulletin "Bacteriological Examination of Canned Foods" in 1917. They discuss leaks, springers, the proper method for opening tin cans for examination, pressure in cans, swells and flat sours and organisms of spoilage. They determined by experiment that it takes a tin can of peas standing, over twenty minutes to reach 248 degrees F. Cans are more uniformly heated when agitated. Organisms found were a large lactic acid bacillus, a coccus form which produced malodor and thermophilic forms in appearance similar to B. tetani and B. botulinus. They state that some organisms require a much higher temperature than others to destroy the spores.

Georgia Spooner Burke (6) in 1918 wrote a bulletin, "The Effect of Heat on the Spores of B. Botulinus." She states that B. botulinus toxin is killed by five minutes of boiling, but the

spores are not killed by boiling for five hours in jars of fruit. They will also survive three and one half hours of boiling in an open kettle or fifteen pounds of pressure in the autoclave for ten minutes. She concluded that the pressure cooking is the only sure method of avoiding spoilage by B. botulinus.

In 1918 Bushnell (7) experimented on the influence of cold shock on the sterilization of foods working with both glass jars and test tubes. He found that cold shock did not promote the keeping qualities of canned food. Exclusion of air, however, prevented aerobic organisms from developing when their spores were present.

J. Weinzirl (20) in his thesis entitled "The Bacteriology of Canned Foods" gives his results from investigation of one thousand and eighteen samples of canned goods. Beside molds and yeasts, he isolated three hundred and ninety two bacteria, representing thirty eight species. The most prevalent organisms found were B. mesentiricus, B. subtilis, B. thermoindifferens, B. vulgatus, and B. cereus.

He concludes that spores may be present in apparently unspoiled canned goods as found on the market. These may be unable to grow due to lack of oxygen. Vacium is essential for the preservation of foods under the present methods of processing. Food poisoning organisms, B. botulinus and B. enteritidis are not found in commercially canned food.

Method of Procedure.

In the summer of 1918 between the first and twentieth of July, in connection with an experiment in soils, thirteen lots

of peas were picked, weighed, and later canned in accordance with the Government methods for cold pack canning. One lot was heated in steam; five were cooked by the hot water bath method; and seven lots were processed in the autoclave at fifteen pounds pressure. Lot A.II.K. is the only one where the canning was done the same day that the peas were picked. In lot H.W.B.I. and A.VI. the peas were kept at room temperature over night. All others were kept in the refrigerator. All of these peas except lot A.II.K. mentioned above, were partly shelled one day, then kept until the next day when the shelling was finished and they were canned.

In two weeks, spoilage began to occur. The following chart shows the data upon this:

Lot	Jar	Size	Sterilization	Time	No.of cans	Spoilage.
A.I.	Mason	Pts.	Autoclave 15 lbs.	40 min.	7	7
A.II.O.	Mason	Pts.	Autoclave 15 lbs.	40 min.	17	1
A.II.K.	Mason	Pts.	Autoclave 15 lbs.	40 min.	33	4
A.III.	Seal fast	Pts.	Autoclave 15 lbs.	40 min.	30	20
A.IV.	Mason	Qts.1Pt.	Autoclave 15 lbs.	1 hr.	21	19
A.V.	Mason	Qts.1 Pt.	Autoclave 15 lbs.	1 hr.	14	14
A.VI.	Mason	Qts.1 Pt.	Autoclave 15 lbs.	1 hr.	4	1
H.W.B.I.	Mason	Pts.,Qts.	Hot water bath	3 hrs.	4	1
H.W.B.II.	Mason	Pts.,Qts.	Hot water bath	3 hrs.	3	3
H.W.B.III.	Seal fast	Pts.	Hot water bath	3 hrs.	21	18
H.W.B.IV.	Mason	Pts.	Hot water bath	3 hrs.	27	19
H.W.B.V.	Seal fast	Pts.	Hot water bath	3 hrs.	17	5
S.I.	Mason	Pts.,Qts.	Steam	3 hrs.	15	11

Percent spoilage of those autoclaved 50.9 percent

Percent " " " cooked in hot water bath 63.8 "

Percent " " " cooked in steam 73.3 "

Five cans were selected for examination from lot A.1. and five from A.V. where there was entire spoilage.

These cans were examined for swells, gas, leakage, and all evidences of spoilage before opening;then the cans were carefully washed with mercuric chloride solution of a one to one thousand dilution. Alcohol was poured over the cover and then burned off. The can cover was then lifted enough to take samples of the juice.

Five cubic centimeters of the juice were titrated to determine the acidity. Two sets of dilution plates of 1 to 100, 1 to 10,000 and 1 to 100,000 were made. One set of these was placed under anaerobic conditions in a Novy jar, filled with hydrogen. One hundredth of a cubic centimeter was smeared uniformly over one square centimeter on a slide and stained for a direct microscopic count and also for the determination of the vegatative forms present as much as possible. Two sets of shakes were made by the loop dilution method into gelatin agar. One set was heated to 80 degrees C. for the determination of the presence of spores.

All cultures were incubated at room temperature. Each organism isolated was inoculated into a tube of dextrose gelatin agar. When this was proved to be a pure culture, transfers were made into two tubes of sterile peas in distilled water. One set was made anaerobic by pouring sterile white paraffin oil over the top of the tube. After the action in this medium was determined, cultures were made in all media used commonly for identification purposes. Cultures were also made in a one percent starch peptone solution to determine the reduction of starch to sugar and the production of acid. The thermal death point of each organism was

determined and stains and measurements made. By comparison of results, the organisms occurring in more than one can were determined.

Known organisms having similar characteristics were then inoculated into aerobic and anaerobic tubes of peas to determine their action in this medium.

Results.

Lot A. I. Sterilized forty minutes in the autoclave at 15 pounds pressure.

Can	A.I.1	A.I.2	A.I.3	A.I.4	A.I.5
Jar	Pint Mason	Pint Mason	Pint Mason	Pint Mason	Pint Mason
Cloudy	+ sediment	+ sediment	+	+	+
Gas	+	-	+	+	+
Swell	-	-	-	-	-
Leak	-	Reautoclaved	-	-	-
Acidity	.028/N	.038/N	.033/N	.025/N	.045/N
Odor	Normal	Nearly Normal	Normal	Normal	Nearly Normal
Bacterial Count per c.c.					
aerobic	2,750	7,156	3,435,333	622,400	295,666
anaerobic	Spreader	Liquefier	24,407,140	Spreader	350,000
Direct microscopic count	14,221,200	569,084,000	43,923,200	1,611,736	47,404
Organisms	3	14	3	3	2

Plate I.

Lot A. I.

Can A.I.1.

Can A.I.2.

Can A.I.3.

Can A.I.4.

Can A.I.5.

Lot A. V. Autoclaved one hour at fifteen pounds pressure.

Can	A.V.10	A.V.11	A.V.12	A. V. 13	A.V.14
Jar	Qt. Mason	Qt. Mason	Qt. Mason	Qt. Mason	Pt. Mason
Cloudy	+	+	+ sediment	+	+
Gas	+	+	+	+	+
Swell	-	+	+	+	-
Leak	+	Resealed not heated	Resealed not heated	+	+
Acidity	.076/N	.086/N	.082/N	.102/N	.073/N
Odor	Nearly Normal	Nearly Normal	Normal	butyric	Normal
Bacterial count per c.c.					
aerobic	361,833	700	300,000	776,400	48,200
anaerobic	360,000	200	Spreader	796,500	8,679,500
Direct microscopic count	3,033,856,000	568,848,000	568,858,000	209,612,000	2,439,772,800
Organisms	2	2	3	4	1

Plate II.

Lot A. V.

Can A.V.10.

Can A.V.11.

Can A.V.12

Can A.V.13.

Can A.V.14.

Organisms Found.

Bacillus A.

Found in cans A.I.1, A.I.5, A.V.10, A.V.14, A.I.3, A.V.1

I. Morphology:

1. Vegetative Cells:

(a) Form - Small rod.

(b) Limits of size - 1.5 - 2 x 0.5 microns.

2. Endospores:

(a) Position - Central.

(b) Form - Oval.

(c) Produce slight enlargement of the rod.

3. Motility:

(a) Actively motile.

4. Staining:

(a) Gram negative.

(b) Stains with ordinary dyes.

II. Cultural Characteristics:

1. Gelatin Stab:

(a) Growth - Best at top.

(b) Liquefaction - Napiform becoming stratiform.

2. Nutrient Broth:

(a) Surface growth, pellicle settles on shaking.

(b) Cloudy.

3. Litmus Milk:

(a) Peptonization - Slow.

(b) Reduction from the bottom upward to a tan colored liquid.

(c) Alkaline.

4. Glycerin Potato:

(a) Abundant, flat.

(b) Dull, finely wrinkled.

(c) Cream colored, becoming tan.

(d) Water in bottom pinkish.

5. Gelatin Agar Colony:

(a) Growth - Rapid.

(b) Flat.

(c) Form - Spreading.

(d) Semitransparent.

III. Physical and Biochemical Features:

1. Fermentation tubes, neutral red broth plus

(a) Dextrose: (Gas - Negative. (Reaction - Acid.

(b) Lactose: (Gas - Negative. (Reaction - Alkaline.

(c) Saccharose: (Gas - Negative. (Reaction - Acid.

2. Peas in distilled water:

(a) Aerobic Tubes:

(Gas.
(Creamy top growth becoming deep red
(underneath; more readily produced at
(37 degrees C.

(b) Anaerobic tubes:

(Gas.
(Not much visible growth, no color.

3. Peptone Solution:

(a) Indol production, slight.

4. Nitrate Peptone Solution:

(a) Nitrate reduction.

(b) Ammonia production.

5. Starch Peptone Solution, neutral:

(a) Starch reduction.

(b) Slight test for sugar.

(c) Slight acid.

6. Temperature Relations:

(a) Growth at room temperature.

(b) Favored by 37 degrees C.

(c) Not killed by heating at 120 degrees C. for ten minutes.

(d) Killed by heating at 120 degrees C. for twenty minutes.

Bacillus B.

(Conforms to description of B. subtilis as given by Ford)

Found in cans A.I.1, A.I.2, A.I.3, A.I.4, A.I.5, A.V.11, A.V.1
A.V.13.

I. Morphology:

1. Vegetative Cells:

(a) Rod.

(b) Size - 2.5 - 4.5 x 0.5 - 0.75 microns.

2. Endospores:

(a) Position - Central or nearly so.

3. Motility:

(a) Actively motile.

4. Staining:

(a) Gram positive.

(b) Stains with ordinary dyes.

II. Cultural Characteristics:

1. Gelatin Stab:

(a) Top growth.

(b) Liquefaction - Nearly crateriform.

2. Nutrient Broth:

(a) Heavy top growth not easily broken.

(b) Broth nearly clear, yellowed.

3. Litmus milk:

(a) Peptonization.

(b) Alkaline.

4. Glycerin Potato:

(a) Very abundant growth.

(b) Dull rugose.

(c) Cream colored.

5. Gelatin Agar Colonies:

(a) Growth-Rapid.

(b) Granular.

(c) Rhizoid.

(d) White.

III. Physical and Biochemical Features:

1. Fermentation tubes, neutral red broth plus

(a) Dextrose: (Gas - None.
(Reaction - Acid.
(Heavy top growth.

(b) Lactose: (Gas - None.
(Reaction - Alkaline.
(Heavy top growth.

(c) Saccharose: (Gas - None.
(Reaction - Acid.
(Top growth.

2. Peas in distilled water:

(a) Aerobic culture:

(Gas production.
(Cream colored wrinkled, growth, darkens.
(Slime production.

(b) Anaerobic culture:

(Gas production.
(Slime.
(Yellowish liquid.

3. Peptone Solution:

(a) Indol formation.

4. Nitrate Peptone Solution:

(a) Nitrate reduction.

(b) Ammonia production.

5. Starch Peptone Solution:

(a) Complete reduction of starch.

(b) Large quantity of sugar.

(c) Solution, slightly alkaline.

6. Temperature Relations:

(a) Grows readily at room temperature.

(b) Favored by 37 degrees C.

(c) Not killed by heating at 120 degrees C. for ten minutes.

(d) Killed by heating at 120 degrees C. for twenty minutes.

Bacillus C.

(Resembles B. botulinus)

Found in can A.I.2.

I. Morphology:

1. Vegetative Cells:

(a) Large rod with rounded ends.

(b) Size - 4 - 6 x 0.75 microns.

2. Endospores:

(a) Position - Polar, making the rod club shaped.

3. Motility:

(a) Actively motile.

4. Staining:

(a) Gram positive.

(b) Stains with ordinary dyes.

II. Cultural Characteristics:

1. Nutrient Broth under oil:

(a) Broth cloudy.

(b) Odor of butyric acid.

2. Gelatin Agar Colonies:

(a) Anaerobic.

(b) Furry.

(c) Whitish, becomes somewhat tan.

3. Dextrose Gelatin Agar Shake:

(a) Gas at 25 degrees C.

(b) No gas at 37 degrees C.

III. Physical Characteristics:

I. Temperature Relations:

(a) Growth best at 25 degrees C.

(b) Slow growth at 37 degrees C.

(c) Not killed by heating at 120 degrees C. for ten minutes.

(d) Killed by heating at 120 degrees C. for twenty minutes.

Bacillus D.

I. Morphology:

1. Vegatative Cells:

(a) Slender rods.

(b) Size - 1.5 - 3 x .5 microns.

2. Endospores:

(a) Position - Central.

(b) Slight enlargement of the rod on sporulation.

(c) Spores - Oval.

3. Motility:

(a) Actively motile.

4. Stain:

(a) Gram negative.

(b) Stains with ordinary dyes.

II. Cultural Characteristics:

1. Gelatin Stab:

(a) Liquefaction, infundibuliform.

2. Nutrient Broth:

(a) No top growth.

(b) Cloudy.

(c) Sediment.

3. Litmus milk:

(a) Peptonization.

(b) Alkaline.

4. Glycerin Potato:

(a) Flat, smooth glistening growth.

(b) Color creamy tan.

5. Gelatin Agar Colonies:

(a) Flat.

(b) Spreading.

(c) White, turning tan in forty eight hours.

III. Physical and Biochemical Features:

1. Fermentation tubes, neutral red broth plus

(a) Dextrose: (Gas - None. ((Reaction - Slightly acid.

(b) Lactose: (Gas - None. ((Reaction - Alkaline.

(c) Saccharose: (Gas - None. ((Reaction - Acid.

2. Peas in distilled water:

(a) Aerobic culture:

(Gas. (Creamy top growth.

(b) Anaerobic culture:

(Gas.

3. Peptone Solution:

(a) No indol production.

4. Nitrate Peptone Solution:

(a) Nitrate reduction.

(b) Ammonia production.

5. Starch Peptone Solution:

(a) None or very slight reduction of starch.

(b) No production of sugar.

(c) Slight acid.

6. Temperature Relations:

(a) Growth both at 25 degrees and 37 degrees C.

(b) Not killed by heating at 120 degrees C. for ten minutes.

(c) Killed by heating at 120 degrees C. for twenty minutes.

Bacillus E.

(Resembles B. ramosus)

Found in A.V.10, and A.V.12.

I. Morphology:

1. Vegetative Cells:

(a) Thick rods.

(b) Size - 2 - 3.5 x 0.75 - 1 microns.

2. Endospores:

(a) Position - Central.

3. Motility:

(a) Motile, not active.

4. Staining:

(a) Gram positive.

(b) Stains with ordinary dyes.

II. Cultural Characteristics:

1. Gelatin Stab:

(a) Liquefaction - Crateriform to stratiform.

(b) Whitish sediment which turns pinkish.

2. Nutrient Broth:

(a) Top growth flaky which settles on shaking.

3. Litmus milk:

(a) Peptonization.

(b) Alkaline.

4. Glycerin Potato:

(a) Growth abundant, raised, contoured.

(b) Moist creamy to pink becoming purplish.

5. Gelatin Agar Colony:

(a) Flat.

(b) Sometimes somewhat spreading.

(c) White, translucent.

III. Physical and Biochemical Features:

1. Fermentation tubes, neutral red broth plus

(a) Dextrose: (Gas - None.
(Reaction - Acid.

(b) Lactose: (Gas - None.
(Reaction - Slightly alkaline.

(c) Saccharose: (Gas - None.
(Reaction - Acid.

2. Peas in distilled water:

(a) Aerobic culture:

(Gas.
(Somewhat rose colored top growth.

(b) Anaerobic culture:

(Gas.

3. Peptone Solution:

(a) Indol formation.

4. Nitrate Peptone Solution:

(a) Nitrate reduction.

(b) Ammonia formation.

5. Starch Peptone Solution.

(a) Slight reduction of starch.

(b) Slight test for sugar.

6. Temperature Relations:

(a) Grows readily at either 25 degrees or 37 degrees C.

(b) Not killed by heating at 110 degrees C. for ten minutes.

(c) Killed by heating at 110 degrees C. for thirty minutes.

Bacillus H.

Found in cans A.I.4, and A.I.3.

I. Morphology:

1. Vegetative Cells:

(a) Rods usually in long chains.

(b) Size - 2.5 - 3 x .5 microns.

2. Motility:

(a) Slowly motile.

3. Endospores:

(a) Central.

4. Staining:

(a) Gram positive.

(b) Stains with ordinary dyes.

II. Cultural Characteristics:

1. Gelatin Stab;

(a) Liquefaction - Crateriform.

2. Nutrient Broth:

(a) No top growth.

(b) Medium cloudy.

(c) Fine precipitate.

3. Litmus milk:

(a) Peptonization.

(b) Reduction.

(c) Alkaline.

4. Glycerin Potato:

(a) Growth - Moderate.

(b) Dull creamy yellow growth.

5. Gelatin Agar Colony:

(a) Granular.

(b) White.

(c) Lacerate edges.

III. Physical and Biochemical Features:

1. Fermentation tubes neutral red, broth plus

(a) Dextrose (Gas - None. (Reaction - Acid. (Green iridescence.

(b) Lactose: (Gas - None. (Reaction - Alkaline.

(c) Saccharose: (Gas - None. (Reaction - Acid.

2. Peas in distilled water:

(a) Aerobic cultures

(Gas. (Very moist growth which developes a (touch of bright tan color.

(b) Anaerobic culture:

(Gas.

3. Peptone Solution:

(a) Indol production.

4. Nitrate Peptone Solution:

(a) Nitrate reduction.

(b) Ammonia production.

5. Starch Peptone Solution:

(a) Starch reduction.

(b) No test for sugar.

6. Temperature Relations:

(a) Grows readily both at 25 degrees and 37 degrees C.

(b) Not killed by heating at 120 degrees C. for ten minutes.

(c) Killed by heating at 120 degrees C. for twenty minutes.

Bacillus K

Found in can A. I. 4.

I. Morphology:

1. Vegetative Cell:

(a) Large rod, usually in short chains.

(b) Size - 2 - 4.25 x 0.75 microns.

2. Endospores:

(a) Position - Central.

3. Motility:

(a) Slowly motile.

4. Staining:

(a) Gram negative.

(b) Stains with ordinary dyes.

II. Cultural Characteristics:

1. Gelatin Stab:

(a) Liquefaction - Crateriform.

2. Nutrient Broth:

(a) Top growth finely wrinkled.

(b) Medium clear.

3. Litmus milk:

(a) Peptonization.

(b) Reduction.

(c) Alkaline.

4. Glycerin Potato:

(a) Growth moderate, controued, raised.

(b) Dry chalky white growth.

5. Gelatin Agar Colony:

(a) Regular.

(b) White with thicker creamy spot in centers.

III. Physical and Biochemical Features:

1. Fermentation tubes, neutral red broth, plus

(a) Dextrose: (Gas- None.
(Reaction - Acid, iridescent.

(b) Lactose: (Gas - None.
(Reaction - Alkaline.

(c) Saccharose: (Gas - None.
(Reaction - Acid.

2. Peas in distilled water:

(a) Aerobic culture:

(Gas.
(Heavy creamy wrinkled top growth.

(b) Anaerobic culture.

(Gas.

3. Peptone Solution:

(a) Indol formation.

4. Nitrate Peptone Solution:

(a) Nitrate reduction.

(b) Ammonia Production.

5. Starch Peptone Solution:

(a) Starch reduction.

(b) No test for sugar.

(c) Reaction - Alkaline.

6. Temperature Relations:

(a) Growth at 25 degrees C.

(b) Favored by 37 degrees C.

(c) Not killed by heating at 110 Degrees C. for forty minutes.

(d) Killed by heating at 120 degrees C. for ten minutes.

Bacillus Y.

Found in Can A. V. 12.

I. Morphology:

1. Vegetative Cells:

(a) Very large rod.

(b) Size - 3 - 5 x 1 micron.

2. Endospores:

(a) Position - Subterminal.

(b) Formed within forty eight hours.

(c) Shape - Slightly oval.

3. Motility:

(a) Slowly motile.

4. Staining:

(a) Gram negative.

(b) Stains with ordinary dyes.

II. Cultural Characteristics:

1. Gelatin Stab:

(a) Top growth, light orange.

(b) Liquefaction - Napiform becoming stratiform.

2. Nutrient Broth:

(a) Top growth, ring.

(b) Medium very cloudy.

3. Litmus milk:

(a) Reduction in twenty four hours.

(b) Coagulation in thirty six hours.

(c) Reaction - Acid.

4. Glycerin Potato:

(a) Growth vigorous.

(b) Dull wrinkled whitish growth.

5. Gelatin Agar Colony:

(a) Tree-like spreading growth.

(b) White in color.

III. Physical and Biochemical Features:

1. Fermentation tubes of broth with neutral red plus

(a) Dextrose: (Gas - None. (Reaction - Acid.

(b) Lactose: (Gas - None. (Reaction - Neutral.

(c) Saccharose: (Gas - None. (Reaction Neutral.

2. Peas in distilled water:

(a) Aerobic culture:

(Gas
(
(Moist growth with touch of bright tan
(
(color.

(b) Anaerobic culture:

(Gas - None.

3. Peptone Solution:

(a) Indol formation.

4. Nitrate Peptone Solution:

(a) Nitrate reduction.

(b) Ammonia formation.

5. Starch Peptone Solution:

(a) Starch reduction.

(b) No sugar.

(c) Reaction - Acid.

6. Temperature Relations:

(a) Growth at both 25 degrees C. and 37 degrees C.

(b) Not killed by heating at 120 degrees C. for ten minutes.

(c) Killed by heating at 120 degrees C. for twenty minutes.

Bacillus Z.

Found in San A. V. 13.

I. Morphology:

1. Vegetative Cell:

(a) Slender rod.

(b) Size - 1 - 2 x 0.5 microns.

2. Endospores:

(a) Produced subterminally.

(b) Shape - Oval.

3. Motility:

(a) Actively motile.

4. Staining:

(a) Gram negative.

(b) Stains with ordinary dyes.

III. Cultural Characteristics:

1. Gelatin Stab:

(a) Liquefaction - Infundibuliform.

2. Nutrient Broth:

(a) Top growth soft and heavy breaks to a cloudy precipitate.

3. Litmus milk:

(a) Increased alkalinity.

(b) No peptonization, curd of reduction.

4. Glycerin Potato:

(a) Potato gray.

(b) Growth flat yellowish gray.

5. Gelatin Agar Colony:

(a) Raised.

(b) Glistening.

(c) Yellowish white.

III. Physical and Biochemical Features:

1. Fermentation tubes of broth neutral red plus

(a) Dextrose: (Gas - None.
(Reaction - Neutral.

(b) Lactose: (Gas - None.
(Reaction - Neutral.

(c) Saccharose: (Gas - None.
(Reaction - Neutral.

2. Peas in distilled water:

(a) Aerobic Culture:

(Gas - None.
(Heavy creamy wrinkled top growth, liquid yellowed.

(b) Anaerobic Culture:

(Gas - None.

3. Peptone Solution:

(a) Indol formation.

4. Nitrate Peptone Solution:

(a) Nitrate reduction.

(b) Ammonia production.

5. Starch Peptone Solution:

(a) Starch reduction.

(b) Test for sugar.

6. Temperature Relations:

(a) Grams at 25 degrees C.

(b) Favored by 37 degrees C.

(c) Not killed by heating at 110 degrees C. for twenty minutes.

(d) Killed by heating at 110 degrees C. for thirty minutes.

Plate III.

Bacillus A.

Bacillus B.

Bacillus C.

Bacillus D.

Bacillus E.

Plate IV.

Bacillus X.

Bacillus K.

Bacillus Y.

Bacillus Z.

Cultural Characteristics in Peas of Common Spore Bearing Bacteria.

The following spore forming bacteria corresponds quite closely in all cultural and morphological characteristics to certain of the bacteria just described. In the first two instances, even to gas formation in peas. As far as can be determined this is a circumstance hitherto unnoted.

I. B. subtilis.

1. Aerobic Culture:
   (a) Gas after forty eight hours.
   (b) Abundant top growth.
   (c) Growth dull, wrinkled cream colored.
   (d) Favored by 37 degrees C.
   (e) Liquid yellowed.
2. Anaerobic Culture:
   (a) Gas formation - None.
   (b) Growth - Below surface of oil.

II. B. ramosus.

1. Aerobic Culture:
   (a) Gas production in twenty four hours.
   (b) Abundant top growth.
   (c) Growth - Moist, white, turning pink then deep red below surface of top growth.
2. Anaerobic Culture:
   (a) Gas production in twenty four hours.
   (b) Growth - Below surface of oil but no color.

III. B. mycoides.

1. Aerobic Culture:

(a) Gas - None.

(b) Top growth slow in developing.

(c) Growth - Moist, white.

(d) Hindered by 37 degrees C.

2. Anaerobic Culture:

(a) Gas - None.

(b) Growth - Below surface of oil.

IV. B. mesentericus vulgatus.

1. Aerobic Culture:

(a) Gas - None.

(b) Top growth slight.

(c) Growth - Light tan in color.

(d) Growth hindered by 37 degrees C.

2. Anaerobic Culture:

(a) Gas - None.

(b) White growth in tube.

Discussion of Results.

This work upon spoiled canned peas has necessarily been limited. For more complete data bacteriological examinations should be made of cans sterilized by the several methods and the work should be carried on for several seasons. This has been impossible in the present instance. However, some very definite conclusions may be drawn.

Since the organisms found in this investigation resemble B. subtilis and other soil organisms, it is reasonable to suppose

that all are from that source. The means of contamination may have been from one or all of three sourses: Dust on the pods may have been transferred to the peas in shelling; there may have been dust on the hands of those shelling the peas which would be transferred to the peas; dust from the air may have settled upon the shelled peas in the room or refrigerator when left over night. As the peas shelled and canned the same day showed a much lower percentage of spoilage than peas partly shelled and left until the next day before canning, this last method of contamination would seem an important one.

The percentage of spoilage was much lower in peas autoclaved than those sterilized by any other method. This doubtless accounts for the higher percentage of spoilage of home canned peas, usually canned by the hot water bath method, in comparison with the commercially canned product.

The commercial canner is especially careful to have his peas canned as soon as possible after cutting. He is also very thorough in washing the product before canning. Doubtless here is the secret of the low percentage of spoilage as his process consists of heating the cans at 112 - 115 degrees C. for 35 to 40 minutes,a temperature withstood by the organisms described in this paper.

The ability to form gas in peas seems to be due to the action of organisms upon some protein or proteins found in peas and not in artificial media. It may be possible to further differentiate between similar organisms by use of peas as a medium since organisms not classified as gas formers were found to produce gas when so tested.

Summary.

1. The lowest percentage of spoilage was found in peas sterilized in the autoclave.

2. Peas canned immediately after shelling had a comparatively low percentage of spoilage.

3. Organisms found were all spore forming bacilli.

4. All organisms withstood from ten to fifteen pounds pressure in the autoclave for ten to twenty minutes.

5. Nearly all organisms reduced starch to sugar.

6. Seven of these organisms caused peptonization in milk.

7. But one organism found failed to produce indol from peptone.

8. Eight of these organisms found in peas produced gas in these medium but not in other media.

9. B. subtilis and B. ramosus produced gas in peas but not in other media.

Conclusion.

The spoilage in cold packed canned peas is largely due to the presence of resistant spore forming organisms which are not killed by the prescribed method for sterilization.

Therefore, before canning vegetables, the product should be very carefully washed to remove all soil or dust and thus remove a greater percentage of organisms.

The time for sterilization of vegetables should be lengthened so that the center of the can may be at a high temperature sufficiently long to kill these more resistant organisms.

Sterilization of all cold packed canned vegetables should be carried out by the pressure method.

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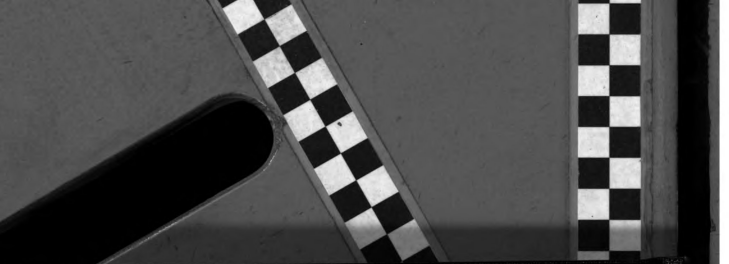

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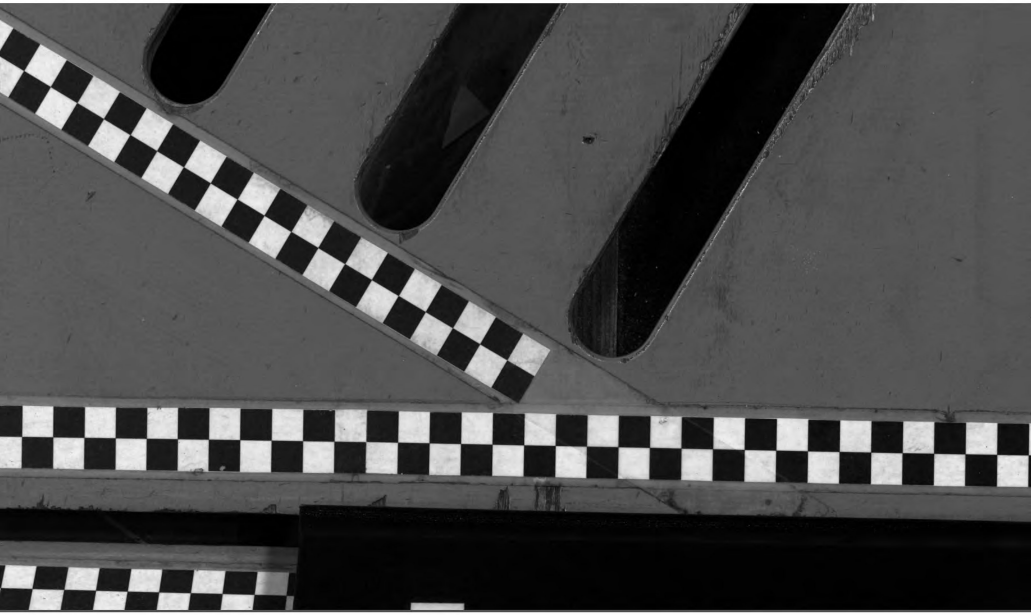


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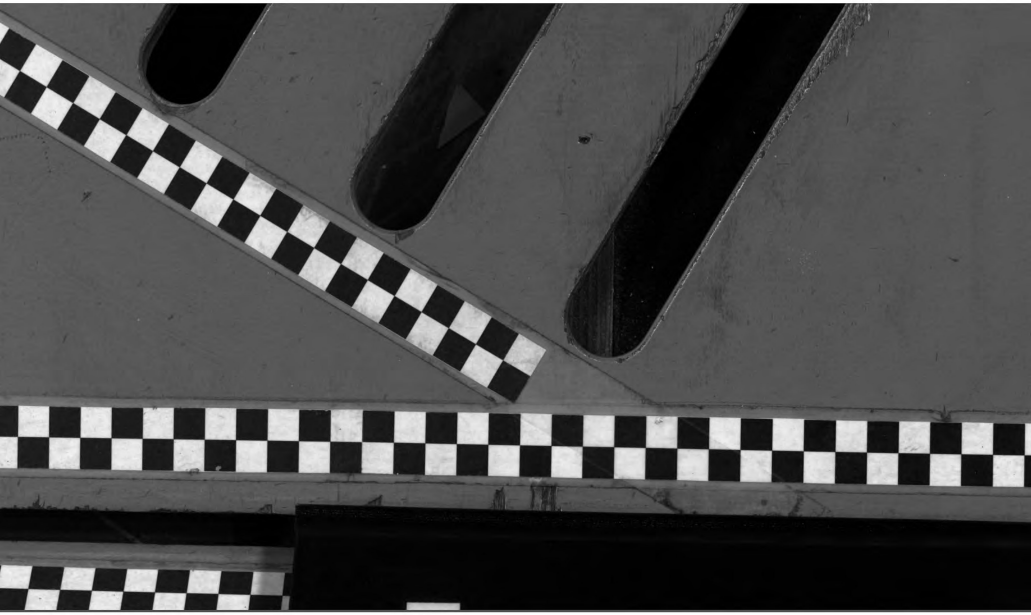

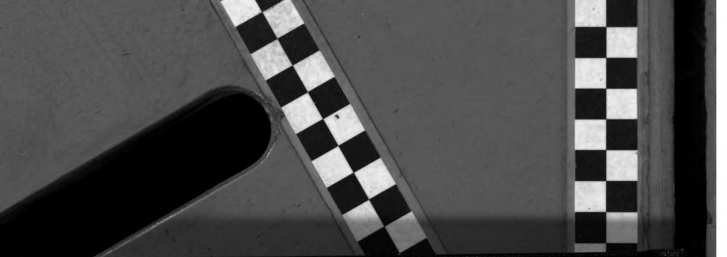
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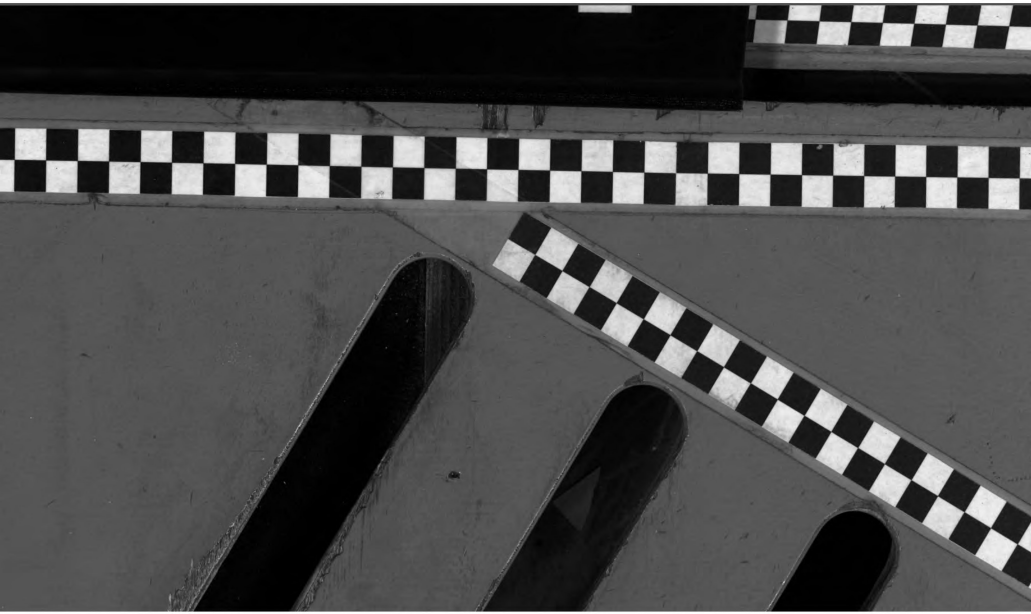

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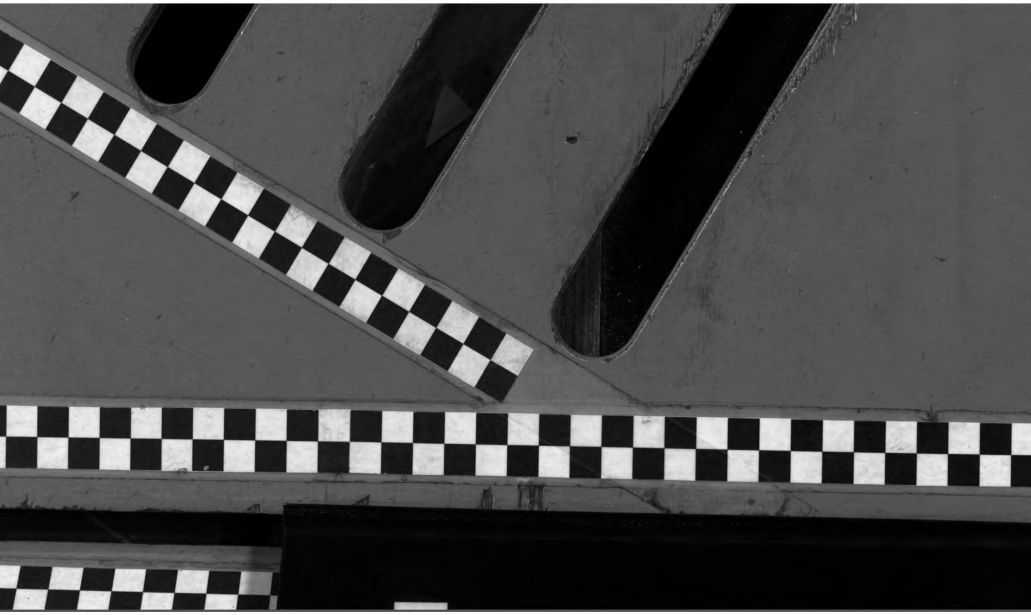

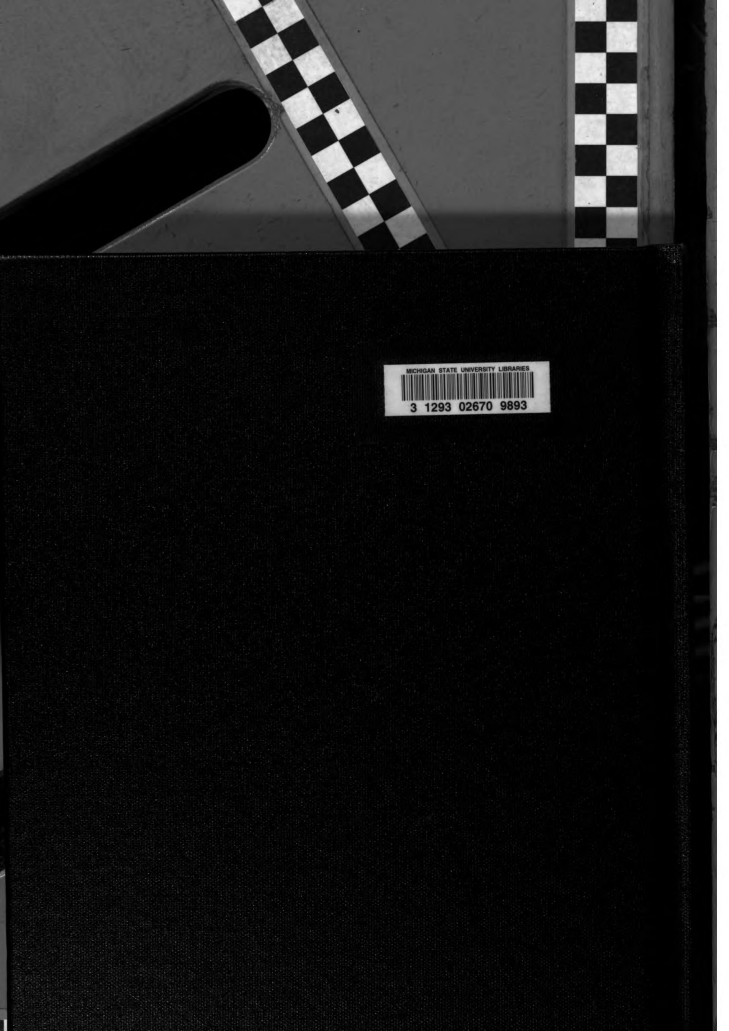
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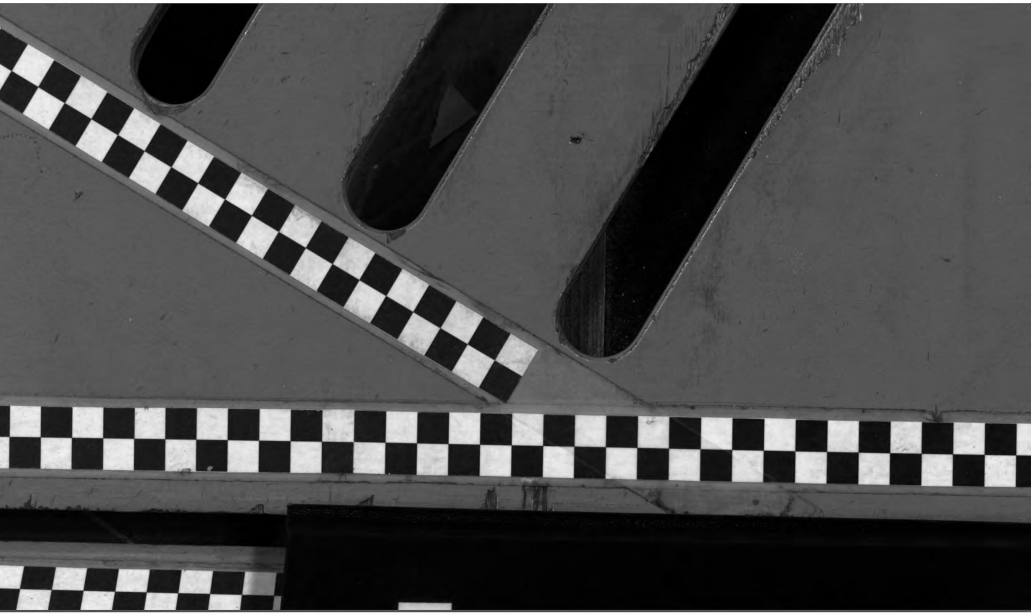

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