THE EFFECTIVENESS OF CERTAIN CHEMICALS IN REDUCING THE PROCESSING TIME AND TEMPERATURE OF CANNED MEAT

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INTRODUCTION

It is well known that low acid foods especially meat, peas, and corn require a long processing time and high temperature to assure successful preservation in canning. Although this treatment preserves these products, the canned product, due to over-cooking, does not compare in color, flavor, and texture with the fresh product after cooking.

The object of this study is to determine whether commercial sterility can be obtained in canning meat by using a chemical preservative with less severe processing. This will reduce the processing time and temperature so that a canned product comparable with the freshly cooked product may be obtained.

No attempt was made to synthesize new compounds nor to modify any part of the molecular constitution of compounds. Also such factors as toxicity of the agents employed and present regulations concerning the use of chemicals in food products were not taken into consideration in this research.

REVIEW OF LITERATURE

Meat Preservation and Microorganisms Involved:

Thermophilic bacteria which have been so important in the spoilage of canned vegetables have not been important in the spoilage of canned meats, at least those to which no cereals are added. Jensen and Hess (19) pointed out that thermophilic sporeforming bacteria have become significant in meat products to which soya flour, grits, or meal have been added.

Salle (31, p. 536) stated that the most pronounced changes in meats are produced by the anaerobic, spore-bearing organisms. Some of the anaerobes that have been isolated from fresh and spoiled meat are Clostridium perfringens, C. tertium, C. bifermentans, and C. sporogenes. These organisms are responsible for putrefactive changes on proteins, resulting in the liberation of foul-smelling compounds and gases.

Jensen (18, p. 281) also indicated that the most prevalent of the species of anaerobes found in canned meats was C. sporogenes.

Haines (11) reviewed the various spoilage problems of meat preservation and the microorganisms involved. Jensen (18) covered this subject completely in his book "Microbiology of Meats." Tanner (35, pp. 838-910) also has

discussed this phase of work rather thoroughly in his book "Microbiology of Foods."

Microbial Inhibition by Food Preservatives:

Wyss (43) limited his review to a discussion of the mechanism of the anti-microbial action brought about by chemical substances and possible applications of this knowledge in the food industries. He stated that in many instances it will be observed that, when the log of the number of surviving bacteria is plotted against the time of exposure to a chemical agent, a straight line results.

Hunhoimer and Fabian (27, p. 1045) showed typical curves of the effect of some acids, sugars, and sodium chloride on strains of food poisoning due to Staphylococci. The curves did not give straight lines when the log of the number of surviving bacteria was plotted against the time of exposure in days.

In looking for a food preservative one must be able to recognize the attributes that make the preservative good or desirable. These attributes have been listed by Gershenfeld and Perlstein (9) as follows:

1- It must be effective against the types of micro-organisms causing decomposition or spoilage.

2- It must be soluble in the concentration used.

3- It must not be toxic in the concentration in

which it is employed.

4- It must be compatible, must not alter the character of the substances to which it is added, must not impart any odor, color, taste, etc. to the product, and it must be practically neutral so that it will not affect the pH of the product.

5- It should be available or potentially available and not prohibitive in cost.

6- The developed inhibiting effect must be lasting and therefore it may not be possible to depend on volatile substances, the effects of which disappear after evaporation.

Among the preservatives which have been of interest are:

1- The phenol derivatives (4, 32).

2- The quaternary ammonium compounds. These were reviewed by Hucker (15, 16, 38) and Hoogerkeide (14). Josyln et al. (21) found that "phemerol" was effectively bactericidal when examined against 10 pathogenic test-microorganisms, and its germicidal action was not reduced when incorporated as the active agent in higher aqueous or tincture formulae. As a result of the work of Rawlins et al. (30) it was concluded that the general configuration of the molecule of the

quaternary ammonium salts was as important as the exact chemical nature of its substituents. The quaternary ammonium compounds and their use in the food industry have been reviewed very recently by Dunn (5).

3- The aliphatic acids and their salts (13, 17). Nunheimer and Fabian (27) found in their studies on food poisoning due to Staphylococci a decreasing order of germicidal action for the acids studied: acetic citric lactic malic tactaric hydrochloric. The decreasing order of antiseptic action was found to be acetic lactic citric malic tartaric hydrochloric. These authors pointed out that the observed effects are due to factors in addition to the hydrogen ion, presumably either the un-ionized molecule or the anion or both. Erickson and Fabian (6) reported that if preserving values of acids are based on pH, the order is acetic citric lactic, but if the number of grams of each acid in 10 ml. of broth is determined the order is lactic acetic citric. For the germicidal values of the acids on bacteria the order is lactic acetic citric if the number of grams of acid in 10 ml. of broth is used as the basis of comparison, whereas if

pH is used as the basis, the order is acetic citric lactic.

4- The benzoic acid derivatives (44). Jensen (18, p. 267) pointed out that extraordinary conditions arose in World War II where adequate refrigerated ship space was no longer available. Lack of refrigeration left the meat industry with chemical preservatives like benzoic acid, boric acid, and their sodium salts for use on meats consigned overseas. It appears that benzoic acid, sodium benzoate and borax are acceptable preservatives and are used. According to Folin (7, pp. 32-42) benzoic acid is not generally considered poisonous as it is eliminated from the body as hippuric acid. However, sodium benzoate is not satisfactory from many points of view. The chief objection according to Rann (29) is its weak preservative effect in neutral or slightly acid food materials.

5- The quinones (24, 26). The quinones and naphthoquinones offer greater challenge to the investigator of food preservatives. There are many naturally occuring quinones and naphthoquinones. Among these are fumagatin, spinulusion, citrinin, actinomycin and puberulic acid. Some

of these are definitely classified as antibiotics (24). Geiger (8) found that the nitrogen analogies of the quinones and hydroquinones retain the physiological activity of the quinones themselves. Wooley (42) reported that 2-methyl-1, 4-naphthoquinone (synthetic vitamin K) acts to negate the antibacteriostatic and antifungistatic action of 2, 3-dichloro-1, 4-naphthoquinone.

6- The miscellaneous compounds. These are represented by a well known food preservative group, the sulfurdioxide liberating compounds such as sodium metabisulfite and sodium sulfite (28).

In order to prevent thermophilic spoilage of canned ground meats containing contaminated soybean flour, Jensen and Hess (19) said that the meat must be cured with 3.5 lb. NaCl, 2 1/3 oz $NaNO_3$ per 100 lb. and the mixture must be retorted in the six pound can at 235°F. for 3 hours and 20 minutes.

After studying the effect of meat-curing solutions on anaerobic bacteria, Tanner and Evans (36) reported that up to 0.92 per cent sodium nitrite could not be relied on alone to prevent toxin formation by C. botulinum, or spoilage by C. putrificum, and C. sporogenes; commercial curing mixtures were very effective. Spoilage of cooked and

uncooked meats was not prevented by 3.5 per cent NaCl in combination with one to two per cent sodium nitrate or 0.1 to 0.2 per cent sodium nitrite.

Klarmann and Wright (22) found that organic matter impaired germicidal performance of disinfectants to a more or less extent. Their tests showed that the reduction in germicidal effect caused by citrated blood and serum was much greater in the case of some of the newer disinfectants such as quaternary ammonium compounds and 4-chlorophenylphenol than in the older disinfectants such as cresol and pine oil.

There are a few sporadic reports on the effectiveness of particular compounds such as proprionates in controlling microbial growth on fruits and vegetables (41). Somewhat more comprehensive studies are reported for animal products where a number of germicides were tested on a comparative basis against mixed cultures of marine flora in fish slime medium (37).

Antibiotics:

Many investigators now believe that there are more than 200 antibiotics. The current emphasis on fundamental research in antibiotics should reveal information required for determining the usefulness of these products for food preservation.

The Western Regional Research Laboratory announced recently (39) the initial use of antibiotics in food preservation. The scientists used in their experiments aureomycin, chloromycetin, lupulon and subtilin with a short cooking time of only ten minutes at 212°F. for canned vegetables. Subtilin was used mostly in these experiments. It was used successfully in extremely minute quantities, namely, concentrations of about ten parts per million based on the weight of the canned material.

Hansens (12) reported that by the dilution method, humulon and lupulon are highly bacteriostatic against gram-positive bacteria, lupulon being nearly 20 times as active as humulon. Neither substance shows activity against top or bottom yeasts, wild yeast, or gram-negative bacteria as Acetobactor contaminant of beer. The activity of lupulon diminishes rapidly with time due to its instability on standing.

A distinction is made between "absolute" and "commercial" sterility of canned meats as is done with other canned foods. "Commercial" sterility is that condition in which viable bacteria are present but they have not developed, to cause spoilage. "Absolute" sterility implies the absence of any viable bacteria. Jones (20) reported that commercially canned meats and fish upon

extended bacteriological examination always contain living organisms which do not necessarily make the food unfit for human consumption. Complete sterility of these products is not to be expected, and the safety and suitability for food must be judged from the wholesomeness.

METHODS AND MATERIALS

1. Preparation of Spores

(A) Preparation of Stock Spores of *Clostridium sporogenes* #7955:

A pure culture of *Clostridium sporogenes* was obtained from the American Type Culture Collection at Washington, D.C. (2). This organism was mentioned by Williams (40) as *C. sporogenes*, strain No. 3679, having a heat resistance somewhat in excess of the recorded maximum for *C. botulinum*. Gross *et al.* (10) used it also in their work because it has commonly been used as an inoculated-pack test organism, and its spores are reported to have resistance to high heat. It was first reactivated by three successive transfers through liver broth media containing ground liver at the bottom of the tubes and sealed with sterile wax after inoculation. These tubes were incubated at 98.6°F. (37°C.) for 72 hours between each successive transfer. The liver broth used was prepared as recommended by Tanner (35, p. 762), for the detection of putrefactive anaerobes.

After this preliminary reactivation of the test organism, stock spores were prepared in liver broth as outlined by Benjamin *et al.* (33).

Each ml. of the stock culture in liver broth contained

1,710,000 spores enumerated by plating various dilutions in thioglycollate agar, incubating the plates at 98.6°F. (37°C.) in standard pyrex desiccator jars under nearly complete vacuum, and counting the resultant colonies.

(B) Preparation of Stock Spores of Bacillus stearothermophilus #7953:

A pure culture of Bacillus stearothermophilus was also obtained from the American Type Collection at Washington, D.C. (2). This culture was reactivated by three successive transfers on nutrient agar slopes. The tubes were incubated at 131°F. (55°C.) for 48 hours between each successive transfer.

After this reactivation, transfers of the pure culture were made onto nutrient agar in 12 Koole flasks. These flasks were then incubated for 72 hours at 131°F. (55°C.), after which the spores were collected as follows:

(1) The 12 Koole flasks were removed from the incubator and the growth on the surface of the agar was collected by first loosening with a sterile needle and then washing with sterile distilled water.

(2) This suspension of the microorganisms (both vegetative and spores) was filtered through sterile cheese cloth to remove any agar particles.

(3) It was then centrifuged in sterile cups for 30 minutes at a speed of 1000 r.p.m.

(4) The clear supernatant fluid was poured off and the cups were refilled with sterile 0.6 per cent K_2HPO_4 and the spores resuspended in this buffer solution by vigorous shaking.

(5) This was centrifuged at 1000 r.p.m. for 30 minutes.

(6) This washing procedure was repeated once again.

(7) Then the washed microorganisms were filtered through a bacterial filter.

(8) The bacterial filter was dried under vacuum at 104°F. (40°C.) for 24 hours. This undoubtedly killed the vegetative forms and left the spores dried on the filter.

(9) The spores were scraped from the filter with a sterile knife into a sterile mortar.

(10) The spores were ground and mixed with dry sterile lactose.

(11) This dry mixture of spores in lactose was packed in a sterile glass weighing bottle and kept in a desiccator for future use.

(12) The number of spores per gram of this dry material was determined as follows:

Ten mg. of the stock spores were suspended in 100 ml. of sterile water for one hour. This

short period is not sufficient to allow sporulation. After this soaking, various dilutions were plated in triplicate. The highest dilution, 1 to 1,000,000, was found satisfactory for counting after incubation at 131°F. (55°C.) for 24 hours. The average number of colonies for the three plates was 202.3 or 202,300,000 spores per gram of the prepared stock spores in lactose.

2. An Attempt to Prepare Sterile Fresh Meat for Experimental Work

The outside surface of a four-pound cut of beef rump was trimmed with a sterile knife to remove surface contamination. This meat was ground once through an Enterprise machine (coarse grind), and then reground with a Wiley machine to produce a fine meat paste. The two machines were first washed thoroughly with soap and water, then rinsed with 95 per cent alcohol and allowed to air dry before use.

Sixteen clean "thermal death time" cans, hereafter designated as T.D.T. cans, (two for each treatment) were filled with the meat paste (20 grams each) and then sealed under 25 inches vacuum. The cans were divided into two lots of eight cans each and incubated for six days at

98.6°F. (37°C.) and 131°F. (55°C.) respectively. The results are tabulated in Table I.

Table I

Suitability of
Slightly Processed Meat as an Experimental Medium

			Incubated (37°C.)				Incubated (55°C.)
Exp. No.	Process. Time 212°F.	Cans Swelled	Count Anaerobes	Exp. No.	Process. Time 212°F.	Cans Swelled	Count Aerobes
1	5 min.	1	500,000/gm.	1'	5 min.	None	20,000/gm.
2	10 min.	2	*	2'	10 min.	None	———
3	20 min.	2	*	3'	20 min.	None	———
4	30 min.	1	376,650/gm.	4'	30 min.	None	Negative

* No counts made since cans were discarded after swelling.

From these preliminary results it may be concluded that this sample of meat was highly contaminated as indicated by bacterial counts and the swelling of the cans due to gasses produced by bacteriological action. It may be impossible to prepare sterile fresh meat for experimental work.

To verify the preliminary results of the first series of experiments the following six experiments were carried out.

About 500 grams were left over from the previous experiments. This sample was placed in a #2 tall tin can, sealed, sharp frozen at -20°F. (-28.9°C.) for 24 hours,

then stored at 0°F. (-17.5°C.) for five days.

The frozen meat was thawed at 32°F. (0°C.) for 24 hours and samples placed in 24 T.D.T. cans (20 grams each) and sealed at 25 inches vacuum. The cans were divided into two lots of 12 cans each and incubated for six days at 98.6°F. (37°C.) and 131°F. (55°C.) respectively. The results in Table II verified those of Table I.

Table II

Verification of Table I

		Incubated (37°C.)				Incubated (55°C.)	
Exp. No.	Process. Time 212°F.	Cans Swelled	Count Anaerobes	Exp. No.	Process. Time 212°F.	Cans Swelled	Count Aerobes
5	5 min.	2	*	5'	5 min.	None	———
6	10 min.	2	*	6'	10 min.	None	———
7	15 min.	2	*	7'	15 min.	None	———
8	20 min.	2	*	8'	20 min.	None	———
9	30 min.	2	*	9'	30 min.	None	———
10	None	1	33,000/gm.	10'	None	None	Negative

* No counts made since cans were discarded after swelling.

To arrive at a conclusion of the possibility of preparing fresh sterile meat for experimentation, the following experiments were done:

A five-pound cut of beef was used. The outside surface was removed by a sterile knife under aseptic conditions. The trimmed meat was then ground in the

Enterprise machine which had been first sterilized at 248°F. (120°C.) for 30 minutes. This (coarsely ground) meat was used in experiments 11 and 12 without further grinding. For further experiments the meat was reground in the Wiley machine (which was only washed with alcohol) to a fine meat paste. The empty cans were autoclaved at 248°F. (120°C.) for 20 minutes before use. The lids were washed well with soap and water and then rinsed with alcohol. Sterilizing the lids at high temperature was not possible as the rubber gaskets were affected by the heat.

The remainder of the meat and the trimmings were mixed together and passed through the Wiley machine. Four #2 short cans were filled with this meat and used in experiment No. 20.

All experiments, 11-20, were divided into two groups and incubated at 98.6°F. (37°C.) and 131°F. (55°C.) respectively for six days. The results are summarized in Table III.

Table III

Further Tests of Suitability of Slightly Processed Meat
(All Experiments Were Run in Duplicate Except No. 17 Which Was In Quadruplicate)

Exp. No.	Process. Time 212°F.	Incubated at 37°C. Cans Swelled	Plate Count Aerobes	Plate Count Anaerobes	Exp. No.	Process. Time 212°F.	Incubated at 55°C. Cans Swelled	Plate Count Aerobes	Plate Count Anaerobes
11	20 min.	2	*	*	11'	20 min.	None	———	———
12	None	1	Negative	50,000/gm	12'	None	None	Negative	Negative
13	5 min.	2	*	*	13'	5 min.	None	———	———
14	10 min.	2	*	*	14'	10 min.	None	———	———
15	20 min.	2	*	*	15'	20 min.	None	———	———
16	30 min.	2	*	*	16'	30 min.	None	———	———
17	None	3	Negative	95,000/gm	17'	None	None	Negative	Negative
18	15 min. 250°F.	None	Negative	Negative	18'	15 min. 250°F.	None	Negative	Negative
19	45 min. 250°F.	None	Negative	Negative	19'	45 min. 250°F.	None	Negative	Negative
20	90 min. 250°F.	None	Negative	Negative	20'	90 min. 250°F.	None	Negative	Negative

* No counts made since cans were discarded after swelling

From the results as shown in the preceding three tables, the conclusion was drawn that it was not possible to prepare sterile fresh meat. It was further decided to use for future work sterile meat processed according to the recommendations of the American Can Company (1, p.550). For #2 tall cans, which we used for our stock meat, 90 minutes at 250°F. (121°C.)(15# pressure) were used. The cans were then cooled and stored at 32°F. (0°C.) for stock use.

3. Effect of Process on Spore-Inoculated, Cooked, Sterile Meat Paste in T.D.T. Cans

The cooked, sterile meat paste which was used in the following experiments, 21, 22, and 23, was from experiment 20. Both organisms, Clostridium sporogenes #7955 and Bacillus stearothermophilus #7953, were added to the meat paste at the level of 10,000 spores per each gram of meat. A summary of these experiments is tabulated in Table IV. When the five cans which were not inoculated were incubated at 131°F. (55°C.), all of them showed no swelling. Plating and incubating them at 131°F. (55°C.) resulted in no growth.

Table IV

Effect of Process on Spore-Inoculated Meat Paste

Exp. No.		Organism Used	No. of Cans	Treatment	Cans Swelled 37°C.	Plating No. of Organisms/gms. 37°C.
21	I	No organism	4	No heat	Swelled	———
21	II	No organism	2	10 min. 212°F.	None	Negative
21	III	No organism	2	15 min. 250°F.	None	Negative
21	IV	No organism	2	45 min. 250°F.	None	Negative
22	I	C. sporogenes	4	No heat	3	2,100,000
22	II	C. sporogenes	2	10 min. 212°F.	1	5,500,000
22	III	C. sporogenes	2	15 min. 250°F.	None	Negative
22	IV	C. sporogenes	2	45 min. 250°F.	None	Negative
23	I	*	4	No heat	None	Negative
23	II	*	2	10 min. 212°F.	None	Negative
23	III	*	2	15 min. 250°F.	None	Negative
23	IV	*	2	45 min. 250°F.	None	Negative

* B. stearothermophilus

These results showed that:

1. Transfer of the sterile, cooked meat to the T.D.T. cans contaminated the meat.
2. Ten minutes at 212°F. (100°C.) proved to be sufficient to get rid of the contamination, if any, during transfer to the T.D.T. cans.
3. The results of the C. sporogenes inoculations showed that the meat medium was suitable for their growth and verified the fact that the culture was still active.
4. On the other hand, the results of the B. stearothermophilus platings were quite unexpected, especially of experiments 23, I and 23, II. They were expected to show high growth.
5. All the cans heated 15 minutes or longer at 250°F. (121°C.) showed no growth.

To find out the cause of the unexpected results of experiments 23, I and 23, II, the vitality of the spores of B. stearothermophilus were checked as a first step. The actual count of the stock spores in dry form with the lactose was repeated. The results showed that the spores were active. The count was nearly the same as originally found: 202,500,000 spores per gram of the dry stock spores.

The next step was to find out if vacuum affected the

spores. The stock spores were suspended in water for only five minutes (instead of one hour) to be sure that the spores would not vegetate, or otherwise we would be dealing with vegetative forms which could be easily destroyed by heat and possibly by vacuum.

Two different series of experiments were done, one without adding any organism, and the other by adding B. stearothermophilus at the rate of 10,000 spores per gram of meat. All cans were incubated at 131°F. (55°C.). The summary of these experiments and their results are found in Table V.

Table V

Test of the Effect of 25" of Vacuum on the Spores of Bacillus stearothermophilus

(Two cans each)

Exp. No.		Micro-organism	Vacuum	Treat-ment	Swelling	Plating No. per Gram Meat for Aerobes
24	Ia	None	Vacuum	No heat	No swelling	Negative
24	Ib	None	None	No heat	No swelling	Negative
24	IIa	None	Vacuum	10 min. 212°F.	No swelling	Negative
24	IIb	None	None	10 min. 212°F.	No swelling	Negative
24	IIIa	None	Vacuum	15 min. 250°F.	No swelling	Negative
24	IIIb	None	None	15 min. 250°F.	No swelling	Negative
25	Ia	*	Vacuum	No heat	No swelling	100
25	Ib	*	None	No heat	No swelling	150
25	IIa	*	Vacuum	10 min. 212°F.	No swelling	100
25	IIb	*	None	10 min. 212°F.	No swelling	200
25	IIIa	*	Vacuum	15 min. 250°F.	No swelling	Negative
25	IIIb	*	None	15 min. 250°F.	No swelling	Negative

* B. stearothermophilus

The results of this series of experiments were not conclusive as the number of bacteria started with, namely ten thousand per gram, were not recoverable.

T.D.T. cans with and without vacuum showed growth, but it was less than 5-10 per cent of what we started with. The growth was weak, and the colonies were very small on the nutrient agar media even after 48 hours incubation at 55°C.

Experiment 24, to which no microorganisms were added, clearly proved that there is no aerobic contamination to the canned meat upon incubation at 55°C.

(A) Test of the Effect of Vacuum on the Spores of Bacillus stearothermophilus in Meat Medium and in Nutrient Broth Medium:

To reach conclusive results about the growth of B. stearothermophilus, the following two series of experiments were done. The sterile cooked meat medium and sterile nutrient broth medium were used. B. stearothermophilus spores were added to both of them at the rate of 10,000 spores per gram of meat or per ml. of nutrient broth. The cans were sealed under vacuum, without vacuum, and not sealed (first roll only). All cans were incubated at 55°C. for three days.

The results of these two series of experiments are summarized in Table VI. Two cans were used in each part

of each experiment without any process heating.

Table VI

The Effect of Vacuum
on the Spores of Bacillus stearothermophilus
in Meat Medium and in Nutrient Broth Medium

Exp. No.		Medium	Vacuum	Swelling	Plating No. of Organism/gm. or 1 ml.
25	Ia	Meat Paste	Vacuum	None	70
25	Ib	Meat Paste	None	None	90
25	Ic	Meat Paste	Not sealed	None	100
26	Ia	Nutrient Broth	Vacuum	None	170
26	Ib	Nutrient Broth	None	None	280
26	Ic	Nutrient Broth	Not sealed	None	17,500

From these results it may be stated:

1. Vacuum (25 inches) in T.D.T. sealed cans had no effect on the microorganisms either in the meat paste or in nutrient broth.
2. The count was almost triple in nutrient broth as compared to that in the meat. In either case it was less than two per cent of the original count.
3. The unsealed cans containing meat paste showed a little higher count but still only a percent of the original count of the stock spores. On the other hand, the unsealed cans with nutrient broth

gave a high count, almost doubling the original count.

4. These experiments indicate that this particular microorganism requires access to free oxygen to continue its growth in the nutrient broth. It is possible that the spores were killed after they started to vegetate and did not find enough oxygen in the sealed T.D.T. cans either under Vacuum or without vacuum.
5. The meat medium could possibly be unsuitable for this microorganism to survive.

To confirm the results of experiments 25, Ia and 25, Ib of the unsuitability of the meat media for this microorganism to survive, the following two experiments were carried out. Five T.D.T. cans were canned in each experiment, one with vacuum and the other without vacuum. Both were inoculated at the rate of 10,000 spores per gram of meat and were not heated after canning. One can of each experiment was plated out after zero hour, 24 hours, and 48 hours from the canning time. The summary of these two experiments is tabulated in Table VII.

Table VII

Test of the Effect of Meat Medium on the Growth of the Spores of Bacillus stearothermophilus With and Without Vacuum

Exp. No.	Vacuum	Count per Gram of Meat Zero Hour	24 Hours	48 Hours	Remarks
27	Vacuum	1350	190	80	2 cans left did not swell
28	None	1680	240	100	2 cans left did not swell

Although the original inoculation was not recovered after zero hour in plating, it is clear from the 24 hour count (in both experiments) that the number of micro-organisms per gram of meat decreased about 85 per cent of the zero hour count. Furthermore, there was another decrease of about 60 per cent on the 48 hour count from that of the 24 hour count in both experiments. These results confirmed the results of experiments 25, Ia and 25, Ib that meat is not a suitable medium for this micro-organism to grow in and the vacuum has no effect on growth.

(B) Control Experiment: Effect of Absence of Meat From Standard Preparation Procedure Upon Recovery of the Bacillus stearothermophilus:

Since it was suggested that the presence of meat may

be the cause of the low recovery of the inoculated microorganism, the following experiment was carried out.

Ten milligrams of the dry stock spores of Bacillus stearothermophilus were suspended in 25 milliliters of sterile water and mixed for five minutes with glass beads.

Then the 25 ml. of the mixed water and spores were transferred to 175 ml. of water, instead of 175 grams of meat as done previously, to make a total of 200 ml. of 10,000 spores of the mieroorganism per ml.

Twenty ml. of this mixture (to represent 20 grams meat) were transferred to a sterile Waring blender, made up to 100 ml. with sterile water, and blended for three minutes exactly. The mixture was then filtered through sterile filter paper and plated in the following dilutions:

1. One ml. of the filtrate was transferred into each of three petri dishes. This dulution represents 1:5 dilution.
2. Then 0.1 ml. of the filtrate was also plated in triplicate representing 1:50 dilution.
3. One ml. of the filtrate was diluted to 100 ml., then 1 ml. of this dilution was plated out in triplicate representing 1:500 dilution. All the plates were incubated at 131°F. (55°C.) and after 48 hours were counted. The results are shown in

Table VIII.

Table VIII

Effect of Absence of Meat

Exp. No.	Dilution	Cal. Inocu-lation	Plate Count per Petri Dish			Average	No. of Bacteria per ml. Calculated
29 a	1:5	2000	X	X	X	X	X
29 b	1:50	200	222	186	194	201	10,050
29 c	1:500	20	15	22	18	18	9,000

X Heavy growth, could not be counted.

From these experiments it seemed evident that the spores of B. stearothermophilus were viable and it was able to recover them quantitatively by using the standard experimental procedure without the meat medium.

It was clear that the meat medium using this technique was not suitable for the growth of these spores.

On the other hand, this technique was evidently suitable for the tests carried on with C. sporogenes. Therefore, all subsequent experiments were limited to this test microorganism.

The thermal death time of the stock spores of C. sporogenes was determined to be seven hours and fifteen minutes at 212°F. (100°C.) in phosphate buffer solution of pH 7.00 containing 10,000 spores of the microorganism per ml.

CHEMICALS TESTED

1. List of Chemicals Tested.

The effectiveness of the chemicals to be used in attempting to reduce the time or temperature of processing was first estimated on an artificial medium. From this screening procedure the most promising compounds were tested in a meat medium in T.D.T. cans (see Section 3).

The chemicals tested were chosen on the basis of their availability, literature reports, and manufacturer's claims. It is realized that the list of potentially effective chemicals is unlimited, but for this study a sizable group of approximately 50 was taken with representatives in several classes as shown below.

1. Phenol Derivatives:
    1. Dowicide A: Orthophenylphenol, sodium salt.
    2. Dowicide B: 2,4,5-Trichlorophenol, sodium salt.
    3. Dowicide C: Chloro-2-Phenylphenol, sodium salt.
    4. Dowicide 1: Orthophenlphenol.
    5. Dowicide 2: 2,4,5-Trichlorophenol.
    6. Santophen 20: Pentachlorophenol.
    7. Pentadecadienylphenol.

8. Pentadecadienyl-anisol.

II. Quaternary Ammonium Compounds:

1. Hyamine No. 1622: Diisobutyl-phenoxy-ethoxy-ethyl-dimethyl benzyl ammonium chloride.
2. Hyamine No. 10: Diisobutyl-cresoxy-ethoxy-ethyl-dimethyl benzyl ammonium chloride.
3. Roccal: Alkyl-dimethyl-benzyl ammonium chloride.
4. Phemerol "crystals": Para-tetra-octyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl ammonium chloride.
5. Onyxide: Alkyl-dimethyl-benzyl ammonium bromide.
6. Cetab: Cetyl-trimethyl ammonium bromide.

III. Aliphatic Acids and Their Salts:

1. Mycoban: calcium propionate.
2. Dehydroacetic acid.
3. Sodium salt of dehydroacetic acid.
4. Iodoacetic acid.
5. Silver lactate.
6. Beta-Iodopropionic acid.
7. Malonic acid.
8. l-Malic acid.
9. Fumaric acid.
10. Sulfamic acid.

IV. Benzoic Acid Derivatives:

1. Sodium benzoate.
2. 2,3,5-Triiodobenzoic acid.
3. 2-Chloro-4-amine-benzoic acid.
4. Ethyl-p-propylamino-benzoate.
5. Methyl-p-propylamino-benzoate.

V. Quinones:

1. 2-Methyl-1, 4-napthohydroquinone, 3-sodium sulfonate.
2. Phygon wettable: 2,3-Dichloro-1, 4-napthoquinone.
3. Hydroxyphygon.
4. Phenanthroquinone.
5. Para-xyloquinone (Eastman kodak).

VI. Miscellaneous:

1. Sodium sulfite.
2. Sodium meta bisulfite.
3. Triethylamine.
4. Eswel (50% sodium sulfite and 50% sodium benzoate).
5. Thiourea.
6. Sodium fumarate.
7. Resorcinol.
8. Hexyl resorcinol.

9. K-5807 (The Dow Chemical Company, Midland, Michigan).
10. Uracil (Nutritional Biochemicals Inc.).
11. 2,4-Dimitrol-naphthol-7-sulfonic acid.
12. Methyl decab.
13. Compound G 4: 2,2'-Dihydroxy,5,5'-dichloro-diphenylmethane.
14. Compound G 11: 2,2'-Dihydroxy-3,5,6-3',5', 6'-hexachloro-diphenylmethane.
15. Para-Hydroxybenzophenone.
16. Butyl ester of Dl-tyrosine.
17. Synkamin HCl: 2-Methyl-4-amino-1-napthol-hydrochloride.
18. Triethyl ammonium bromide.
19. Polyethylene glycol 300.

2. Preliminary Estimate of the Effectiveness of Various Compounds on Clostridium sporogenes #7955 on an Artificial Medium

This test is a modification of the Lamanna and Shapiro Method (23). After the preparation of the sterile thioglycollate medium the test was run as follows:

1. The medium was inoculated with the spores of C. sporogenes #7955 at the rate of 10,000 spores per ml. after it had been cooled to about 113°F. (45°C.).

2. About 10 ml. of the inoculated medium were poured into each petri dish and left to solidify.
3. Each chemical was tested at four levels of concentration: 0.1 per cent, 0.05 per cent, 0.01 per cent and 0.005 per cent.
5. When the medium solidified, two discs (6 mm. diameter) of No. 7 Whatman filter paper were dipped in each concentration of the tested chemical and then placed on the thioglycollate medium surface. Another two discs were used as a control and were dipped in the solvent. Plate I illustrates how the discs were distributed on the medium surface and the effect of four chemicals.
5. The plates were incubated under vacuum of about 28 inches at 98.6°F. (37°C.) for three days.
6. The effectiveness of the chemical is reported in mm. of the diameter of the inhibition zone.

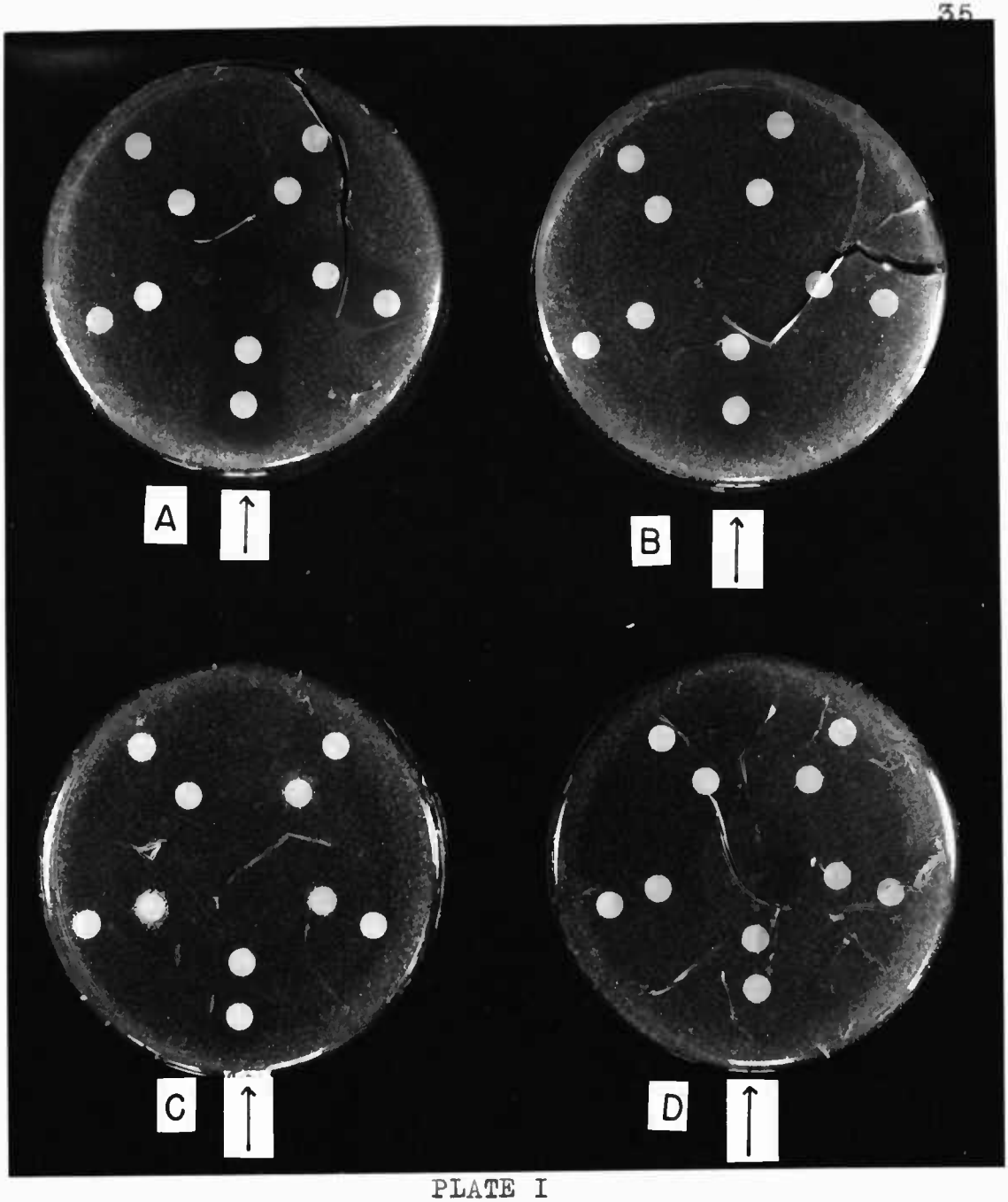


PLATE I

Preliminary Estimate of the Effectiveness of 4 Chemical Compounds on C. sporogenes #7955 on an Artificial Medium.

A. Hamine 1622.
B. Sodium meta-bisulfite.
C. Sodium benzoate.
D. Iodoacetic acid.

The concentrations of the chemicals beginning at six o'clock and proceeding counter clockwise are:

0.10%, 0.05%, 0.01%, 0.005%, and 0.00%.

Results:

1. Chemicals that showed no inhibition of growth:
   A. Water soluble:
      1. Iodoacetic acid.
      2. Malonic acid.
      3. l-Malic acid.
      4. K-5807.
      5. Fumaric acid.
      6. Sulfonic acid.
      7. Uracil.
      8. 2,4-Dinitro-1-naphthol-7-sulfonic acid.
   B. Soluble in 95 per cent alcohol, water insoluble:
      1. Dowicide 2.
      2. 2-Chloro-4-amine-benzoic acid.
      3. Methyl-p-propylamino benzoate.
      4. Para-xyloquinone.

II. Chemicals that showed inhibition of growth are tabulated in Table IX according to their inhibition action.

Table IX

Inhibition of Growth
Caused by 39 Chemicals on an Artificial Medium

No.	Chemical	Zone Inhibition Diameter in mm. at % Concentrations. 0.1	0.05	0.01	0.005
A-	Water Soluble				
1	Hyamine 1622	17	13	10	8
2	Roccal	10	9	8	7
3	Hyamine 10x	10	8	7	6
4	2-Methyl-1, 4-napthohydro-quinone, 3-Sodium sulfonate	9	8	7	7
5	Phemerol crystals	9	8	7	6
6	Sodium benzoate	8	7	7	6
7	Sodium meta-bisulfite	8	7	6	6
8	Thiourea	8	7	6	6
9	Onyxide	8	7	6	6
10	Sodium fumarate	7	6	6	6
11	Mycoban (calcium propionate)	7	6	6	6
12	Cetab	7	6	6	6
13	Beta-2-Iodopropionic acid	7	6	6	6
14	Resorcinol	7	6	6	6
15	Sodium sulfite	7	6	6	6
16	Eswel	7	6	6	6
17	Dowicide A	7	6	6	x
18	Dowicide B	7	6	6	x

Table IX (continued)

No.	Chemical	Zone Inhibition Diameter in mm. at % Concentrations. 0.1	0.05	0.01	0.005
19	Dowicide C.	7	6	6	x
20	Methyl decab.	7	6	6	x
21	Hexyl resorcinol	7	6	x	x
22	Silver lactate	6	x	x	x
B-	Soluble 95% Alcohol				
1	Triethyl ammonium bromide	9	8	7	6
2	Polyethylene glycol 300	8	7	7	6
3	Santophen 20	8	7	6	6
4	Pentadecadienyl phenol	8	7	6	6
5	Phygon wettable	8	7	x	x
6	Synkamin HCL	7	7	6	6
7	Butyl ester of Dl-tyrosine	7	6	6	6
8	2,3,5-triiodobenzoic acid	6	6	6	6
9	Triethylamine	6	6	6	x
10	Compound G 4	6	6	x	x
11	Compound G 11	6	6	x	x
12	Dowicide I	6	6	x	x
13	Ethyl-p-propylamine benzoate	6	6	x	x
14	Hydroxyphygon	6	6	x	x
15	Phenanthroquinone	6	x	x	x

Table IX (continued)

No.	Chemical	Zone Inhibition Diameter in mm. at % Concentrations. 0.1	0.5	0.01	0.005
16	Para-hydroxybenzophenone	6	x	x	x
17	Pentadecadienyl anisol	6	x	x	x

x No inhibition zone at all.

3. General Procedure for the Estimation of the Effectiveness of the Most Promising Chemicals on Clostridium sporogenes #7955 with Sterile Ground Beef Media in T.D.T. Cans

The following routine procedure was established to determine the effect of about 37 different chemicals on a sterile beef medium. These chemicals were chosen on the basis of their inhibitory action against the test microorganism grown on artificial media.

1. Four chemicals were tested at a time by using the sterile canned meat prepared previously for this purpose, placed in #2 tall cans, processed at 250°F. (121°C.)for 90 minutes (American Can Company recommendations)(1, p. 550), and held in storage at 32°F. (0°C.).
2. Each #2 tall can contained 500 grams of meat. Therefore, each can contained sufficient meat to fill 24 T.D.T. cans with 20 grams each. That is five T.D.T. cans for each of the four chemicals,

totaling 20 T.D.T. cans. The remaining four T.D.T. cans were used as controls- two T.D.T. cans of sterile meat alone and two T.D.T. cans inoculated with the test organism without the addition of any chemical.

3. The unopened #2 tall can of meat was heated in a water bath at 158°F. (70°C.) for 15 minutes, then it was opened under sterile conditions. The entire content was emptied into the sterilized bowl of a heavy duty food mixer, covered with a sterile towel, and stirred for 15 minutes at moderate speed.
4. After this preliminary mixing, two 20-gram portions of meat were removed with a sterile spoon and weighed into the two T.D.T. control cans.
5. The rest of the meat (i.e., 460 grams) was inoculated with the spores of the test microorganisms at the rate of 10,000 spores per gram of meat. The meat was stirred again mechanically for five minutes to insure uniform distribution of the spores throughout.
6. Now two T.D.T. cans were filled with 20 grams each of the inoculated meat to constitute the second control.

7. An aliquot of 100 grams of the inoculated meat was transferred to a sterile mortar and pestle to facilitate mixing with the chemical under test. As the chemicals were prepared in one per cent solutions, it was found convenient to add this solution at the rate of two ml. per 18 grams of meat. The chemical was mixed in thoroughly with the pestle.
8. The five T.D.T. cans were filled with 20 grams each of this preparation.
9. This procedure was followed also for the remaining three chemicals.
10. All cans were sealed under 25 inches of vacuum.
11. Then the cans were cooked in boiling water (approximately 212°F.)(100°C.) for 10 minutes, after which they were immediately cooled in cold running water.
12. All cans were incubated for 72 hours at 98.6°F. (37°C.).
13. Then three of the five cans from each of the four chemicals tested (12 cans) were sampled as follows:
    a. Each T.D.T. can was opened under sterile conditions as recommended by E.J. Cameron (3).

b. Then the contents were transferred to a sterile Waring blender and the can and its cover rinsed with 80 ml. of sterile distilled water to increase weight to 100 grams or 1:5 dilution.

c. This material was blended for exactly three minutes.

d. Then the blended material was filtered through sterile folded filter paper No. 193 of the Eaton-Dikeman Company and the filtrate collected during approximately a 15-minute period of time.

e. From each filtrate collected the following plating procedure was carried out:

(1) Into each of two sterile petri dishes was pipetted 0.15 ml. of the filtrate. This dilution was arrived at by presupposing that the chemical would have very little inhibiting effect on the spores. As each T.D.T. can contained 18 grams of meat (10,000 spores/gram), the can would contain 180,000 spores. The filtrate contained a 1:5 dilution of the can contents or 1,800 spores per ml. of filtrate. Therefore, 0.15

ml. of the filtrate would contain 270 spores, which was considered an optinum number for counting. If the count after 72 hours of incubation under anaerobic conditions at 98.6°F. (37°C.) is 270 or less, then one may conclude that the chemical has an inhibiting effect on the spores and warrants further experimental work. However, if the count exceeds 270 colonies per plate, then one may rightly conclude that the chemical has very little or no effect on the spores, and work with this chemical should be discontinued.

(2) Also at this time a second dilution was made by pipetting 0.5 ml. of the filtrate into each of two sterile petri dishes. This was intended to give a more accurate count for those chemicals showing decided germicidal effects.

f. Three sets of controls were plated with each experiment:

(1) Each of the two T.D.T. cans of sterile meat was treated as described for the cans containing the chemicals except

that lower dilutions were made. Into each of two dishes was pipetted 0.5 ml. of the filtrate and into each of two more was pipetted one ml. of the filtrate.

(2) Each of the two T.D.T. cans containing the inoculated meat without the chemical was treated exactly as the cans containing the added chemical.

(3) Duplicate plates of the medium alone were also poured to act as a partial check on sterile procedure.

g. All dishes were incubated in desiccators under 29 inches of vacuum at 98.6°F. (37°C.) for 72 hours.

Cans #4 and #5 of each treatment which did not swell were also plated out as outlined above after 30 days and 6 months incubation respectively at 98.6°F. (37°C.)

The results are summarized in Table X. They showed that sodium meta-bisulfite and sodium sulfite were the only water soluble compounds effective in reducing canning time and temperature.

Some alcohol soluble compounds which were effective are 2,3,5-triiodo-benzoic acid, synkamin hydrochloride, hydroxyphygon and dehydroacetic acid.

Table X

Effect of Chemicals Upon The Spores of *Clostridium sporogenes* #7955 in Canned Meat

Exp. No.	Chemical	Conc. of Chem. (% can contents)	Bacterial Count No's./gm. meat (Initial 9,000 spores/gm.) 72 hrs. Inc. at 37°C. (Ave. cans) 1,2,3	% Reduction in Count	30 days Inc. at 37°C. (Can 4)	% Reduction in Count	6 months Inc. at 37°C. (Can 5)	% Reduction in Count
32	Sodium benzoate	0.1 (1)	13,700	growth	swelled	growth	swelled	growth
33	Hyamine 1622	0.1 (1)	*	growth	swelled	growth	swelled	growth
34	Hexyl Resorcinol	0.1 (1)	*	growth	swelled	growth	swelled	growth
36	Thiourea	0.1 (1)	*	growth	swelled	growth	swelled	growth
37	Sodium meta-bisulfite	0.1 (1)	3,780	58.0	88	99.02	60.	99.33
38	Sodium fumarate	0.1 (1)	*	growth	swelled	growth	swelled	growth
39	Mycoban	0.1 (1)	*	growth	swelled	growth	swelled	growth
40	Cetab	0.1 (1)	13,867	growth	swelled	growth	swelled	growth

Table X (Continued)

Exp. No.	Chemical	Conc. of Chem.(% can contents)	Bacterial Count No's./gm. meat (Initial 9,000 spores/gm.) 72 hrs. Inc. at 37°C. (Ave. cans) 1,2,3	% Reduction in Count	30 days Inc. at 37°C. (Can 4)	% Reduction in Count	6 months Inc. at 37°C. (Can 5)	% Reduction in Count
41	2-Methyl-1, 4-naptho-hydro-quinone 3-sodium sulfonate	0.1 (1)	8,400	6.67	swelled	growth	swelled	growth
42	beta-Iodo-propionic acid	0.1 (1)	*	growth	swelled	growth	swelled	growth
43	Dowicide A	0.1 (1)	*	growth	swelled	growth	swelled	growth
44	Dowicide B	0.1 (1)	*	growth	swelled	growth	swelled	growth
45	Dowicide C	0.1 (1)	*	growth	swelled	growth	swelled	growth
46	Phemerol "crystals"	0.1 (1)	*	growth	swelled	growth	swelled	growth
47	Resorcinol	0.1 (1)	*	growth	swelled	growth	swelled	growth
48	Hyamine 10X	0.1 (1)	*	growth	swelled	growth	swelled	growth

Table X (Continued)

Exp. No.	Chemical	Conc. of Chem. (% can contents)	Bacterial Count No's./gm. meat (Initial 9,000 spores/gm.) 72 hrs. Inc. at 37°C. (Ave. cans) 1,2,3	% Reduction in Count	30 days Inc. at 37°C. (Can 4)	% Reduction in Count	6 months Inc. at 37°C. (Can 5)	% Reduction in Count
49	Sodium sulfite	0.1 (1)	820	90.89	415	95.39	swelled	growth
50	Esvel	0.1 (1)	1,710	81.00	10,450	growth	swelled	growth
51	Methyl decab	0.1 (1)	2,370	73.67	swelled	growth	swelled	growth
52	Ethyl alcohol	9.5	7,033	21.86	3,980	55.78	2,117	76.48
53	2,3,5-Triiodo-benzoic acid	0.1 (2)	2,120	76.44	1,470	83.67	430	95.22
54	Santophen 20	0.1 (2)	7,067	21.48	3,690	59.00	1,327	85.26
55	Butyl ester of D-tyrosine	0.1 (2)	7,677	15.70	16,000	growth	5,300	41.11
56	Ethyl alcohol	4.75	8,567	4.81	4,940	45.11	3,933	56.30
57	Synkamin HCl	0.1 (3)	8,266	8.16	700	92.22	273	96.97
58	Pentadecadienyl-phenol	0.1 (3)	4,600	48.89	4,200	53.33	2,667	70.37

Table X (Continued)

Exp. No.	Chemical	Conc. of Chem.(% can contents)	Bacterial Count No's./gm. meat (Initial 9,000 spores/gm.) 72 hrs. Inc. at 37°C. (Ave. cans) 1,2,3	% Reduction in Count	30 days Inc. at 37°C. (Can 4)	% Reduction in Count	6 months Inc. at 37°C. (Can 5)	% Reduction in Count
59	Trimethyl ammonium bromide	0.1 (3)	8,300	7.78	4,480	50.22	1,633	81.86
60	Polyethylene glycol 300	0.1 (2)	4,870	45.89	6,190	31.22	3,600	60.00
61	Triethyl-amine	0.1 (2)	4,490	50.11	7,400	17.78	4,967	44.81
62	Phygon wettable	0.1 (2)	1,840	79.56	4,440	50.67	1,600	82.22
63	Compound G4	0.1 (2)	8,633	4.08	7,870	12.56	4,600	48.89
64	Compound G11	0.1 (3)	9,300	growth	3,830	57.44	2,300	74.44
65	Dowicide I	0.1 (3)	4,740	47.33	2,790	69.00	1,013	88.74
66	Ethyl-p-propylamino benzoate	0.1 (3)	8,800	2.22	3,750	58.33	1,767	80.34
67	Hydroxyphygon	0.1 (3)	5,700	36.67	690	92.33	337	96.26
68	Ethyl alcohol	2,357	*	growth	swelled	growth	swelled	growth

Table X (Continued)

Exp. No.	Chemical	Conc. of Chem. (% can contents)	Bacterial Count No's./gm. meat (Initial 9,000 spores/gm.) 72 hrs. Inc. at 37°C. (Ave. cans) 1,2,3	% Reduction in Count	30 days Inc. at 37°C. (Can 4)	% Reduction in Count	6 months Inc. at 37°C. (Can 5)	% Reduction in Count
69	Ethyl alcohol	1.19	*	growth	swelled	growth	swelled	growth
70	Roccal	0.1 (1)	*	growth	swelled	growth	swelled	growth
71	Onyxide	0.1 (1)	*	growth	swelled	growth	swelled	growth
72	DHA	0.1 (2)	1,790	80.11	900	90.00	400	95.56
73	DHA-S	0.1 (1)	*	growth	swelled	growth	swelled	growth

* Over 30,000
(1) The chemical was added as 2 ml. of a one per cent water solution.
(2) A one per cent solution of the chemical was made up in 95 per cent ethyl alcohol and 2 ml. of this added to 18 grams of meat.
(3) A one per cent solution of the chemical was made up in 47.5 per cent ethyl alcohol and 2 ml. of this added to 18 grams of meat.

4. Intensive Investigations of Sodium Meta-bisulfite

(A) Effect of 0.1 per cent Sodium Meta-bisulfite, Processing Time at 212°F., and Incubation Period at 98.6°F. (37°C.) on the spores of Clostridium sporogenes #7955 in Canned Meat in T.D.T. Cans

Sodium meta-bisulfite was used in these experiments at 0.1 per cent of the can contents. Four different treatments were carried out, each using 16 T.D.T. cans of inoculated cooked sterile meat, at the rate of 9,000 spores of Clostridium sporogenes #7955 per gram of meat. As a control for each treatment, six T.D.T. cans of the inoculated meat were used without adding the chemical. In canning all these treatments the outlined routine procedure given in experiment 3 was followed exactly.

Processing times were 10, 20, 40 and 60 minutes at 212°F. respectively for each treatment and its control. All cans were incubated at 98.6°F. (37°C.). The 24 control T.D.T. cans of the four treatments swelled after three days incubation. That showed that the four different processing times did not have any inhibitory effect on the spores of the microorganism.

Table XI summarizes the results of the four treatments. Each result is the average of testing two T.D.T. cans.

Graph I shows the rate of destruction curves of these four treatments.

The destruction curves did not give straight lines when the log of the number of surviving bacteria was plotted against the incubation time in months. That agrees with the results of Nunheimer and Fabian (27, p. 1045) on strains of food poisoning due to Staphylococci. Their curves did not give straight lines to show the effect of some acids, sugars, and sodium chloride on these strains when plotted as in these experiments.

Although there were still some viable bacteria left in the canned meat, the wholesomeness of the product seemed to be unimpaired. That proved that "commercial" sterility was possible by using 0.1 per cent sodium metabisulfite and reducing the processing time and temperature.

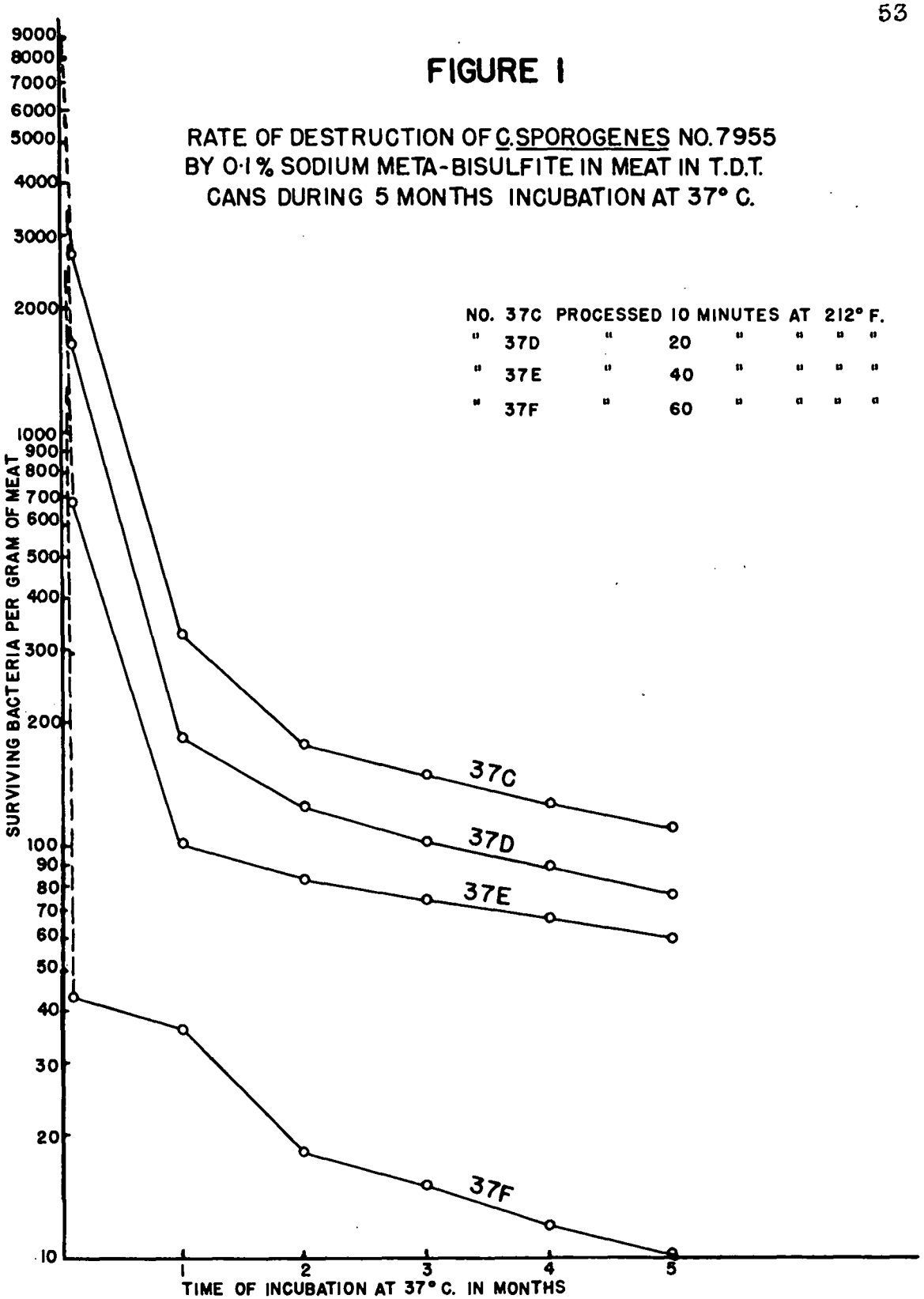
Table XI

Effect of 0.1 per cent Sodium Meta-Bisulfite, Processing Time at 212°F., and Incubation Period at 98.6°F. on the Spores of *Clostridium sporogenes* #7955 in Canned Meat in T.D.T. Cans

Exp. No.	Process. Time at 212°F.	Average count per gram of meat after incubation at 37°C.						
		Initial	3rd day	1st. mo.	2nd. mo.	3rd. mo.	4th. mo.	5th. mo.
37 C	10 min.	9,000	2,755	322	177	150	127	113
37 D	20 min.	9,000	1,063	183	127	103	90	77
37 E	40 min.	9,000	680	100	83	75	67	60
37 F	60 min.	9,000	43	36	18	15	12	10

FIGURE 1

RATE OF DESTRUCTION OF C. SPOROGENES NO. 7955 BY 0·1% SODIUM META-BISULFITE IN MEAT IN T.D.T. CANS DURING 5 MONTHS INCUBATION AT 37° C.



(B) Effect of 0.1 per cent of Sodium Meta-bisulfite and 60 minutes Processing at 212°F. in Preserving Commercial, Sanitary, Fresh Meat in T.D.T. Cans

A five and a half pound cut of beef rump was bought from a local meat market. All the lean parts of it were ground once through an Enterprise machine (coarse grind), and then reground with a Wiley machine to produce a fine meat paste. The two machines and other equipment which was used for the preparation of the meat were only washed thoroughly with soap and water, but were not sterilized. The trimmings were also ground separately in the same manner.

From the lean paste the following experiments were made:

37G- 24 T.D.T. cans with 0.1 per cent* chemical in water.

37H- 24 T.D.T. cans with 0.1 per cent* dry chemical.

Control- 24 T.D.T. cans without chemical.

With the thought that the trimmings and fat would be heavily contaminated naturally, paste of this source was treatdd separately as follows:

37I- 14 T.D.T. cans with 0.1 per cent* chemical in water.

Control- 14 T.D.T. cans without chemical.

---

*p.1 per cent of weight of can contents.

All cans were sealed under 25 inches of vacuum, processed 60 minutes at 212°F. (100°C.) and were incubated at 98.6°F. (37°C.).

The cans were carefully observed for evidences of spoilage. When any can swelled, it was discarded after being recorded.

Table XII summarizes the results of these experiments.

Table XII

Effect of 0.1 per cent of Sodium Meta-bisulfite and 60 minutes Processing at 212°F. on Preserving Commercial, Sanitary, Fresh Meat in T.D.T. Cans

Exp. No.	No. of T.D.T. cans	Chemical Added	No. of T.D.T. cans swelled during Incubation at 37°C.			
			First Month	Second Month	Third Month	Fourth Month
37 G	24	0.1% in water	None	None	None	None
37 H	24	0.1% dry chemical	None	None	None	None
Control	24	None	4	3	15	None
37 I	14	0.1% in water	None	None	None	None
Control	14	None	3	2	6	None

This shows that 0.1 per cent sodium meta-bisulfite was very satisfactory in reducing the processing time and

temperature for canning commercially sanitary fresh meat which was contaminated naturally.

5. Intensive Investigation of Sodium Sulfite

(A) Effect of 0.1 per cent of Sodium Sulfite, Processing Time at 212°F., and Incubation Period at 98.6°F. on the Spores of Clostridium sporogenes #7955 in Canned Meat in T.D.T. Cans

Sodium sulfite was used in the following experiments at 0.1 per cent. Four different treatments were also carried out, each using 14 T.D.T. cans of inoculated cooked meat, at the rate of 9,000 spores of Clostridium sporogenes #7955 per gram of meat. As a control, for each treatment, six T.D.T. cans of the inoculated meat were also used without adding the chemical. The canning and processing time were the same as that of experiment 4A. All cans were incubated at 98.6°F. (37°C.). The 24 T.D.T. cans of the controls, of the four treatments, also swelled after three days' incubation. That confirmed the previous results of experiment 4A, that the four different processing times did not have any effect on the spores of the microorganism.

Table XIII summarizes the results of the four treatments. Each result is the average of testing two T.D.T. cans.

Graph II shows the rate of destruction curves of

these four treatments.

The destruction curves did not give a straight line also when the number of surviving bacteria was plotted against the incubation time in months. That correlates with the results of experiment 4A, and those of Nunheimer and Fabian (21, p. 1045).

Table XIII

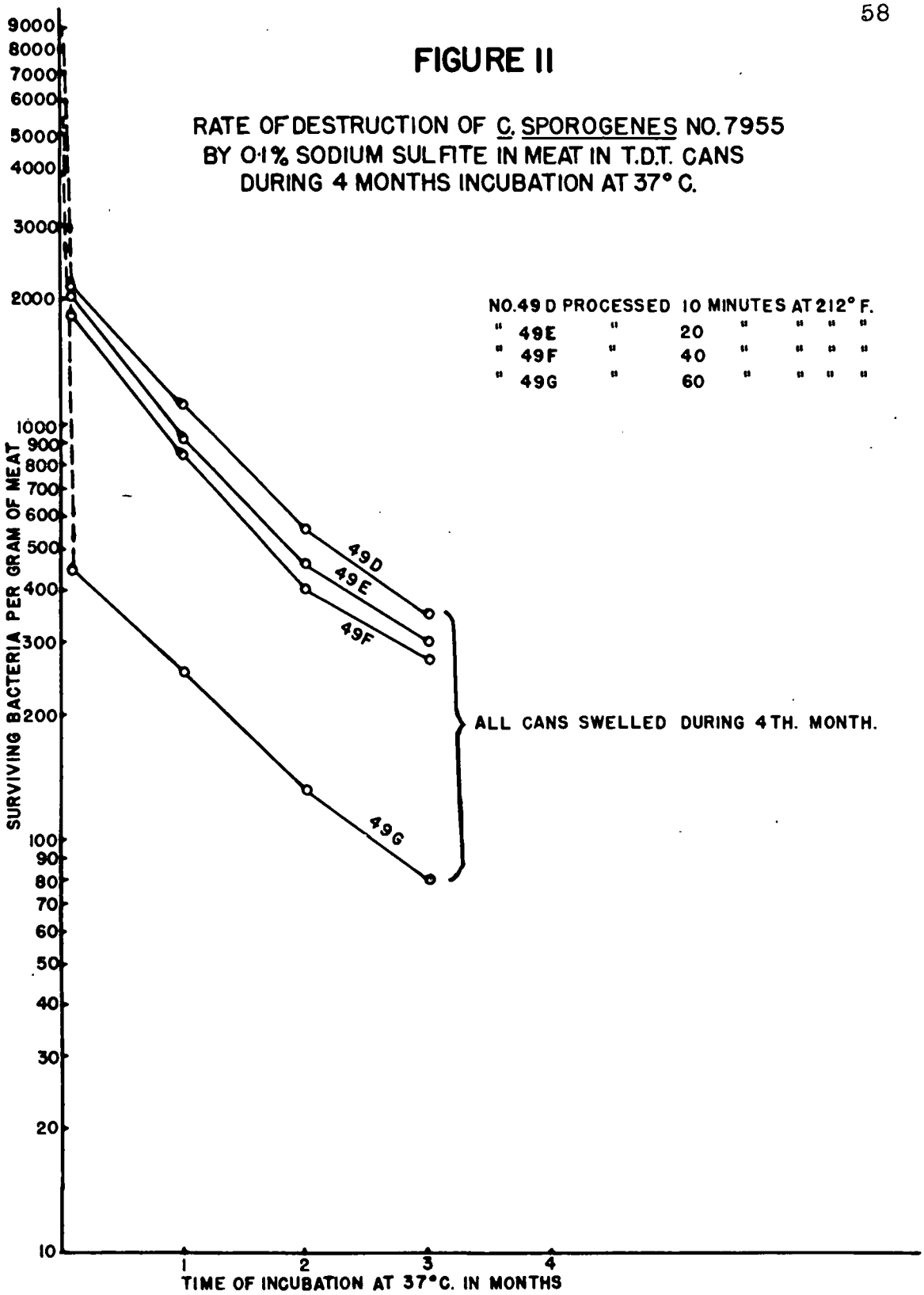
Effect of 0.1 per cent of Sodium Sulfite, Processing Time at 212°F., and Incubation Period at 98.6°F. on the Spores of *Clostridium sporogenes* #7955 in Canned Meat in T.D.T. cans

Exp. No.	Process. Time at 212°F.	Average count per gram of meat after incubation at 37°C.					
		Initial	3rd. Day	1st. Mo.	2nd. Mo.	3rd. Mo.	4th. Mo.
49 D	10 min.	9,000	2,175	1,125	550	350	x
49 E	20 min.	9,000	2,058	927	460	300	x
49 F	40 min.	9,000	1,850	854	400	270	x
49 G	60 min.	9,000	446	252	130	80	x

x All the 24 T.D.T. cans left, 6 of each experiment, swelled within 4 months' incubation and were discarded.

FIGURE II

RATE OF DESTRUCTION OF C. SPOROGENES NO. 7955 BY 0·1% SODIUM SULFITE IN MEAT IN T.D.T. CANS DURING 4 MONTHS INCUBATION AT 37° C.



The inhibiting effect of sodium sulfite on the spores was very good up to three months' incubation. During the fourth month all the 24 T.D.T. cans, six of each experiment, left under incubation swelled. That showed that the bacteriostatic action of sodium sulfite disappeared. It may be concluded that 0.1 per cent sodium sulfite is not satisfactory in reducing the processing time and temperature of canned meat.

(B) Effect of 0.1 per cent of Sodium Sulfite, and 60 minutes Processing at 212°F. on Preserving Commercial, Sanitary, Fresh Meat in T.D.T. Cans

A seven-pound cut of beef rump from a local meat market was prepared exactly as in experiment 4B.

From the lean paste the following experiments were made:

49H- 24 T.D.T. cans with 0.1 per cent* chemical in water.

49I- 24 T.D.T. cans with 0.1 per cent* dry chemical.

Control- 24 T.D.T. cans without chemical.

From the trimmings and fat paste the following experiments were made:

49J- 24 T.D.T. cans with 0.1 per cent* chemical in water.

49K- 24 T.D.T. cans with 0.1 per cent* dry chemical.

* 0.1 per cent of weight of can contents

Control- 24 T.D.T. cans without chemical.

All cans were processed 60 minutes at 212°F. (100°C.) and were incubated at 98.6°F. (37°C.)

The cans were also carefully examined for evidences of spoilage. When any can swelled, it was discarded after being recorded.

Table XIV summarizes the results of these experiments. This shows that 0.1 per cent sodium sulfite was not satisfactory in reducing the processing time and temperature for canning commercially sanitary meat which was contaminated naturally.

Table XIV

Effect of 0.1 per cent of Sodium Sulfite, and 60 Minutes Processing at 212°F. on Preserving Commercial, Sanitary, Fresh Meat in T.D.T. Cans

Exp. No.	No. of T.D.T. Cans	Chemicals Added	No. of T.D.T. Cans swelled during incubation (37°C.) within 1st. Mo.	2nd. Mo.	3rd. Mo.	4th. Mo.
49 H	24	0.1% in water	None	None	1	4
49 I	24	0.1% dry chemical	None	None	4	2
Control	24	None	2	3	2	2
49 J	24	0.1% in water	None	None	None	4
49 K	24	0.1% dry chemical	None	None	None	4
Control	24	None	1	3	None	4

6. The Effectiveness of Antibiotics in Reducing Processing Time and Temperature of Canned Meat

(A) Lupulon: A sample of lupulon was secured from the Western Regional Research Laboratory. Since it had been stored several months in our refrigerator before use, the active substance was determined by ultra-violet spectrophotemetry. The method of Lewis et al. (25) was followed. The active substance of lupulon was found to be 66 per cent by weight of the original sample.

Ten milligrams of lupulon were first dissolved in a measuring flask in five milliliters (added by volumetric pipette) of 95 per cent ethyl alcohol. Then it was made up to 25 milliliters with sterile distilled water. That gave a solution of ca. 400 parts per million lupulon in 19 per cent ethyl alcohol. This solution was used in preparing the following experiments:

74 A- Seven T.D.T. cans of 40 p.p.m. crude lupulon or 26.40 p.p.m. of the active substance and 1.9 per cent ethyl alcohol of the whole content of each can.

74 B- Seven T.D.T. cans of 4 p.p.m. crude lupulon or 2.64 p.p.m. of the active substance and 0.19 per cent ethyl alcohol of the whole content of each can.

74 C- Seven T.D.T. cans of 2 p.p.m. crude lupulon or 1.32 p.p.m. of the active substance and 0.095 per cent ethyl alcohol of the whole content of each can.

Control- Two T.D.T. cans without lupulon.

All cans were prepared according to the established routine procedure. The meat was also inoculated with the spores of Clostridium sporogenes #7955 at the rate of 9,000 spores per gram. The cans were incubated at 98.6°F. (37°C.), after being seamed under 25 inches vacuum, processed 10 minutes at 212°F. (100°C.) and immediately cooled in cold running water. The results of these experiments are tabulated in Table XV. Each figure is the average of testing two T.D.T. cans.

Table XV

Effect of Lupulon on the Spores of Clostridium sporogenes #7955 in Sterile Cooked Meat in T.D.T. Cans

Exp. No.	p.p.m. of active lupulon	Average pH of meat after incubation at 37°C. for			Average count per gram of meat after incubation		
		0-days	3-days	33-days	Initial	3-days	33-days
74 A	26.40	5.70	5.72	5.73	9,000	273	90
74 B	2.64	5.70	5.87	*	9,000	x	*
74 C	1.32	5.70	6.21	*	9,000	x	*

x Very high count
* Cans swelled and were discarded

The results of these experiments showed that 26.40 p.p.m. of the active lupulon was very effective. The average destructions of the Clostridium sporogenes #7955 spores per gram of meat after 3 days' and 33 days' incubation at 98.6°F. (37°C.) were 96.96 per cent and 99.00 per cent respectively. This is the highest percentage destruction obtained in any experiment done in this research during the same time of incubation. However that does not prove that lupulon is a good preservative. The bacteriostatic action may diminish considerably after longer incubation periods as pointed out by Hansens (12). At that time the spores may be able to vegetate and spoil the meat medium. More work is now underway using lower amounts of lupulon and at longer periods of incubation in order to reach more comprehensive conclusions.

(B) Subtilin: A sample was also obtained from the Western Regional Research Laboratory. The active substance in the sample was 70 per cent as labeled on the package.

As it is claimed by The Western Regional Research Laboratory (39) that about 10 parts per million of subtilin was effective in preserving canned vegetables under vacuum with a short cooking period of only 10 minutes at 212°F. (100°C.), the antibiotic was first tried out in similar quantities. It was dissolved in distilled sterile

water at the concentration of 215 parts per million. The following experiments were prepared:

75 A- Nine T.D.T. cans of 21.5 p.p.m. subtilin or 15 p.p.m. of the active substance

75 B- Nine T.D.T. cans of 14.33 p.p.m. subtilin or 10 p.p.m. of the active substance.

75 C- Nine T.D.T. cans of 10.75 p.p.m. subtilin or 7.5 p.p.m. of the active substance.

Control- Two T.D.T. cans without subtilin.

All cans were prepared as usual according to the established routine procedure. The meat was inoculated with 9,000 spores of Clostridium sporogenes #7955 per gram. The cans were sealed under 25 inches vacuum, cooked 10 minutes at 212°F. (100°C.) then immediately cooled in running cold water and were incubated at 98.6°F. (37°C.)

All cans swelled within 48 hours' incubation and were discarded.

Another series of experiments was done with higher concentrations of subtilin. Four different concentrations were carried out as follows:

75 D- Five T.D.T. cans of 71.4 p.p.m. subtilin or 50 p.p.m. of the active substance.

75 E- Five T.D.T. cans of 57.1 p.p.m. subtilin or 40 p.p.m. of the active substance.

75 F- Five T.D.T. cans of 42.9 p.p.m. subtilin or 30 p.p.m. of the active substance.

75 G- Five T.D.T. cans of 28.6 p.p.m. subtilin or 20 p.p.m. of the active substance.

Control- Four T.D.T. cans without subtilin.

The cans were prepared as indicated in the previous experiments and were incubated at 98.6°F. (37°C.).

All cans swelled also within 48 hours incubation and were discarded.

These results showed clearly that subtilin was not effective up to 50 parts per million of the active substance against our test microorganism. These results are contrary to those of the Western Regional Research Laboratory for vegetable without pure culture inoculation. That may be due to the fact that the microorganisms which are involved in the spoilage of canned meat are different from those which are responsible for the spoilage of canned vegetables. It is believed that if the antibiotic is not effective up to 50 parts per million, it is seemingly not of value as a meat preservative against this organism.

SUMMARY AND CONCLUSIONS

This dissertation presents the results of investigations on the effectiveness of 55 chemicals in reducing the processing time and temperature of canned beef in thermal death time (T.D.T.) cans (American Can Company).

A review of the literature showed that little work had been done to reduce the processing time and temperature of canning by the addition of chemicals in concentrations of 1,000 p.p.m. or less. The Western Regional Research Laboratory (39) recently made the first announcement of experiments using antibiotics in canned vegetables. The scientists used a short cooking time of only 10 minutes at 212°F. The literature, however, does show that a few chemicals, such as quaternary ammonium halides or quinones, have bacteriostatic effects in small concentrations on artificial media as distinguished from canning operations.

The spores of Clostridium sporogenes #7955 and those of Bacillus stearothermophilus #7953 were prepared as the test microorganisms.

Three different groups of experiments were run in an effort to determine if sterile fresh meat could be prepared for use during subsequent experimentation. The conclusion was made that it was not possible to prepare sterile fresh meat from commercial cuts. It was then decided to use for

experimentation sterile meat processed according to the recommendation of the American Can Company (1, p. 550).

The results of the experiments showed that the transfer of the sterile, cooked meat to the T.D.T. cans contaminated the meat. Ten minutes' processing at 212°F. (100°C.) proved to be sufficient to destroy the contamination, if any, during transfer to the T.D.T. cans as determined by the fact that all controls showed negative plate counts. Replicate controls were used in every experiment throughout this investigation. By experimentation on spore inoculated meat paste in T.D.T. cans it was determined that meat medium was suitable for growth of C. sporogenes. On the other hand, the results of B. stearothermophilus inoculations in different experiments proved clearly that the meat medium was not suitable for the growth of these spores. Therefore succeeding experiments were limited to C. sporogenes.

The effectiveness of the chemicals used in attempts to reduce the time of processing at 212°F. (100°C.) was first estimated on an artificial medium. Four concentrations of the chemicals were employed: 0.1 per cent, 0.05 per cent, 0.01 per cent, and 0.005 per cent. From this screening procedure of using an artificial medium, the most promising compounds were then tested in a meat medium in T.D.T. cans.

The conclusions from this research are:

1. At 212°F. (100°C.) 0.1 per cent sodium metabisulfite reduced the process time maximally in T.D.T. cans of sterile meat paste inoculated with 9,000 spores of C. sporogenes #7955 per gram from 7.15 hours (thermal death time in phosphate buffer solution) to 10 minutes. This was determined by reduction of plate counts by 99.33 per cent and retention of the wholesomeness ("commercial" sterility) up to 6 months incubation at 98.6°F. (37°C.).
2. Longer processing times up to 60 minutes at 212°F. (100°C.) with 0.1 per cent sodium metabisulfite reduced counts somewhat more than above (99.89 per cent reduction in counts).
3. All 62 T.D.T. cans (100 per cent) or the uninoculated commercial, sanitary, fresh meat also retained their wholesomeness (" commercial" sterility) up to 4 months incubation at 98.6°F. (37°C.) by adding 0.1 per cent sodium metabisulfite to the can contents and processing 1 hour at 212°F. (100°C.). Shorter heating times have not been investigated.
4. Lupulon was effective in its inhibitory action

on C. sporogenes at 26 p.p.m. of can contents during one month incubation. Further incubation of the cans containing lupulon is presently underway.

5. Subtilin was not effective up to 50 p.p.m. against our test microorganism. It is believed that if the antibiotic is not effective up to 50 p.p.m., it is seemingly not of value as a meat preservative against this organism.

6. Some of the alcohol soluble compounds which showed high effectiveness against the test microorganism were 2,3,4-triiodo-benzoic acid, synkamin, hydrochloride, hydroxyphygon and dehydroacetic acid. They produced a reduction of about 95 per cent of the initial 9,000 spores per gram of meat during 6 months incubation with 0.1 per cent concentration of can contents and only 10 minutes processing at 212°F. All of these cans evidenced commercial sterility and wholesomeness.

7. The rate of destruction curves with 0.1 per cent sodium meta-bisulfite, processing times of 10, 20, 40, and 60 minutes at 212°F., and incubation periods up to 5 months at 98.6°F. (37°C.) on C. sporogenes #7955 in canned meat in T.D.T. cans

are presented. The destruction curves did not give straight lines when the log of the number of surviving bacteria was plotted against the incubation time in months.

ADDENDUM

The experimental work to date has shown progress toward the ultimate goal of commercial application. However, this work has been done on a laboratory scale and cannot be applied to commercial operation at this time. Further work should be done covering the inhibitory effects on other organisms, involving additional chemical compounds, and aiming towards commercial operating conditions. Also such factors as toxicity of the agents employed and present regulations concerning the use of chemicals in food products should be carefully investigated in the cases of those compounds which are effective in reducing the time and temperature of the retorting process.

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