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Heat Resistance and Growth Characteristics of Bacterial Spores in Canned Hams and Luncheon Meats.

W. L. Brown and C. Vinton
John Morrell & Co.
U.S.A.

Meat is thermally processed to prevent spoilage and to preserve the desirable qualities of flavor, texture and appearance. The major problem is to destroy or prevent germination of heat resistant bacterial spores present in the product.

The purpose of our work was to determine the lethal effect of a wide range of thermal processes on bacterial spores. We were also interested in the spores that survived sub-lethal heat treatment. These spores were incubated in product to study spore inhibition over extended periods of time.

MATERIALS & METHODS

Unprocessed cans of luncheon meat were obtained from a commercial canning line at random over a twenty-year period. Product shipped from another plant was selected in the same way and immediately packed with dry ice, sufficient to deliver to destination with a small surplus. The cans of frozen product were defrosted by running tap water for a sufficient time to defrost, but keeping the temperature under 40°F. Two hours were found to be sufficient for defrosting a 6-lb. can.

The cans were opened using good aseptic bacteriological technique to avoid contamination. The top portion was removed and a sterile Alemite gun filled from the center of the can. The desired number of clean sterile 10 x 75 mm chemical test tubes were filled with approximately one gram of meat. The tubes were sealed in a blast lamp. A five-minute preliminary process in an oil bath at 175°F. was used to insure that all tubes were the same initial temperature when processing began, Stumbo et al 1945 (1). Processing was accomplished by an oil bath controlled to plus or minus 0.1°C. Upon removal the tubes were immediately placed in cooling water at 70°F. Part of the tubes at each heating level were subcultured into glucose brain broth, and the remainder were held at 25° to 30°C. incubation temperature for a minimum of five years.

The number of tubes used varied at different periods of the investigation but was never less than six. The results of subculture were judged by microscopic examination. The tubes under incubation were checked by visual inspection at gradually increasing time intervals. Any tube not appearing normal was classified as "suspect" and opened. By organoleptic tests, microscopic examination and subculture, a decision was made whether there had been any bacterial activity or leakage. At the end of five or more years under incubation all tubes were opened and rated for acceptability. The processing values used were expressed in terms of F_o units. F_o units were calculated according to the procedures presented by Ball (1928) (2).

DATA and RESULTS

Table I shows the lethal effect of different processes.

Table I - Effect of Thermal Processing - Luncheon Meat

Period	Samples	Per cent Samples Positive for Viable Bacteria Processing Level in F_o Values					
		0.05	0.2	0.6	1.0	2.0	Negative
1944-47	380	98.0	87.0	67.0	40.0	19.0	2.0
1949	337	79.0	43.0	11.0	0.0	0.0	21.0
1950-51	218	78.0	43.0	9.0	1.4	0.0	22.0
1952	94	75.5	44.0	0.6	0.0	--	24.4
1953	74	67.5	29.0	2.9	0.0	--	32.4
1954	96	77.0	44.0	8.0	0.0	--	23.0
1955	84	91.6	66.0	19.0	0.0	--	8.3
1956	96	71.8	36.0	3.0	0.0	--	28.1
1957	25	64.0	14.0	1.0	4.0	--	36.0
1958	39	56.4	25.0	1.0	0.0	--	43.5
1959	51	54.0	15.0	21.0	9.0	--	45.0
1960	40	65.0	30.0	5.0	0.0	--	35.0
1962	14	57.0	28.0	0.0	0.0	--	43.0
1963	9	66.6	44.4	0.0	0.0	--	33.3

A portion of this data was presented at the Fourth Research Conference of the American Meat Institute Foundation meeting in 1952. Please refer to Reference (3).

The number of samples showing viable organisms depended upon the level of processing. During the years 1944-47, a heavy process was necessary to obtain a high per cent sterility. Our examination of these samples indicated that many of the samples contained spores of PA3679. The incidence of PA3679 decreased from approximately 40 per cent in 1944-1947 to 5 per cent in 1949 and 0.9 per cent in 1950-1951, and remained at less than 1 per cent until 1959, when an increase was observed. This increase was traced to an equipment change in the plant permitting a build-up of spores.

The extra tubes not subcultured immediately after processing were held under 25-30°C. incubation for a minimum of five years. Any tubes discolored or for any reason appeared to have changed in appearance were listed as "suspects" and examined in detail by organoleptic tests and microscopic slides. The tubes were then subcultured into glucose brain broth to check viability.

All samples examined microscopically and by organoleptic tests were found to be unaltered and there was no evidence of bacterial growth.

Table 2 gives the results of the subculture of "suspect" tubes compared with the results of subculture immediately after processing.

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Table 2 - Viability of Organisms - "Suspect" Tubes

Process F_o	Months of Incubation	Viability of Organisms Present			
		Immediately after Process		After Incubation	
		Yes	No	Yes	No
0.05	30-32	6	0	4	1
0.2	15-24	9	3 *	4	8
	27-31	3	0	2	1
	37-40	2	0	0	2
	74-78	0	3	0	3
0.6	18-21	4	5 *	4	5
	26-31	2	2	1	3
	36-39	2	1	1	2
1.0	18-24	2	5	2	5
	28-32	5	8	2	11
	50-60	0	2	0	2
	70-81	0	7	0	7
2.0	6-12	1	3	0	4
	25	1	1	1	1
	62-70	0	6	0	6

* Samples which were negative immediately after processing and positive after incubation.

In two instances after incubation viable organisms were present in tubes which were negative immediately after processing. Except for the two above-mentioned exceptions, there appears to be a loss of viability after prolonged incubation.

To further test this theory, tubes were selected from samples which had shown viable organisms on subculturing immediately after processing. These samples were subcultured and the results are given in Table 3.

Table 3 - Viability of Organisms After Prolonged Incubation

Process F_o	No. of Samples	Viability of Organisms Present	
		Immediately After Process	Subcultured after 51-87 mos. Incubation
0.05	10	10	0
0.2	12	12	0
0.6	21	21	0
1.0	38	38	0
2.0	9	9	0

The data indicate that viable spores at all processing levels lost their viability during the 51 to 87-months incubation. Total counts indicate that in most meats the spore load of putrefactive anaerobes is very low. Jansen and Aschehoug (4) observed that aerobic spore formers of the genus bacilli are important factors in the spoilage of canned meats. Jensen(5) has reported that such organisms are important in the spoilage of the perishable class of canned meats. Table 4 lists the per cent of aerobic spores present in pasteurized canned luncheon meat for the years 1952 through 1962.

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Table 4 - Total Aerobic Spore Load per gram of Luncheon Meat

Year	No. of Samples	Per Cent					
		Samples Lost	Less than 10	10-100	100-1000	1000-10,000	Above 10,000
1952	94	1.0	10.0	58.5	24.4	5.3	0
1953	74	2.7	2.7	52.7	41.8	0	0
1954	96	0	0	51.0	47.9	1.0	0
1955	84	1.1	5.9	41.6	47.6	3.5	0
1956	96	2	12.0	54.0	30	1.0	0
1957	25	0	12.0	52.0	28	8.0	0
1958	39	0	28.2	38.0	30.7	2.0	0
1959	51	0	53.0	25.4	19.5	1.0	0
1960	40	0	42.5	27.5	22.5	5.0	2.5
1962	14	0	14.3	64.3	21.4	0	0
Average	61	1.0	14.6	47.9	33.6	2.6	0.2

Data indicate that the aerobic spore load is relatively high in the meats used to prepare cured canned luncheon meat.

In 1957, canned hams were included in the study. The same technique was used in handling the ham as employed with luncheon meat except the ham was ground before filling into the small test tubes. A sterile hand grinder was used for preparing the ham for analysis.

Table 5 shows the lethal effect of different processes on ham.

Table 5 - Effect of Thermal Processing - Ham

Period	Samples	Per Cent of Samples Positive for Viable Bacteria — Processing Level in F_o Values 0.05	0.3	0.6	1.0	3.0	Negative
1957	48	*--	64.6	--	45.0	20.0	35.0
1958	19	--	68	--	31.0	5.0	31.5
1959	21	66.0	42.7	38.0	19.0	--	33.0
1960	49	60.0	32.6	18.0	14.0	--	38.8
1961	52	40.3	28.8	15.4	5.0	--	59.6
1962	43	44.1	25.6	11.6	0.0	--	55.8
1963	52	50.0	26.9	7.6	10	--	50.0

* Indicates no data obtained at this processing level.

The extra tubes not subcultured immediately after processing were held at 30°C. incubation. Any tubes discolored or for any reason changed in appearance were examined. Spoilage was evident in some of the ham tubes after two months incubation and continuing as long as 20 months after the start of incubation. Microscopic examination of the spoiled tubes indicated that aerobic spore formers caused the spoilage. The spores were more resistant in ham than luncheon meat.

Table 6 lists the per cent of aerobic spores present in canned hams. Table 7 gives the anaerobic spore load of hams used for canning.

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Table 6 - Total Aerobic Spore Load per gram in Canned Hams

Period	No. of Samples	Per cent			
		Less than 10	10-100	100-1000	1000-10,000
1957	48	6	50.0	37.5	6.2
1958	19	21	21.0	36.8	21.0
1959	21	14	57.0	14.0	14.0
1960	49	31	34.6	21.8	12.8
1961	52	37	25.0	25.0	13.0
1962	43	48	39.0	9.3	2.3
1963	52	19	48.0	26.0	5.0

Table 7 - Total Anaerobic Spore Load per gram in Canned Hams

Period	No. of Samples	Per cent		
		Less than 1	1-5	Over 5
1959	21	47.0	41.0	11.0
1960	49	76.0	17.6	5.8
1961	52	73.0	23.0	3.8
1962	43	74.4	25.5	0.0
1963	52	84.6	5.7	9.6

Additional information was obtained on the ability of the spores to remain viable in meat. The aerobic spore formers were studied by filling small potted meat cans (109 x 109) with portions of the same unprocessed ham and luncheon meat used for thermal

resistance studies. The cans were sealed and processed in water bath at 165°F. to give an internal temperature in the can of 153°F. After processing, the cans were cooled in tap water at 70°F.

One half the cans of pasteurized luncheon meat were placed in a 70°F. incubator and the remainder were placed at 40-45°F. and incubated. The cans were inspected at gradually increasing time intervals. At the end of 15 months incubation the cans at 40-45°F. incubation showed no signs of spoilage. The cans were transferred to 95°F. incubation to determine whether the spores would germinate and cause spoilage. Nine months after the cans were placed in 95°F. incubation, spoilage was evident and the cans continued to spoil for two years with 57 per cent of the cans at 95°F. spoiling from growth of bacteria. At 70°F., 45 per cent of the cans spoiled due to the growth of bacteria--0.4 per cent taking place in the first 12 months, and the remainder within three years. Organoleptic changes in the meat presumably from enzymes not inactivated caused the rest of the cans to be discarded after nine years.

One can of ham from each thermal resistance study since January 1, 1962, has been incubated at 95°--one can at 80° and one at 40-45°F. These cans have been inspected at different intervals to see if viable organisms would germinate and grow.

Table 8 shows when spoilage was evident in canned hams held at 95 and 80°F.

Table 8 - Canned Ham Spoilage

Date	No. of Samples	Total Aerobic Spore Count Per cent Less than 10	10-100	100 Above	Total Anaerobic Spore Count Per cent Less than 1	1-5	Average Days Before Spoilage, Incubation-- at 95°F.	at 80°F.
1962	52	48.0	38.46	13.4	80.8	19.0	55 days	247 days
1963	43	9.3	55.80	34.8	79.0	20.9	125	161
1964	14		50.00	50.0	92.8	7.0	34	57

At 95°F., 66 per cent of the cans spoiled in the first six months and 12 per cent in the last six months with five per cent still satisfactory through June 1964. At 80°, 44.9 per cent of the cans spoiled in the first six months with 25.6 per cent during the last six months and 18.3 per cent still satisfactory.

Spoilage was not evident in the cans held at 40-45°F. Therefore, after 18 months incubation these cans were opened and aerobic and anaerobic counts made. Dextrose Tryptone Agar with yeast extract (0.5%) was used for aerobic spore counts and Eugon agar for anaerobic spore counts. Counts were incubated at 95°F. from 48 to 72 hours.

Table 9 gives the counts at the time of processing and counts on the same product after 18 months.

Table 9 - Spore Germination at 40-45°F.

No. of Cans	At Time of Processing - Counts					After 18 Months Incubation at 40-45°F.				
	Aerobic Spores % of Samples			Anaerobic Spores % of Samples		Aerobic Spores % of Samples			Anaerobic % Samples	
	0-10	10-100	100 Over	0-1	1-5	0-10	10-100	100 Over	0-1	1-5
52	48	38.46	13.4	80.8	19	77	11	12	80	20

Data indicate very little spore germination in pasteurized canned meats held at 40-45°F. There was no indication of putrefaction but organoleptic changes had taken place. These organoleptic changes were again due to enzymatic degradation and in some cases breakdown in the can enamel.

SUMMARY

The spore load and spore heat resistance was determined in unprocessed canned hams and luncheon meat over an extended period of time. A processing level greater than F_o 3 is necessary to obtain complete sterility in these products. Product stability (commercial sterility) is possible with much less total heat. The spores remain viable over extended periods of time in the processed meats. Spoilage of underprocessed meats will take place over many months.

Organoleptic changes in some of the cans from extended storage were evident without bacterial spoilage. These changes were presumed to be due to enzymatic degradation and breakdown of can enamel.

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JOHN MORRELL & CO.
Ottumwa, Iowa, USA

SUMMARY: (English)
The spore load and spore heat resistance was determined in unprossed canned hams and luncheon meat over an extended period of time. A processing level greater than F_3^9 is necessary to obtain complete sterility in these products. Product stability (commercial sterility) is possible with much less total heat. The spores remain viable over extended periods of time in the processed meats. Spoilage of underprocessed meats will take place over many months.

Organoleptic changes in some of the cans from extended storage were evident without bacterial spoilage. These changes were presumed to be due to enzymatic degradation and breakdown of can enamel.

(German)
Die Sporenmenge und der Sporenhitzewiderstand wurde durch verlängerte Zeitspanne in unprozessierten (unzubereiteten) eingemachten Schinken und "luncheon meat" (Aufschnitt) festgestellt. Ein Niveau des Prozessierens (Zubereitens) grösser als F_3^9 ist nötig, um vollständige Sterilität in diesen Produkten zu erlangen. Die Stabilität des Produkts (Handelssterilität) ist mit viel weniger Gesamthitze möglich. Die Sporen bleiben durch verlängerte fähig. Unterprozessiertes (unterzubereitetes) Fleisch wird über viele Monate verderben.

Organische Veränderungen wurden in einigen der Konservenbüchsen während verlängerter Lagerung bewirkt, aber ohne bakterielle Verderbung. Diese Veränderungen wurden vermutlich durch enzymatischen Zusammenbruch und den Zusammenbruch des Büchsenschmelzes veranlasst.