THE EFFECT OF SALT, VINEGAR, AND HEAT ON THE COLIFORMS IN OYSTERS

By ENRIQUE S. SALAFRANCA
Of the Bureau of Fisheries, Manila

FOUR TEXT FIGURES

Oysters obtained from the areas adjoining the City of Manila have been shown to be invariably polluted (Clague, 1950; Division of Public Health Laboratory, 1952; Salafranca, 1952). Corrective measures have, accordingly, been recommended. Realizing the danger posed by these polluted oysters to the health of the people partaking of them, the Secretary of the Department of Health created in 1951, the Committee on Shellfish Industry. The main function of this body is to make experimental studies on the development of an efficient and practical method of purifying oysters. The plans laid out for this purpose have not been put to trial owing to certain difficulties. In the meantime, the experiments reported in this paper were conducted to determine to what extent the common practices observed in preparing oysters for the table affect their bacterial contents, particularly the coliforms.

The effects of salt and vinegar, which are the most common ingredients used, and heat were investigated. These were determined by periodic checks on the total bacterial counts, (TBC) and coliform counts (CC) of the oyster samples.

MATERIALS AND METHODS

The oysters used in these experiments were obtained from the Binakayan oyster farm of the Bureau of Fisheries. The oysters were thoroughly cleaned and shucked asceptically as described in a previous work (Salafranca, 1952).

Salt.—Two series of experiments using salt were conducted. In one series, granulated rock salt with 99.45 per cent sodium chloride (moisture content, 0.006 per cent) was used, and in the other, granulated solar salt with 82 per cent sodium chloride (moisture content, 9.9 per cent). Salt concentrations of 10, 15, 20, and 25 per cent (W/W) were tried at room temperature (RT)[1], 37° C., and 45° C. For seasoning, a 10 per cent salt concentration is high even for people who like their food salty

[1] Room temperature ranged from 23.5° to 27°C. at about 8:00 A.M. and from 27° to 30°C. at about 3:00 P.M. during the period of these experiments.

However, our initial trials with 10 per cent salt showed that even with this amount of salt the oysters spoiled before they were rid of their coliform contents, hence, higher concentrations were tried.

Vinegar.—Vinegar with an acetic acid content of 3.47 per cent was used. Concentrations of 10, 15, 20, 25, and 30 per cent (W/W) at RT, 37° C, and 45° C were tried. Observations similar to those made in the trials with salt determined our choice of concentrations higher than 10 per cent.

Heat.—The effect of various low degrees of heat, including RT, 37° C., 45° C., 59°–61° C. and 99°–101° C. (boiling) were tried. Experiments were conducted at RT, 37°C., and 45°C. out of curiosity inasmuch as these degrees of heat do not approximate any of the heat treatments to which oysters intended to be served fresh are subjected. An electrically heated water bath whose temperature oscillated between 61°–65° C. was employed to heat oyster specimens inside beakers to between 59°–61° C. This water bath treatment gave us the nearest approach possible with our facilities to the temperature to which the oyster meat is subjected when the oyster is steamed to open the shell and is served in this manner. A temperature of 99°–101° C. in the center of the mass was obtained by boiling over an open flame. This was employed to approximate the heat to which oysters are subjected when they are blanched prior to serving.

Culture media.—ZoBell's (1946) 2216 plating medium was employed for determining the TBC.

Lactose broth, EMB agar, Dunham's peptone broth, Clark and Lub's medium, and Koser citrate broth were used for the various cultural tests for coliforms.

In each trial performed in the course of these experiments, no less than 100 grams of oyster meat with the corresponding nectar were used. The TBC and CC of the fresh, untreated material were determined to obtain the initial counts which served as the basis of comparison for the subsequent counts of the corresponding treated portions of the specimens.

A sample of at least 10 grams was employed for each check on the TBC and CC of the treated material. An amount of sterile aged sea water sufficient to make a 10 per cent emulsion was added and the whole run through a Waring blendor for about 30 seconds. The coarse particles were allowed to settle and the emulsified sample was used for the various bacteriological cultures.

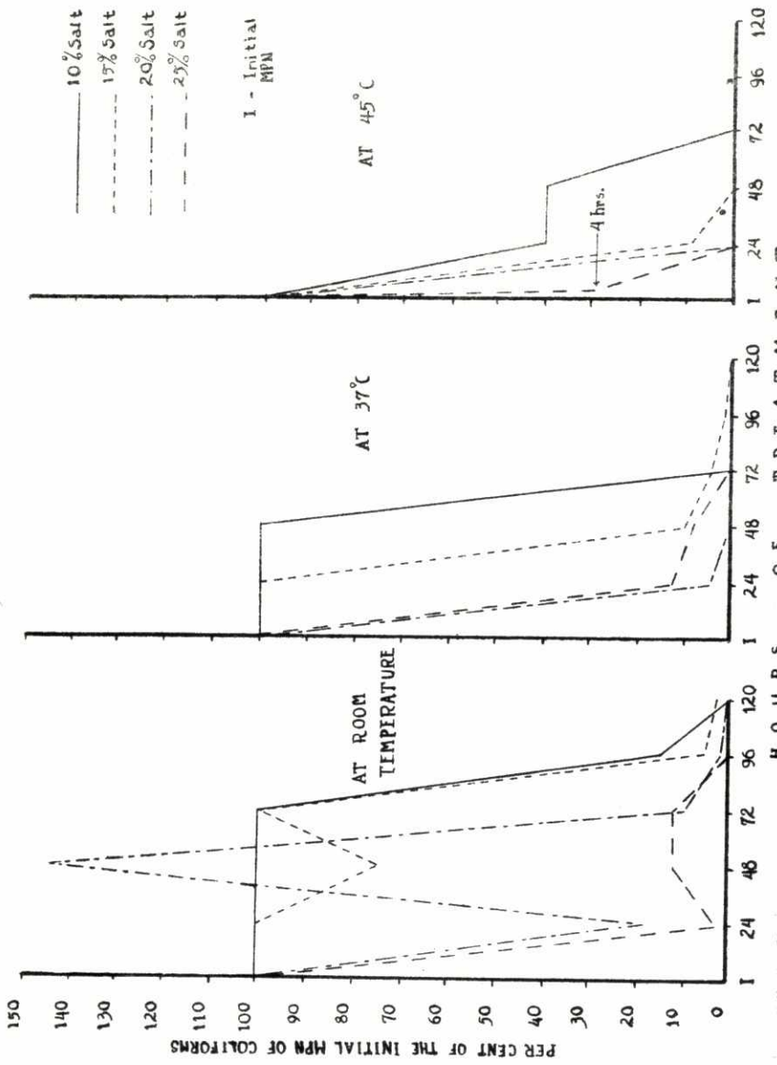

FIG. 1. Trends in the MPN of coliforms in oysters treated with granulated rock (99.45 per cent)

The procedures and techniques adopted for enumeration and computation of the TBC from the plate cultures, the coliform presumptive and confirmatory tests, and the determination of the most probable number (MPN) of coliforms were exactly those described in a previous work in connection with the bacteriological (survey of oysters at the Binakayan oyster farm (Salafranca, 1952).

The initial counts of the fresh oysters were assigned a value of 100 per cent. The bacterial counts obtained in the periodic checks were expressed as percentages of the corresponding initial counts. The counts thus expressed are easily comparable.

RESULTS AND DISCUSSIONS

1. Granulated rock salt (99.45 per cent NaCl and 0.006 per cent moisture).—The results obtained in the trials using this quality of salt are summarized in Table 1. The initial counts have been omitted. The counts in the periodic checks of the treated samples, as stated above, are expressed as percentages of the corresponding initial counts.

Oyster specimens treated with 10, 15, and 20 per cent salt in many instances, reached much greater TBC levels than the corresponding initial counts; with 25 per cent, the TBC were much lower. With a given salt concentration, the counts were generally higher at RT and 37° C. than those at 45° C.

The CC obtained in this series with one exception, were never higher than the corresponding initial counts (fig. 1).

Oysters with 10 and 20 per cent salt concentrations, at RT, were rid of coliforms in about 120 hours; with 15 per cent, the oysters spoiled before a negative CC could be obtained; and with 25 per cent the oysters were rid of coliforms in about 96 hours. At 37° C., 10, 20, and 25 per cent salt concentrations required about 72 hours and 15 per cent about 120 hours to destroy the coliforms. At 45° C., the time required to destroy the coliforms shortened with the increase in salt concentrations: about 72 hours for 10 per cent, 48 hours for 15 per cent, and about 24 hours each for 20 and 25 per cent (fig. 3).

2. Malabon solar salt (82 per cent NaCl and 9.9 per cent moisture).—In general, higher counts were obtained in specimens with lower salt concentrations than those obtained in specimens with higher salt concentrations and lower germicidal power was demonstrated with 15 per cent salt than that demonstrated with 10 per cent salt of this quality (Table 2 and fig. 2).

TEXT FIGURES

TABLE 1.—*Effect of granulated rock salt (99.45 per cent NaCl with 0.006 per cent moisture) on the total bacterial and coliform counts of oysters.*

Treatment	Bacterial counts												Remarks
	4 hours[a]		24 hours		48 hours		72 hours		96 hours		120 hours		
	TBC	CC	TBC	CC	TBC	CC	TBC	CC	TBC	CC	TBC	CC	
10 per cent at R. T.	------	------	[b]1,455	100	24,442	100	27,302	100	45,913	15	52,263	0	One trial, Developed off—odors after 48 hours.
10 per cent at 37° C	------	------	19,830	100	42,797	100	18,816	0	21,574	0	7,346	0	One trial. Developed off—odors after 48 hours.
10 per cent at 45° C	------	------	358	40	137	40	14,202	0	2	0	0.25	0	One trial. Developed off—odors after 48 hours.
15 per cent at R. T.	------	------	10	100	7	75	16	100	52	5	68	3	One trial.
15 per cent at 37° C	------	------	24,883	100	340	10	2,280	5	2,391	1	2,426	0	One trial.
15 per cent at 45° C	------	------	32	10	48	0	80	0	689	0	------	------	One trial.
20 per cent at R. T.	------	------	10	19	19	144	1,112	10	633	1	1,887	0	Average of 4 trials.
20 per cent at 37° C	------	------	355	6	102	0.1	39	0	60	0	------	------	Average of 5 trials.
20 per cent at 45° C	------	------	5	0	76	0	138	0	------	------	------	------	Average of 3 trials.
25 per cent at R. T.	------	------	2	3	3	12	4	12	0.5	0	0.1	0	Average of 2 trials.
25 per cent at 37° C	------	------	1	13	2	9	1	0	1	0	------	------	Average of 2 trials.
25 per cent at 45° C	2	29	1	0	1	0	1	0	1	0	0 0.4	0	Average of 2 trials.

[a] Number of hours of treatment and storage at temperatures indicated.

[b] All figures represent per cent of the "TBC" and "CC" of the corresponding fresh, untreated oyster specimens. TBC—Total bacterial counts; CC—coliform counts.

TABLE 2.—*The effect of Malabon solar salt (82 per cent NaCl with 9.9 per cent moisture) on the total bacterial and coliform counts of oysters.*

Treatment	Bacterial counts												Remarks
	4 hours[a]		24 hours		48 hours		72 hours		96 hours		120 hours		
	TBC	CC	TBC	CC	TBC	CC	TBC	CC	TBC	CC	TBC	CC	
10 per cent at R. T.	[b]142	75	595	109	26,900	24.7	114,000	8.43	------	------	------	------	One trial. Putrid after 96 hours.
10 per cent at 37° C	10.2	15.3	655	20	825	24.6	28,867	40	------	------	------	------	One trial. Putrid after 96 hours.
10 per cent at 45° C	7	109.3	54	0	539	0	4,286	0	------	------	------	------	One trial.
15 per cent at R. T.	293.26	76.88	1,069.41	8.11	6,583.14	4.78	37,548.15	22.95	6.52M	0	2.15M	0	Average of 3 trials.
15 per cent at 37° C	353.11	82.74	339.5	6.3	194,868.88	49.57	20,055.21	0.61	190.5	0	------	------	Average of 3 trials.
15 per cent at 45° C	772.96	39.33	1,792.76	6.8	10,681.77	0	30,572.49	0	190	0	------	------	Average of 3 trials.
20 per cent at R. T.	11.36	68.73	14.54	7.7	21.81	16.25	10.9	0	2,909.09	0	------	------	One trial.
20 per cent at 37° C	21.81	0	18.18	0	81.81	0	1,563.63	0	5,481.81	0	------	------	One trial.
20 per cent at 45° C	15.41	1.6	7.56	0	12.35	0	973.59	0	0.59	0	------	------	Average of 2 trials.
25 per cent at R. T.	22.30	7.6	2.69	11.41	103.84	0	7.16	0	1.30	0	------	------	One trial.
25 per cent at 37° C	11.53	22.28	10.38	21.73	22.3	0	0.53	0	0.71	0	------	------	One trial.
25 per cent at 45° C	3.84	9.23	2.3	0	7.11	0	0.67	0	------	------	------	------	One trial.

[a] Number of hours of treatment and storage at temperatures indicated.

[b] All figures represent per cent of the "TBC" and "CC" of the corresponding fresh, untreated oysters specimens. TBC—Total bacterial counts; CC—coliform counts; M—million.

At RT and 37° C., it was not possible to determine the approximate period 10 per cent salt concentration would require to destroy the coliforms inasmuch as the oysters were putrid after 96 hours and were discarded. At 45° C. about 24 hours were required to destroy the coliforms. Oysters with 15 per cent salt stored at RT and 37° C., respectively, required about 96 hours; at 45°C., about 48 hours. With 20 per cent salt the results were irregular: at RT, about 72 hours; at 37° C., about 4 hours; and at 45° C., about 24 hours. While no attempt was made to determine the cause of this irregularity, several possibilities were noted: variation in the size and nutritive condition of the individual oyster and possible variation in the number and species of their coliform content. Oysters with 25 per cent salt and stored at RT and 37° C., respectively, were negative for coliforms after about 48 hours; at 45° C., after about 24 hours.

In general, the observations in connection with the germicidal power of salt confirm those made by other investigators who have worked along similar aspects of this problem. Salle (1948) states that low concentrations of salt tend to stimulate bacterial growth, whereas, higher concentrations are toxic. Baumgartner (1946) states that the effect of salt on the resistance of microorganisms to heat varies with the concentration employed. Viltjoen, as cited by Baumgartner (1946), found that amounts up to 4 per cent protected the spores of several microorganisms heated in canned pea liquor. Sherman and Holm, as cited by Salle (1948), found that low concentrations of NaCl produced accelerating effect on the growth of *Escherichia coli*. They found that 0.02 M (about 1.16 per cent)[2] to be optimum. A period of equilibrium between the death rate and the rate of multiplication of coliforms is evident in the trials at RT and 37°C. with 10 per cent rock salt (fig. 1) and a period of mild stimulation in the trials at RT and 45°C. with 10 per cent solar salt (fig. 2). This period is about 72 hours and about 4–24 hours, respectively. The presence of great amounts of organic matter in the specimens treated has apparently inactivated the greater portion of the initial amounts of the salt dissolved. Salle (1948) states that salts are more germicidal in distilled water than in solutions containing protein; the organic matter greatly inactivating the salt. It takes sometime, therefore, before the coliforms are exposed to the

[2] Figure in parenthesis is based on our computation.

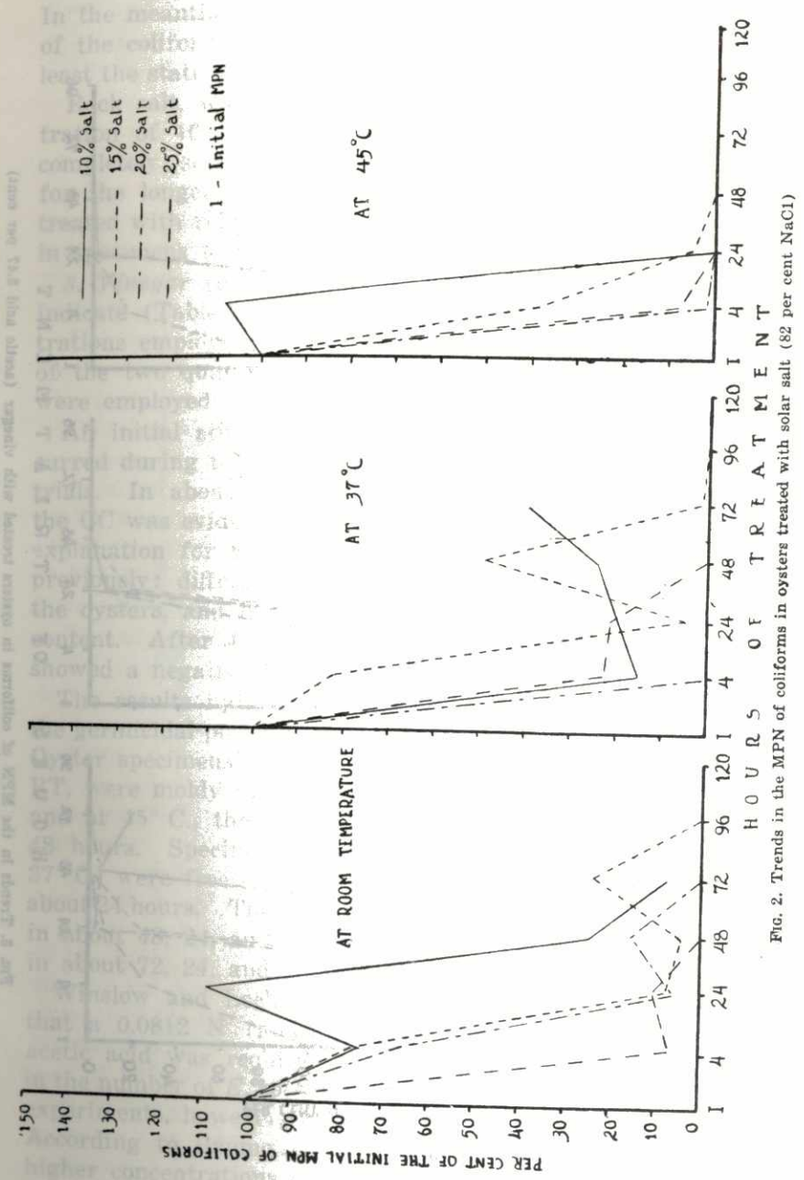

Fig. 2. Trends in the MPN of coliforms in oysters treated with solar salt (82 per cent NaCl)

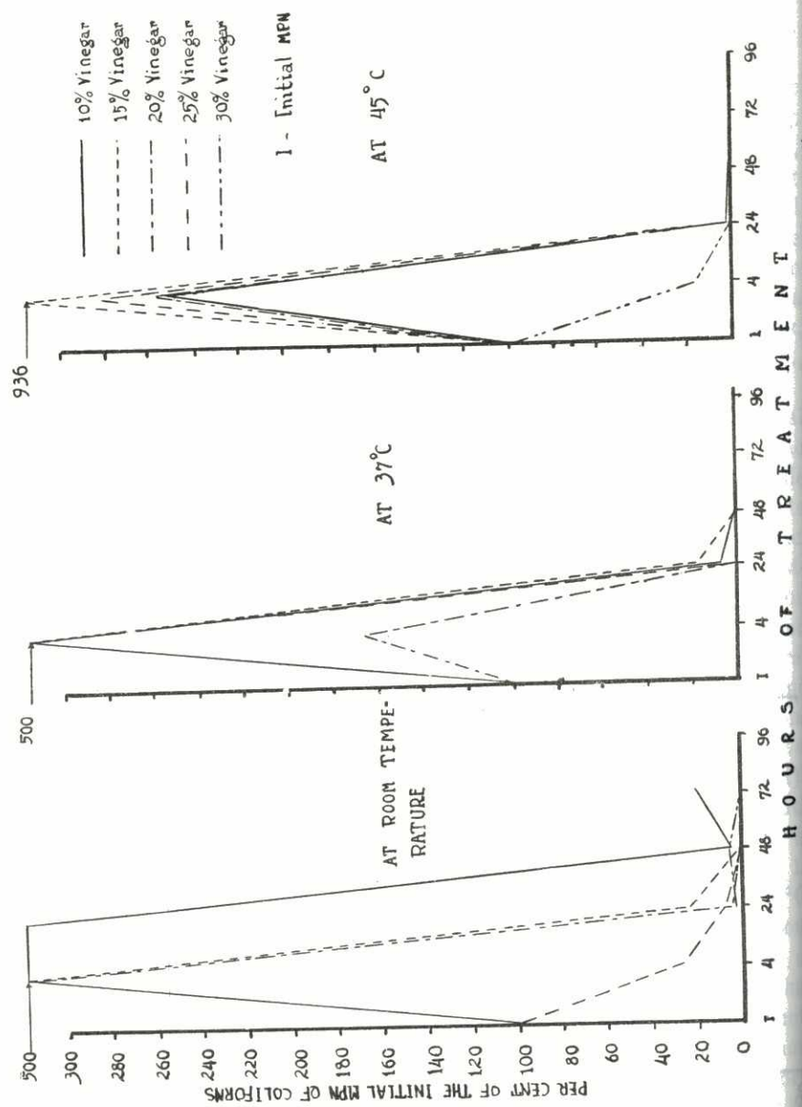

Fig. 3. Trends in the MPN of coliforms in oysters treated with vinegar (acetic acid 3.47 per cent)

full concentration attainable from the amount of salt added. In the meantime, the low concentration stimulates the growth of the coliforms resulting in the periods of stimulation or at least the state of equilibrium noted.

Rock salt, when added in amounts to make a final concentration of 10 per cent, required about 18 hours to dissolve completely; solar salt about, 4 hours. This, probably, accounts for the longer periods of equilibrium observed in specimens treated with rock salt than the periods of stimulation observed in specimens treated with solar salt.

3. Vinegar (*acetic acid content 3.47 per cent*).—The results indicate (Table 3) that this quality of vinegar in the concentrations employed, is a more powerful bactericide than either of the two qualities of salt used, in the concentrations they were employed (Tables 1 and 2).

An initial stimulation of the growth of the coliforms occurred during the first 4 hours, or thereabouts, in all but two trials. In about 24 hours, however, a marked reduction in the CC was evident in all but one trial (fig. 3). The possible explanation for the variations have already been pointed out previously: differences in the size and nutritive condition of the oysters, and in the number and species of their coliform content. After 48 hours, the specimens in all but two trials showed a negative CC.

The results indicate that raising the temperature enhances the germicidal power of any of the vinegar concentrations tried. Oyster specimens with 10 per cent vinegar, after 48 hours at RT, were moldy and no further checks were made; at 37° C. and at 45° C., the specimens were rid of coliforms in about 48 hours. Specimens with 15 and 25 per cent at RT and 37° C., were free of coliforms after 48 hours; at 45° C., after about 24 hours. Those with 20 per cent at RT, 37°C., and 45°C., in about 48, 24, and 48 hours, respectively; with 30 per cent, in about 72, 24, and 24 hours, respectively.

Winslow and Lochridge, as cited by Salle (1948), showed that a 0.0812 N (approximately 0.49 per cent)[3] solution of acetic acid was required to produce a 99 per cent reduction in the number of *E. coli* after an exposure of 10 minutes. These experiments, however, were conducted using laboratory media. According to Baumgartner (1946), for commercial purposes, higher concentrations of about 1.5 to 2 per cent are required. In our trials with vinegar, the concentrations of 10, 15, 20,

[3] Figure in parenthesis is based on our computations.

TABLE 3.—*The effect of vinegar (acetic acid 3.47 per cent) on the total bacterial and coliform counts of oysters*

Treatment	Bacterial counts												Remarks
	4 hours[a]		24 hours		48 hours		72 hours		96 hours		120 hours		
	TBC	CC	TBC	CC	TBC	CC	TBC	CC	TBC	CC	TBC	CC	
10 per cent at R. T.	10.3	500	16.8	500	16.8	6.8	127	20	-----	-----	-----	-----	One trial. Moldy after 72 hours.
10 per cent at 37° C	30.3	500	16.3	9	9.5	0	17.3	0	-----	-----	-----	-----	One trial. Moldy after 72 hours.
10 per cent at 45° C	2	250	3.7	1.2	1.6	0	1.3	0	0.07	0	-----	-----	Average of 2 trials. Markedly hydrolyzed after 72 hours.
15 per cent at R. T.	4.8	500	2.5	25.3	1.08	0	0.1	0	0.8	0	-----	-----	One trial. Markedly hydrolyzed after 72 hours.
15 per cent at 37° C	7.5	500	6.8	15	0.2	0	0.7	0	1.1	0	-----	-----	One trial. Hydrolyzed markedly after 72 hours.
15 per cent at 45° C	1.6	936	3.4	0	0.77	0	0.25	0	0.13	0	-----	-----	Average of 2 trials. Completely hydrolyzed.
20 per cent at R. T.	18	287.5	.67	1.1	2	0	10.33	0	4	0	-----	-----	One trial. Oysters hydrolyzed.
20 per cent at 37° C	16.7	168.7	.67	0	7.3	0	11.3	0	7.7	0	-----	-----	One trial. Oysters hydrolyzed.
20 per cent at 45° C	10.7	257.5	11.7	1.7	3.3	0	4.3	0	1.8	0	-----	-----	Average of 2 trials. Markedly hydrolyzed.
25 per cent at R. T.	2.8	28.7	1.35	3.4	0.5	0	6.5	0	-----	-----	-----	-----	One trial. Moldy. Hydrolyzed.
25 per cent at 37° C	1.6	500	3.2	5.3	6.2	0	1.1	0	0.2	0	-----	-----	One trial. Moldy. Hydrolyzed.
25 per cent at 45° C	39.2	284.3	21.1	0	4.58	0	1.1	0	0.25	0	-----	-----	Average of two trials. Completely hydrolyzed.
30 per cent at R. T.	77.7	500	8	.56	25	7.5	2	0	5.7	0	-----	-----	One trial. Digestion eivdent after 24 hours.
30 per cent at 37° C	34.2	500	5.14	0	13	0	9	0	5.7	0	-----	-----	One trial. Digestion evident after 24 hours.
30 per cent at 45° C	4.2	10	3.87	0	5.3	0	13.33	0	0.3	0	-----	-----	Average of two trials. Digestion more marked than at R. T. and 37° C.

[a] Number of hours of treatment and storage at temperatures indicated.

[b] All figures represent per cent of the original "TBC" and "C" of the corresponding fresh, untreated oyster specimens. TBC—Total bacterial counts; CC—coliform counts.

25, and 30 per cent correspond to approximately 0.35, 0.52, 0.69, 0.86, and 1.04 per cent acetic acid, respectively. Considering the dense mass of the oyster meat, the concentrations tried were really much lower than those suggested by Baumgartner.

4. Heat.—Table 4 summarizes the results of the trials with heat. An initial period of stimulation for the multiplication of coliforms is evident in all of the specimens kept at the various temperatures tried, the period varying with the temperature of exposure. The higher the temperature, the shorter was the period and less marked the stimulation: at RT and 37° C., over 24 hours; at 45° C., about 24 hours; at 59°–61° C., about 5 minutes; and at 99° 101°C., about ½ minute (fig. 4).

The length of time that the coliforms remained alive in oysters kept at RT and at 37° C. has not been determined. The specimens were markedly decomposed after 48 hours and no further checks were made. At 45°C., oyster specimens were negative for coliforms after about 48 hours; at 59°–61° C., after about 1 hour. At 99°–101° C., the specimens were generally negative after about 1 minute. In one of the trials, however, not all the coliforms were destroyed after 10 minutes at 99°–101° C. The possible explanations for this irregularity have already been enumerated elsewhere in this paper.

In contradistinction to the observations made on the CC, an initial decline in the TBC was observed. At RT, 37° C., and 45° C. this decline was followed by a considerable increase in subsequent checks; at 59°–61° C. and at 99°–101° C. the decrement in the TBC was more or less progressive.

This behaviour of the TBC may be explained by the predominance of marine species of microorganisms in the specimens used. The optimum temperature for the growth of these forms is rather low: few of them will grow at temperatures higher than 25° C. (ZoBell, 1946). At RT, 37° C., and 45° C. a great number of them were inhibited or destroyed resulting in the initial decline in the TBC. In the meantime, reproduction of the soil, air, and other contaminants which were also present in the specimens was stimulated. As a result, a marked increase in the TBC is subsequently noted. At 59°–61° C. and at 99°–101° C., the marine forms, as well as most of the contaminants, were destroyed so that at these temperatures a more or less progressive decline in the TBC is noted.

Wilson and Miles (1946) stated that most members of the coliform and *Salmonella* group are killed by exposure to temperatures of 55° C. for about an hour or of 60° C. for 15–20

TABLE 4.—*The effect of heat on the total bacterial and coliform counts of oysters.*

Degree of heat applied	Bacterial counts										Remarks
	4 hours[a]		24 hours		48 hours		72 hours		96 hours		
	TBC	CC	TBC	CC	TBC	CC	TBC	CC	TBC	CC	
R. T.	[b]39.37	500	54,842	500	------	------	------	------	------	------	One trial each only. Markedly decomposed.
37° C	48.12	500	13,093	500	------	------	------	------	------	------	Bloated after 48 hours.
45° C	18.75	500	11,708	56.25	10,312.5	0	11,250	0	30,625	0	Fermented after 20 hours. One trial.
	5 minutes[a]		15 minutes		30 minutes		45 minutes		1 hour		Average of 7 trials.
Water bath	TBC	CC	TBC	CC	TBC	CC	TBC	CC	TBC	CC	
59° C–61 C	29.4	326.8	6.09	71.42	1.8	49.5	5.6	62.5	8.5	0	
	½ minute[a]		1 minute		5 minutes		10 minutes		15 minutes		Average of 4 trials.
Direct heat boiling	TBC	CC	TBC	CC	TBC	CC	TBC	CC	TBC	CC	
99° C–101° C	3.01	287	4.49	0	0.753	0	7.41	0	1 054	0	

[a] Number of minutes or hours exposure to degrees of heat indicated.
[b] All figures represent per cent of the original "TBC" and "CC" of the corresponding fresh untreated oyster specimens. TBC—Total bacterial counts; CC—coliform counts.

minutes; that the various species comprising the coliform group do not differ significantly from one another, although *E. coli* tends to have a slightly higher resistance. It may be presumed, therefore, that when the check on a heat-treated specimen in-

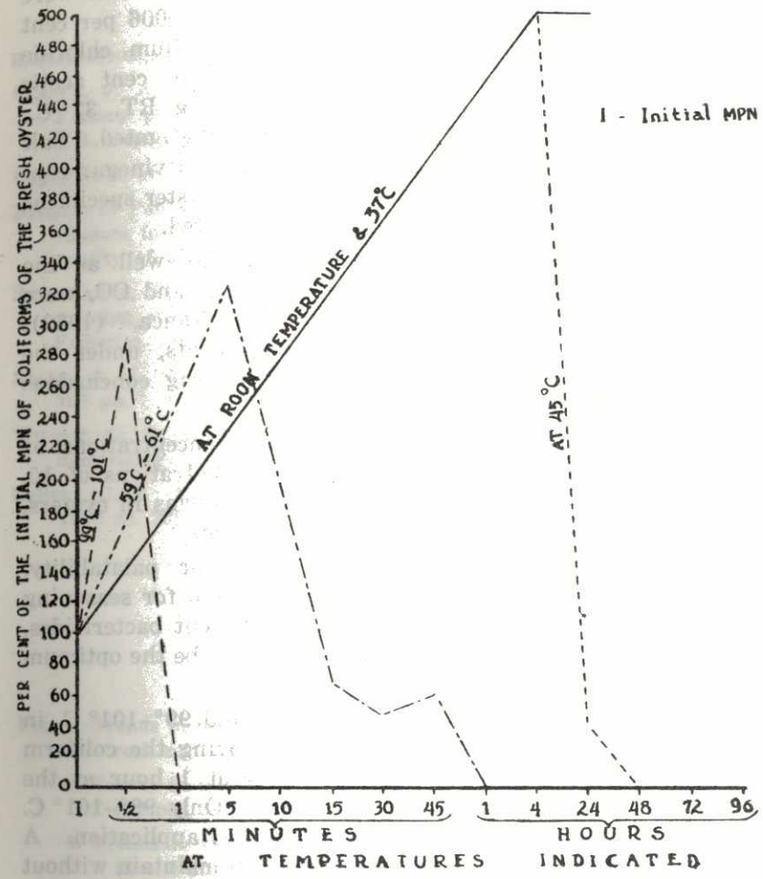

FIG. 4. Trends in the MPN of coliforms in heat-treated oysters.

dicates a negative CC, the members of the *Salmonella* group of organisms which might have been present, were destroyed. The results of the trials in which the oysters were subjected to 59°–61° C. (Table 4) are similar to those observed by Wilson and Miles.