STUDIES ON THE MICROBIAL SPOILAGE OF CANNED FOOD

II. Effect of Heat, H-ion Concentration and Chemicals on the Spoilage Bacteria

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IN the earlier studies (Rangaswami and Venkatesan, 1959), seven bacterial cultures were isolated from spoiled food cans, their harmful effect established, and their systematic position determined. These organisms were found to vary widely in the degree and type of damage caused to the canned food and also in many of their physiological properties. In order to further study their physiological properties and to understand the vulnerable area to combat them the present investigations were undertaken. The H-ion concentration of the food material is known to exert a great influence on the establishment and growth of the contaminant in the cans (Foster and Randall, 1921; Levine and Fellers, 1940; Nolte and Loesecke, 1940; Salle, 1953). Killing the contaminants by heat treatment or creating conditions unfavourable for their growth by the addition of higher concentrations of salt or sugar and other chemical preservatives to food are some of the methods being followed in the canning industry (Wyant and Normington, 1920; Spiegelberg, 1940; Lal and Sadasivan, 1942; Cruess, 1948; Johar, 1951; Williams and Zimmermann, 1951; Baumgartner and Hersom, 1956). The seven bacterial isolates were studied in relation to the above factors and the results are reported here.

MATERIAL AND METHODS

The bacterial isolates, *viz.*, *Bacillus circulans* Jordan, *B. brevis* Migula, *B. subtilis* Cohn, *B. coagulans* Hammer, *B. licheniformis* (Weighmann) Chester, *Clostridium histolyticum* (Weinberg and Seguin) Bergey *et al.* and *Lactobacillus fermenti* Beijerinck obtained in the earlier studies (Rangaswami and Venkatesan, 1959) were used in all these studies.

The thermal death rate of the bacteria was determined by following the method given by Esty and Meyer (1922) and subsequently modified by

Tanner and McCrea (1923). The bacterial suspension was poured in test-tubes and heated in a water-bath for various lengths of time and the surviving bacteria estimated by the dilution plate method. To determine the thermal death point of the bacteria the suspension was heated in a Pressure Heater for one minute at different pressures as recommended by Bigelow and Esty (1920). To determine the thermal death time of the bacteria the suspension was heated in a Pressure Heater for various lengths of time at different pressures. The surviving bacteria were estimated by the dilution plate method and the thermal death time was obtained by plotting the results on a semi-log paper.

To study the effect of H-ion concentration of the medium on bacterial growth, nutrient broth was adjusted to various pH levels with a Beckman pH meter using N/10 citric acid or N/10 KOH solution. The media were distributed in equal quantities into test-tubes, sterilized in the usual manner and inoculated with the bacterial suspension. To another set of the media agar was added and poured in petri dishes after sterilizing. The bacteria were inoculated in the usual manner and their growth examined after three days.

In order to study the effect of salt, sugar and other chemical preservatives on the growth of bacteria, required quantities of the chemical were added to nutrient broth or agar media and tested in the usual manner in test-tubes or in petri dishes. The growth of the test organism was examined after three days.

EXPERIMENTAL RESULTS

Thermal death rate.—The seven bacterial isolates in water suspension were heated at 212° F. in a water-bath. The results obtained are illustrated in Fig. 1.

L. fermenti was found to be least resistant to heat as the cells were killed by a moment's heating at 212° F. Of the spore formers, *Cl. histolyticum* was killed in 90 minutes as compared to 130–400 minutes required for the species of *Bacillus*. The rate of death of the bacterial population also varied with the species; 90 per cent. of the population was killed in three minutes in the case of *Cl. histolyticum*, whereas 24 minutes were required to destroy the same percentage of population in *B. subtilis*.

Thermal death point.—The thermal death points of the seven isolates were determined by treating them for one minute in a Pressure Heater at various pressures ranging from 0–15 lb./sq.in. The estimates of the bacterial population after the treatments are represented in Fig. 2.

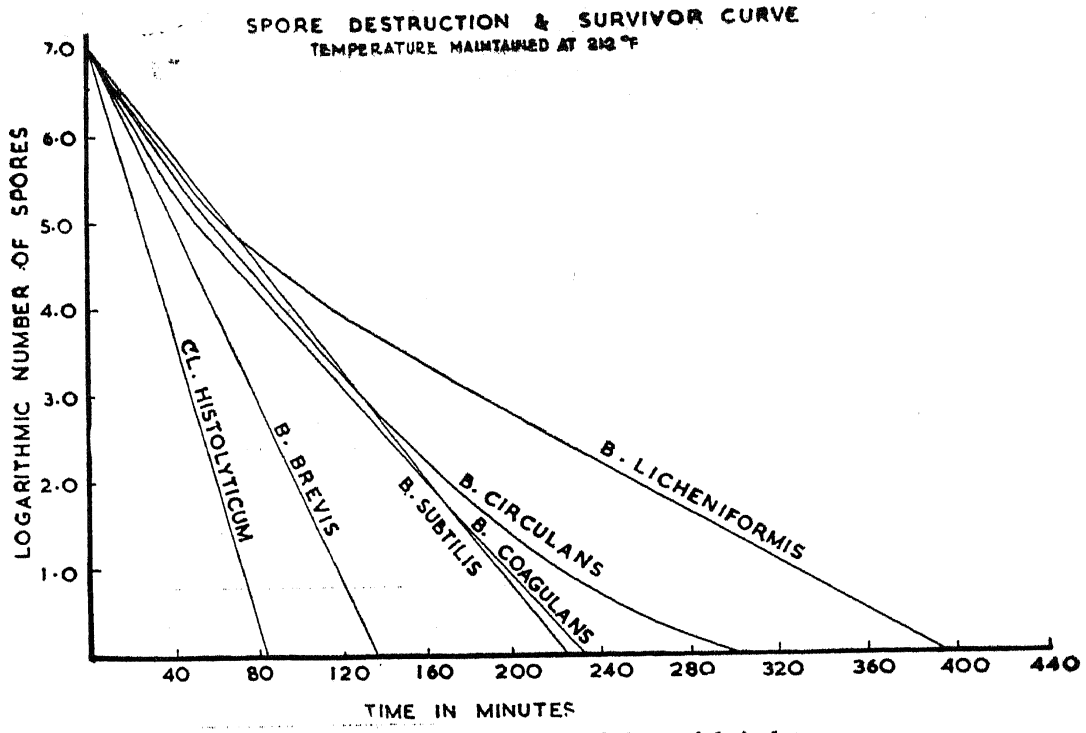

FIG. 1. Thermal Death Rate of bacterial isolates.

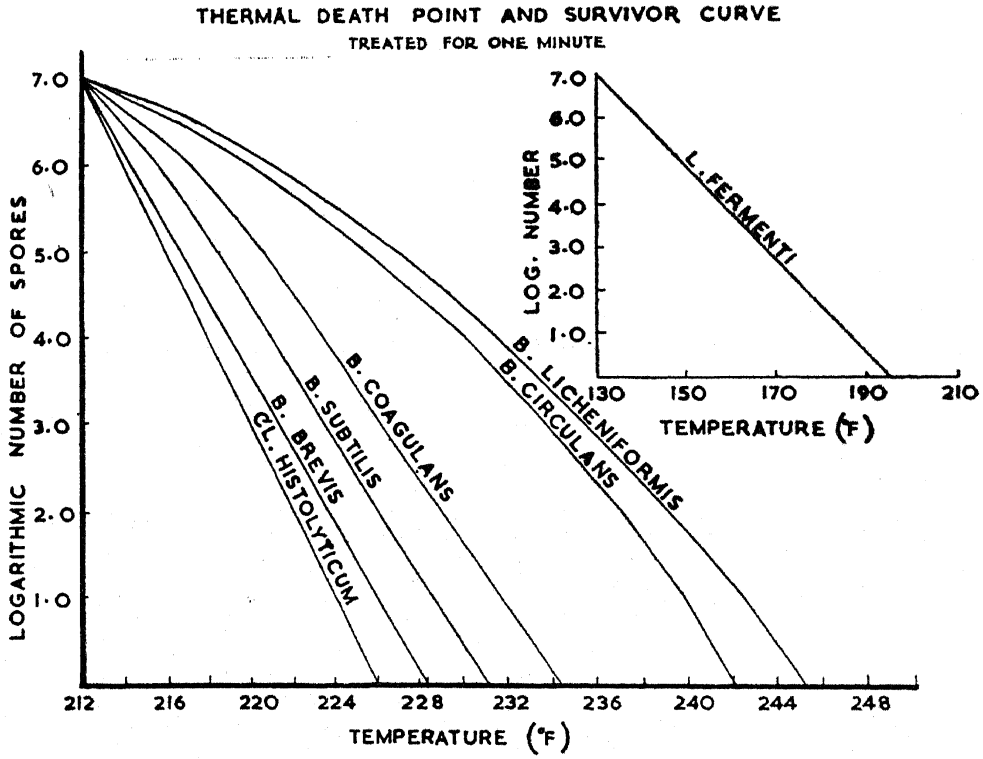

FIG. 2. Thermal Death Point of bacterial isolates.

Again *L. fermenti* was found to be most labile to the treatment as it was killed in one minute at 195° F. The temperature required to kill the other organisms varied from 226–246° F., *B. licheniformis* being the most resistant.

Thermal death time.—The thermal death time for the two of the isolates, *Cl. histolyticum* and *B. licheniformis*, was determined following the procedure described earlier. The time and temperature combinations required to kill the two organisms were determined. The results obtained are represented on a semi-log paper (Fig. 3). The time taken by the bacteria to traverse one log cycle was determined from the graph.

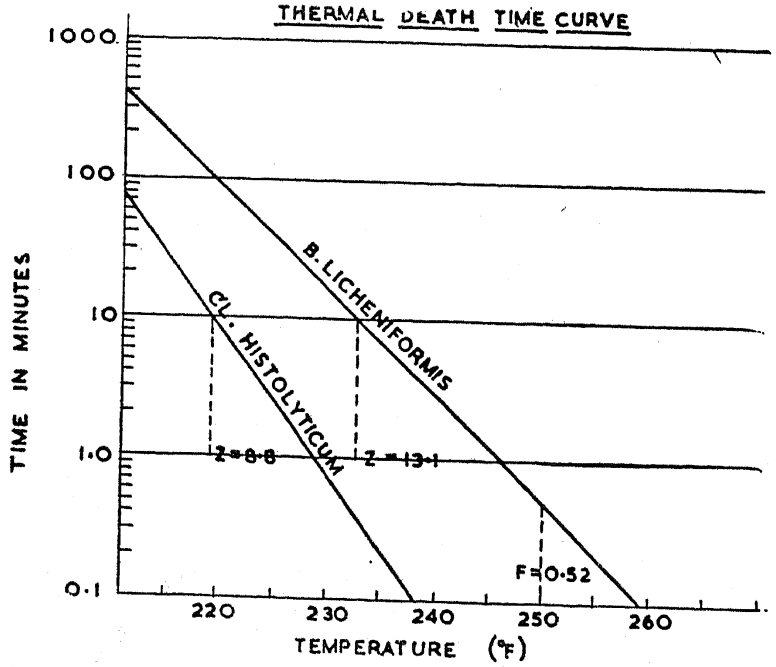

FIG. 3. Thermal Death Time of *B. licheniformis* and *Cl. histolyticum*, represented on a semi-log paper (Z = the slope of the thermal death time curve expressed as ° F. passed over in traversing one log-cycle; F = the number of minutes required to destroy the organism at 250° F.).

From the above results the ' retart heating time ', which is an indication of the resistance put forward by the bacterium to heat, was calculated for *B. licheniformis* by applying the formula given by Stumbo (1948) $U = Z(\log a + p)$, where U = retart heating time for a specific bacterium, Z = the slope of the thermal death time curve expressed as ° F. passed over in traversing one log-cycle, a = initial number of the organism, p = log of the reciprocal of the number of organisms remaining viable at the end of the heating time. The retart heating time (U) for *B. licheniformis* works out to 91·7 minutes, whereas the formula is not applicable to *Cl. histolyticum* as the organism was relatively less resistant to heat.

Effect of H-ion concentration on growth.—The optimum pH levels for the growth of the seven bacterial isolates were determined by growing them in nutrient broth and nutrient agar adjusted to different pH levels.

All the isolates grew in the pH range of 4·5–9·5, but they failed to grow at pH 3·0 except *L. fermenti*, which was found to grow well at that pH. The optimum pH for the spore formers was found to be between 5·5–8·5 and for *L. fermenti* it was between 3·5–8·0.

Salt tolerance.—The salt tolerance capacities of the bacterial isolates were tested in nutrient broth and in nutrient agar media containing different concentrations of sodium chloride.

L. fermenti did not multiply even at 4 per cent. salt concentration, and *Cl. histolyticum* and *B. brevis* at 6 per cent. concentration, whereas the other organisms could tolerate even higher concentrations, *B. licheniformis* being capable of growing at 13 per cent. concentration.

Sugar tolerance.—The sugar tolerance capacities of the bacteria were examined in nutrient broth and nutrient agar media containing different concentrations of cane sugar.

The results indicate, that in general, 40–45° B. is required to inhibit the bacterial growth, but *Cl. histolyticum* was relatively less tolerant as it failed to grow even at 30° B.

Effect of chemical preservatives on the bacteria.—Two chemical preservatives, *viz.*, Na-benzoate and potassium meta-bisulphite (K.M.S.), commonly used in the industry, were tested for their capacity to inhibit the bacterial isolates. The results are summarized in Table I.

It was found that 1,300 p.p.m. of Na-benzoate or 450 p.p.m. of K.M.S. are required to completely inhibit all the bateria tested, but 700 p.p.m. of the former or 150 p.p.m. of the latter is sufficient to reduce the growth of the bacteria. Of the seven isolates, *L. fermenti* was most susceptible to both the chemicals, *B. subtilis* and *B. coagulans* most resistant to Na-benzoate and *B. circulans* and *B. subtilis* most resistant to K.M.S.

Influence of H-ion concentration and sugar content on the action of chemical preservatives.—In order to study the effect of the two chemicals, alone and in combination with each other, on the bacteria, as influenced by the H-ion concentration and sugar content of the substratum, another experiment was conducted. *Cl. histolyticum*, *L. fermenti* and *B. licheniformis*, belonging to three different genera, were selected for the purpose. Five hundred p.p.m. of Na-benzoate and 200 p.p.m. of K.M.S., the concentrations found in the previous experiment to be inhibitory to the three organisms,

TABLE I

Inhibitory action of chemical preservatives on bacterial isolates

(Tested in nutrient agar medium for three days)

Bacterial culture	Inhibitory conc.: p.p.m.	
	Na-benzoate	Potassium meta-bisulphite
B. circulans ..	1,000	500
B. brevis ..	900	400
B. subtilis ..	1,300	500
L. fermenti ..	700	300
Cl. histolyticum ..	1,000	400
B. coagulans ..	1,300	400
B. licheniformis ..	1,200	400

and a combination of half the strength of the two substances were tested on the bacteria in nutrient broth and nutrient agar media containing different concentrations of sugar and adjusted to various pH levels. The minimal inhibitory concentration of sugar at various pH levels was obtained and the results are represented in Fig. 4.

It was found that the chemicals were effective in acid and alkaline ranges than at neutral range. Also when the chemicals were tested together at half the concentrations, they were more effective indicating thereby a synergestic action on the bacteria. Also when the chemicals were tested at various sugar concentrations it was found that the bacteria were completely inhibited at lower pH and sugar combinations.

DISCUSSION

It is well known that the spore-forming bacteria withstand adverse environmental conditions, especially heat, better than others. Though this was found to be true in the present investigations, wide ranges in the heat resistance and chemical tolerance qualities were found among the species. Reynolds and Lichtenstein (1952) analysed the data on heat destruction of

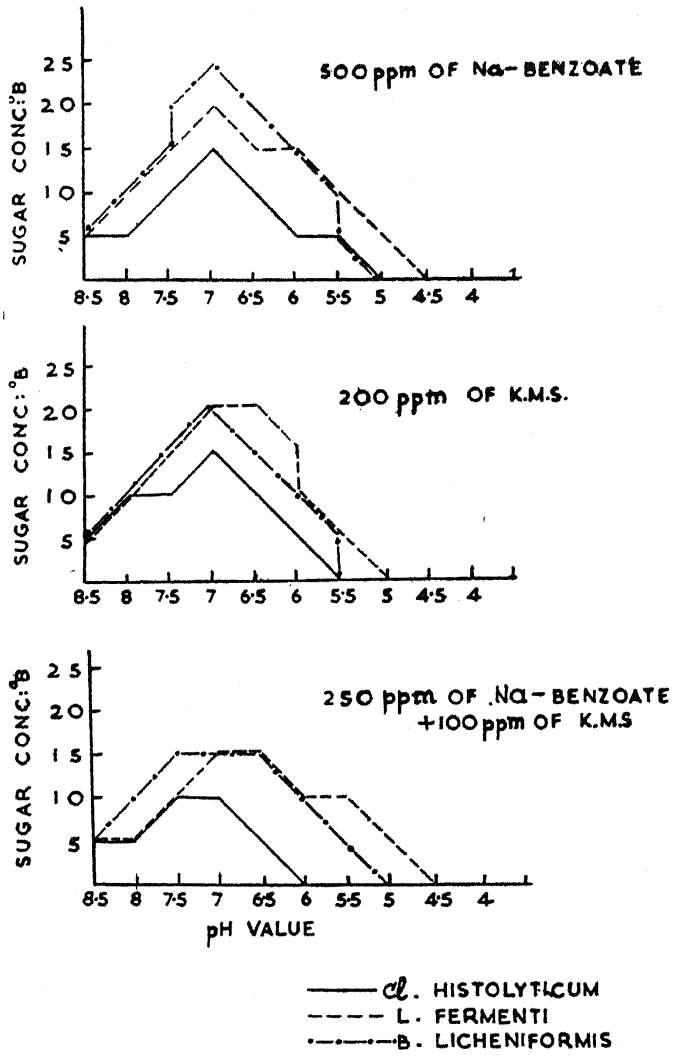

FIG. 4. The influence of H-ion concentration and sugar content of the medium on the action of Na-benzoate and potassium meta-bisulphite (K.M.S.), alone and in combination, on three bacterial species.

many spore-forming bacteria and differentiated three phases in their death rate; an initial phase of accelerated death, a second phase of logarithmic destruction and a final phase of decreasing death rate. The rate of destruction of the spores of *B. licheniformis*, *B. circulans* and *B. coagulans* seem to follow a similar pattern but in *L. fermenti*, *Cl. histolyticum* and *B. brevis* there were no distinct phases of death rates.

The results obtained in the studies on the pH relationships indicate that all the seven bacterial contaminants are capable of causing damage to canned food in the pH range of 4·0–9·0. Most of the vegetable products including tomato and fruit juices have a pH range between 4·0–6·0 and so are capable

of multiplying even at pH 3·0 and so is more dangerous than the rest as it can destroy even highly acidic products like citrus juice and pickles.

The studies on the salt tolerance of the bacteria have clearly brought out that *L. fermenti* and *Cl. histolyticum* are highly sensitive to salt and so can be checked in the industry by increasing the salt percentage of the food medium wherever possible. But *B. licheniformis* is capable of living in high salt concentrations and so is to be carefully watched in foods containing high salt concentrations.

Regarding sugar tolerance, all the seven isolates are inhibited at 40–45° B.; but this concentration is much lower than those required to inhibit yeasts and moulds (Tanner, 1944). *Cl. histolyticum* seems to be easily inhibited by sugar as it failed to grow at 30° B. and so this property can be advantageously utilized to check the spreading of the organism in the food preservation industry.

The two chemical preservatives tested showed that they act in a synergestic manner when combined together as they are much effective in checking the organisms than when tested individually. The influence of sugar content and H-ion range on their effectiveness also indicates very encouraging results. Though more detailed investigations are needed to understand the reasons for the synergestic effect and for the influence of H-ion and sugar contents on the effectiveness of chemicals, the results obtained here indicate that when once the pH of the food material to be preserved is known, a combination of the two chemicals together with calculated quantities of sugar can be used to check the microbial spoilage. Other chemical preservatives have been recently tested in India on spoilage moulds of mango squash (Anand *et al.*, 1958). Work in this line to test various chemical preservatives on the bacterial contaminants in a given set-up will be of much value.

SUMMARY

1. Seven bacterial species, *viz.*, *Bacillus circulans* Jordan, *B. brevis* Migula, *B. subtilis* Cohn, *B. coagulans* Hammer, *B. licheniformis* (Weighman) Chester, *Clostridium histolyticum* (Weinberg and Seguin) Bergey *et al.* and *Lactobacillus fermenti* Beijerinck, isolated earlier from spoiled canned foods, were studied for their physiological properties in relation to heat treatment, H-ion concentration of the substratum and susceptibility to chemical preservatives.

2. The species of *Bacillus* were highly resistant to heat treatment, *Cl. histolyticum* less resistant and *L. fermenti* labile to heat. The rate of death of the bacterial population in *B. licheniformis*, *B. circulans* and *B. coa-*

gulans followed three distinct phases, firstly a major population was destroyed in a relatively short period, followed by a rapid logarithmic destruction and finally a decreasing rate of death. In *B. brevis, B. subtilis, Cl. histolyticum* and *L. fermenti* the population was destroyed in an almost uniform pattern.

3. All the seven bacterial species were found to thrive well in a wide range of pH and *L. fermenti* was capable of multiplying even at pH 3·0 indicating that it can spoil highly acidic food products.

4. *L. fermenti* and *Cl. histolyticum* were highly sensitive to salt concentration of the medium, whereas *B. licheniformis* was found to be a facultative halophile as it could grow even in 13 per cent. salt concentration.

5. In general all the bacteria were inhibited at a sugar concentration of 40–45° B., but *Cl. histolyticum* was relatively less tolerant as it failed to grow even at 30° B.

6. Na-benzoate and potassium meta-bisulphite, when tested on the bacteria, were found to have a synergestic effect when combined together than when tested alone. They were more effective in acid and alkaline ranges of the substratum than in neutral range. Also, when the combination of the chemicals was tested at various sugar concentrations of the medium, only half the concentration of the sugar could suffice to inhibit the bacteria.

7. On the basis of the results it is suggested that *L. fermenti* could be easily eliminated by heat treatment or addition of salt to the product, *Cl. histolyticum* by heat, or addition of salt or sugar and the species of *Bacillus* by a combination of Na-benzoate and potassium meta-bisulphite in a sugar medium acidified with edible acids.

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