THE VIABILITY OF AEROBIC THERMOPHILIC BACTERIA IN THE PRESENCE OF VARYING CONCENTRATIONS OF ACIDS, SALT AND SUGARS

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Horace D. Graham

1951

THESIS

This is to certify that the

thesis entitled

The Viability of Aerobic Thermophilic Bacteria in the Presence of Varying Concentrations of Acids, Salt and Sugars

presented by

Horace D. Graham

has been accepted towards fulfillment of the requirements for

M.S. degree in Bacteriology & Public Health

F. W. Fabian

Major professor

Date June 27,,1952.

O-169

THE VIABILITY OF AEROBIC THERMOPHILIC BACTERIA IN THE PRESENCE OF VARYING CONCENTRATIONS OF ACIDS, SALT AND SUGARS

By

HORACE D. GRAHAM

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Bacteriology and Public Health

1951

ACKNOWLEDGEMENTS

Dr. F.W. Fabian, professor of Bacteriology and Public Health, gains the deepest appreciation of the author for his expert guidance and keen interest in this project throughout the period of investigation.

Thanks are also due to messrs. Ralph C. Zalkan, Hilliard Pivnick and Sam Rosen, graduate students in this laboratory, for useful suggestions and guidance at various times.

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INTRODUCTION

Despite the importance of thermophilic bacteria in the food industry, especially in the spoilage of canned foods, a survey of the literature reveals a paucity of information on their classification and physiological characteristics. Schmidt[1] significantly remarked "The group of organisms known as obligate thermophiles has been completely neglected".

Thermophilic spoilage has for years been a constant and vexing problem of food canners, food technologists and food bacteriologists. The ubiquitous nature of the thermophilic bacteria attested to by the great variety of sources from which they have been isolated, and the extraordinarily high heat resistance of their spores, greatly increase the probability of spoilage in industry. They may occur in food ingredients such as sugar and starch and the spoilage of sugar in storage and during shipment has been attributed to their action. Several cases of wholesale loss of inadequately processed canned foods due to thermophilic spoilage have been reported. Tomatoes, beans, peas, corn, non-acid foods in general- are very susceptible. In all such cases the flat sour obligate thermophiles in the sense of the definition of Cameron and Esty[2] are of particular importance.

The retreat of the Allied armies in the North African campaign during World War II was allegedly due largely to a shortage of

rations incurred as a result of thermophilic spoilage of canned foods stored in the warm African desert. Similar spoilage of canned foods occured in the Pacific. With the Pacific theatre now being regarded as a stragetic area large shipments of edible materials to these areas may be susceptible to thermophilic spoilage. Further close study of the behavior of these organisms may therefore be profitable.

Acids, salt and sugar occuring either naturally or added in varying proportions play an important role in food preservation. This preservative effect was recognised even by primitive man.

The hydrogen-ion concentration of food influences its deterioration by micro-organisms. Concentrated solutions of salt and sugars are used to preserve certain foods by repressing development of micro-organisms; onions, peppers and cauliflowers being preserved by salt and fruits by sirups.

The influence of acids, salt and sugars on thermophilic bacteria has not been extensively studied. As a result, opinions differ widely as to the kind and quantity of any of these ingredients to be used for effective but, at the same time, economical preservation. The phenomenal expansion of the food industry and the present emphasis on the microbiological aspects of foods suggest the necessity for further study.

Objectives of this investigation

The general objective of these studies was to ascertain the trend of growth of thermophilic bacteria in the specific medium which had been adjusted to different pH levels with different acids or to which increasing concentrations of salt or sugar had been added and, ultimately, to determine their possible use in the preservation of foods which are susceptible to thermophilic spoilage. Specifically, the objectives were to obtain the following information:

1- The order of effectiveness, bacteriostatically and bactericidally, of the acids, salt and sugars against thermophilic bacteria. The acids were used to adjust the pH of the medium to the same pH level while the salt and sugars were added in increasing concentrations.

2- The minimum or probable time for which the medium so treated and inoculated with thermophilic cultures would have to be incubated in order to reduce the bacterial population.

3- The relative rates of death of thermophilic bacteria isolated from various sources when incubated for varying periods in a medium so treated.

LITERATURE REVIEW

A very lucid review of the literature on the nature of thermophiles is given by Gaughran[3].

Thermophilic bacteria have been characterized by different investigators in a variety of ways. They may be simply defined as unicellular spore-forming micro-organisms depending for their existence on animal or plant tissues and which grow at relatively high temperatures. Prickett[4] after sifting the various definitions accepted the one given by Cameron and Esty[2]. It is as follows:

(A)- Obligate Thermophiles

Cultures growing at 55°C but not at 37°C or showing no growth at 37°C but growing at 45°C and higher.

(B)- Facultative Thermophiles

Cultures growing both at 55°C and 37°C. Prickett further observed that, though about sixty(60) different species of aerobic thermophilic bacteria are named and described in Bergey's Manual of Determinative Bacteriology, the data on the species of this group are so meager that it is not possible to offer a rational system of classification. Furthermore, many of the characteristics used for separating the various species of thermophilic bacteria are probably as variable as they are in mesophiles.

Imsenecki and Solnzeva[5] have divided them, on a temperature basis, into two main groups:

1- Stenothermal Thermophiles

These develop at 60°C but do now show any growth during several days at 28-30°C. The amylolytic bacterium, Bacillus diastaticus, a potent starch hydrolyser, which grows vigorously at 60°C on potato decoction, but does not grow at 28-30°C on the same medium is an example of this class.

2- Eurithermal Thermophiles

Representatives of this group also exhibit a growth optimum at about 60°C but slight growth may also be evident at such low temperatures as 28-30°C. Though high temperature is a primary requisite for the optimal growth of thermophilic bacteria, several workers (5,6,7,8) have shown that thermophiles are not necessarily inactive at relatively low temperatures.

Bergey[9] divides thermophiles into two groups:

1- True thermophiles

These show optimum growth at 60°C to 70°C and only feeble or no growth below 40°C or 45°C.

2- Facultative Thermophiles

These develop at room temperature- about 20°C, have their optimum temperature at about 50°C and their maximum at about 60°C.

Gaughran[3] points out that the multiple connotations of the terms used to describe thermophiles have led to much misinterpretation. He thinks that bacteria with any optimum growth temperature of between 50°C and 60°C may be called thermophiles and suggests that, if necessary, the terms of Imsenecki and Solnzeva[5] should be used for defining the magnitude of the temperature range for growth.

The Effect of Acids:

Erickson and Fabian[10] showed that thermophiles are more susceptible to increasing concentrations of acids than mesophiles.

Anderson and co-workers[11] demonstrated that lactic, acetic, and citric acids in increasing concentrations reduced the thermal destruction rates of Bacillus thermoacidurans, the relative order of efficiency being lactic > citric > acetic.

Pederson and Backer[12] concluded that there seems to be a significant difference between the minimum pH for the growth of vegetative cells and the minimum pH for the germination of spores of Bacillus thermoacidurans; vegetative cells initiated growth in broth of pH as low as 4.30, while heat resistant spores were incapable of germination at pH below 5.0.

Nunheimer and Fabian[13] found that on a pH basis, the order of effectiveness for the germicidal action of the acids against strains of food poisoning staphylococci was acetic > citric

lactic > malic > tartaric > HCl. The order for growth inhibiting properties was the same except that citric and lactic acids changed places in the series.

Levine and Fellers [14] noted that acetic acid was much more effective than either hydrochloric or lactic acid against Saccharomyces cerevisiae or Salmonella aertrycke.

Wyeth[15] reported that on a pH basis the order of effectiveness against coliform bacteria was acetic > lactic > hydrochloric.

Shillinglaw and Levine [16] found that in a concentration of 0.02N the order of effectiveness of the edible acids as germicides against Escherichia coli at 30°C was tartaric > lactic > acetic > citric.

The Influence of Salt:

The influence of salt on the viability and thermal resistance of micro-organisms has commanded the interest of various investigators who have reported both increased lethality and a definite protective effect.

Viljoen[17] found that concentrations of sodium chloride up to 3.5 percent in pea liquor gave a protective effect against heat to certain micro-organisms including thermophiles. The greatest protection was observed at 1.0- 2.5 percent while 4.0 percent either gave no protective action or else had a killing effect.

Anderson et al[11] showed that the addition of increasing amounts of salt to tomato juice resulted in a progressive lowering of the hydrogen ion concentration and a concomittant more rapid destruction rate for Bacillus thermoacidurans.

The Influence of Sugars:

Erickson and Fabian[10] demonstrated clearly that thermophiles are much less resistant to the effects of sugars than other micro-organisms such as Streptococcus lactis and Streptococcus liquefaciens. They found, for three cultures of thermophiles, a preservative action in the presence of as low as 17.5 percent fructose, 27.5 percent dextrose and 40 to 45 percent sucrose.

Anderson et al[11] found that increasing concentrations of sucrose and dextrose in tomato juice enhanced the thermal resistance of Bacillus thermoacidurans.

Robertson[18] observed an increasing protective action for several micro-organisms including Streptococcus thermophilus, when heated in increasing concentrations of sugars.

Several workers including Baumgartner and Wallace[19] and Fay[20] found that the thermal resistance of some micro-organisms when heated in hypertonic solutions of sucrose, glucose, lactose and other sugars is increased.

PROCEDURE

The underlisted cultures of thermophilic bacteria, obtained from the National Canners Association, were used in this investigation:

Type	N.C.A. Number	Source
Obligate thermophile-flat sour	1373	Hominy
" "	26	Corn
" "	1503	peas
Facultative thermophile	1401	pumpkin
" "	1518	corn

Culture No. 1373 became contaminated during the experiment and was omitted from the later portions.

The food ingredients whose effect on the viability of the above thermophilic bacteria was studied and their sources are as follows:

(A)- Acids: Acetic- Glacial, C.P. grade

Citric- Baker's analysed, C.P. grade.

Lactic- Edible lactic acid, 50 percent.

B1- Salt Sodium chloride, Merck.

C1- Sugars Dextrose, DIFCO, Bacto dextrose, anhydrous

Sucrose, C.P.

Fructose (Levulose) C.P.

Preliminary experimentation:

Growth curves were run on cultures 26, 1401, 1503 and 1518 in order to determine their rate of growth and death in dextrose tryptone broth and the approximate time intervals at which platings were to be done throughout the experiment.

Procedure:

50 ml. portions of sterile dextrose tryptone broth were preheated to 55°C to afford optimal temperature from the very start. Each was inoculated with 1 ml. of a suspension of each organism, washed from a 48 hour dextrose tryptone agar slope and incubated in a water bath maintained at 55 ± 1°C. Plate counts were made at 0,2,4,6,8,10,12,14,16,18,20,24,30,36,42,48, 72 and 96 hours.

Preparation of Test solutions:

(A)- Acid solutions:

A sterility test was run on each batch of acid as follows. A drop of the acid was added to a test tube containing 10 ml. of dextrose-tryptone broth. The tube was then incubated at 55°± 1°C for 48 hours. In no instance, however, did thermophiles appear in any of the acids used.

Using aseptic precautions throughout all manipulations, sterile dextrose tryptone broth was adjusted with the aid of a Beckman pH meter, to the required pH values shown in Tables 2, 3 and 4. Fifteen ml portions were then dispensed into sterile test tubes.

B- Salt solutions:

Sodium chloride in concentrations of 0, 5, 1.5, 2.5, 5.0, 10, 15, 20 and 25 percent by volume was added to dextrose tryptone broth and the mixture heated in the steamer to dissolve all the ingredients. The broth was then dispensed in 15 ml. quantities into test tubes and the tubes autoclaved at 121° C (250° F) for fifteen minutes.

C- Sugar solutions:

In the case of dextrose amounts equivalent to 5, 10, 20, 30, 40 and 50 per cent by volume were added to dextrose tryptone broth and the procedure repeated exactly as in the case of the salt solutions. For fructose and sucrose 100 per cent ~~weight~~ by volume solutions were made up and autoclaved at ten pounds pressure for ten minutes. Enough of these stock solutions were then aseptically pipetted into sterile flasks to make up 100 ml. of each of the concentrations required. From these flasks 15 ml. portions were then aseptically pipetted into sterile test tubes.

Preparation of stock cultures:

All cultures were grown on dextrose tryptone agar slants for 48 hours. This allowed the use of cultures of a standard age of 48 hours throughout the experiment.

Inoculation and plate counts:

All the tubes of broth for the acid, salt or sugar test were pre-heated at 55°C for two hours. The 48 hour cells were washed off the dextrose tryptone agar slopes using 10 ml. of sterile distilled water. Into each of the tubes, 1. ml of the well-shaken cell suspension was inoculated, kept in a water bath maintained at 55 ± 1°C during this process, and tubes then incubated at 55 ± 1°C. Plating on dextrose tryptone agar by the pour plate method was done from each tube after 0,6,12, 24, 48 and 96 hours of incubation and plates incubated at 55 ± 1°C for 36 hours . All inoculations of tubes and platings from tubes were done in duplicate.

RESULTS

Table 1 shows the number of viable cells for cultures 26, 1401, 1503 and 1518 after increasing periods of incubation. Growth curves based on the above numbers are shown on Fig. 1. Table 2 shows the effect of acids on the viability of the organisms. Table 3 shows the effect of increasing concentrations of sodium chloride while the effect of increasing concentrations of dextrose, fructose and sucrose are shown in Table 4.

Explanation of Tables:

Tables 2-8 inclusive.

+ = That percentage increase over original inoculum after period of incubation indicated. e.g. + 300 = an increase of 300 per cent = a threefold multiplication.

- = That percentage decrease or destruction based on the original inoculum e.g. - 59 = 59 per cent of the original inoculum died.

o = Bacteriostasis - no increase or decrease relative to the initial inoculum.

Fig. 1 Normal logarithmic growth curves of test organisms in dextrose tryptone broth at 55° C.

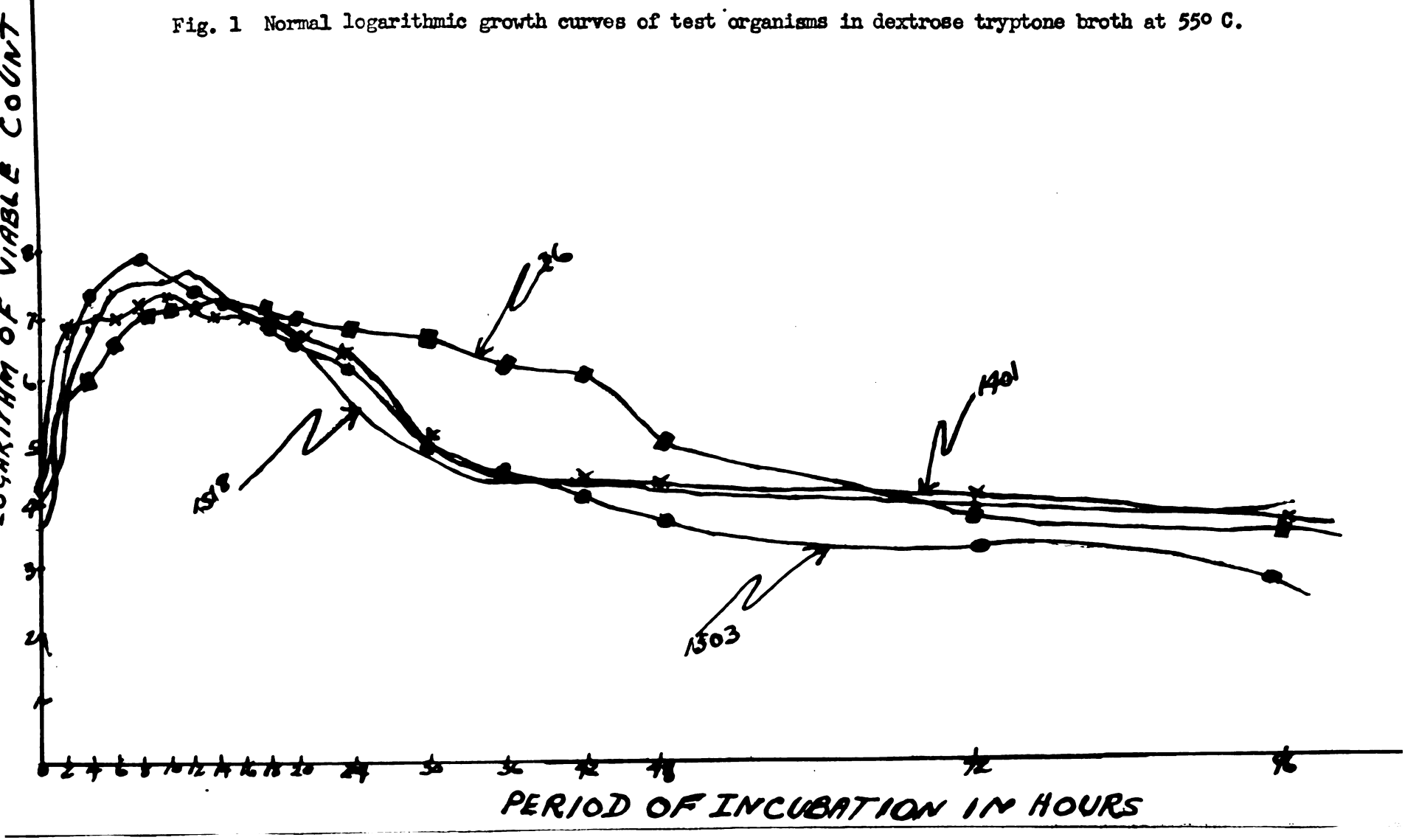

TABLE 1- The Growth of Thermophilic bacteria viable count after increasing periods of incubation in dextrose tryptone broth at 55°C ± 1°C.

		Period of incubation in hours.																
	0	2	4	6	8	10	12	14	16	18	20	24	30	36	42	48	72	96
Culture Number 26	Number of cells 15,000	750T	875T	6M	13M	15M	18M	20M	16M	15M	12M	8M	5M	2M	1.5M	100T	6T	4T
	Log of No: of cells 4.18	5.86	5.94	6.78	7.11	7.18	7.26	7.3	7.20	7.1	7.08	6.9	6.70	6.3	6.18	5.0	3.8	3.6
1401	number of cells 14000	7.5M	9M	11M	16M	19M	14M	12M	10M	9M	6M	3M	100T	36T	24T	20T	11T	4T
	Log of No: of cells 4.15	6.9	6.95	7.04	7.20	7.28	7.15	7.08	7.0	6.96	6.77	6.47	5.00	4.55	4.39	4.30	4.04	3.6
1503	Number of cells 5000	5M	31M	50M	69M	50M	23M	16M	10M	7M	4M	1.5M	90T	40T	15T	6T	2T	600
	Log of cells 3.69	6.69	7.49	7.67	7.83	7.69	7.36	7.2	7.0	6.85	6.6	6.18	4.96	4.6	4.17	3.77	3.3	2.77
1518	Number of cells 8000	57T	8M	22M	29M	36M	43M	24M	15M	11M	4M	420T	83T	32T	28T	20T	9T	7T
	Log of No: of cells 3.9	5.76	6.90	7.34	7.46	7.56	7.63	7.38	7.18	7.04	6.6	5.6	4.91	4.5	4.45	4.3	3.95	3.85

Note- M = million
T = Thousand

Table 2- Average percentage increase or decrease over initial inoculum of thermophilic bacteria in dextrose tryptone broth adjusted to varying pH levels with citric, acetic and lactic acids.

Culture No	PH	Init. in. per ml X 10^4	CITRIC ACID Incubation period in hours 6	12	24	48	96	ACETIC ACID Incubation period in hours 6	12	24	48	96	LACTIC ACID Incubation period in hours 6	12	24	48	96
	cont.no acid ad:																
x	6.7	1.8	+9600	+1424	+ 363	+ 51	- 95	+4900	+31150	+6150	+1150	+650	+461500	+70000	+3000	+2300	+1820
26	6.0	1.8	+14534	+9600	+ 805	+244	+127	+59900	+ 600	+ 50	0	- 20	+150000	+76900	+3747	+2600	+ 130
	5.5	1.8	- 92	- 97	- 97	- 98	- 99	+ 300	+ 650	+ 500	+ 500	+100	+ 1515	+2670	+1970	+1823	+ 140
L	5.0	1.8	- 97	- 99	- 99	- 99	- 99	- 40	- 45	- 50	- 50	-52	+ 207	- 23	-84	-92	+ 98
	4.5	1.8	- 97	- 98	- 99	- 99	- 99	- 40	- 40	- 50	- 50	-50	- 88	- 92	-94	-96	- 99
	4.0	1.8	- 98	- 99	-99	-99	-99	- 55	- 60	- 70	-80	-85	-	-	-	-	-
	C.N.A.A:	1.3	+114	+4462	+1400	-55	-62	+1900	+3900	+ 400	-28	-36	+42750	+3760	+2560	+1688	+1688
x	6.7																
	6.0	1.3	+ 430	+186	-30	-90	-93	+3100	+4700	+2400	+148	-84	+8830	+ 900	-88	-91	-94
	5.5	1.3	-93	-96	-96	-97	-99	+19900	+15900	+9900	-80	-94	+ 70	+ 57	-91	-94	-98
1373	5.0	1.3	-95	-96	-97	-97	-97	-4	-56	-88	-93	-97	-91	-94	-96	-97	-97
	4.5	1.3	-97	-98	-98	-99	-99	-84	-88	-94	-97	-97	-94	-94	-96	-97	-97
	4.0	1.3	-98	-98	-98	-99	-99	-85	-92	-95	-98	-98	-94	-95	-96	-97	-98
	C.n.a.a:																
x	6.7	2.2	+3170	+728	-14	-15	-28	+ 200	+ 900	+100	+ 70	0	+40000	+740	+250	+200	+100
	6.0	2.2	+ 14	+271	+2186	-43	-65	+1200	+ 200	- 40	- 60	-60	+23900	+140	- 80	- 88	- 88
1401	5.5	2.2	- 28	- 36	- 50	-77	-81	+7400	+ 66	- 50	- 70	-70	+ 96	- 56	- 80	- 88	- 88
	4.5	2.2	- 71	- 92	- 97	-60	-60	- 70	- 70	- 80	- 76	-80	- 76	- 80	- 84	- 92	- 96
	4.0	2.2	-71	- 71	- 77	-95	-98	- 60	- 60	- 70	- 80	-80	- 82	-83	- 84	- 88	- 96

NOTE: x = control

Table 2- (continued) **Average percentage increase or decrease over initial inoculum of thermophilic bacteria in dextrose tryptone broth adjusted to varying pH levels with citric, acetic and lactic acids.**

Culture No.	pH	Init. in. per ml X 10^4	CITRIC ACID Incubation period in hours 6	12	24	48	96	ACETIC ACID Incubation period in hours 6	12	24	48	96	LACTIC ACID Incubation period in hours 6	12	24	48	96
1503	x 6.8	1.5	+69130	+ 4709	+ 46	- 61	-80	+ 160	+ 267	+ 100	+ 33	+ 33	+ 177	+3900	+400	+212	+212
	6.0	1.5	+15300	+46050	+4700	+285	-70	+ 333	+566	+ 33	+ 17	- 33	+ 163	+ 400	+ 70	+ 36	+ 33
	5.5	1.5	- 52	- 61	- 80	- 90	-93	+ 1567	+56566	+12400	+633	+433	- 10	- 35	- 50	- 54	- 54
	5.0	1.5	- 52	- 70	- 80	- 87	-92	+ 100	+133	+ 100	0	- 33	- 35	- 63	- 63	- 63	- 70
	4.5	1.5	- 70	- 77	- 80	- 90	-92	+ 100	+ 100	+ 66	0	- 34	- 60	-63	- 70	- 75	- 88
	4.0	1.5	- 77	- 80	- 88	- 95	-97	+ 66	+ 33	+ 33	0	- 50	- 60	-65	- 70	- 83	- 95
1518	x 6.8	1.7	+1900	+ 140	- 90	- 95	-95	+ 117	+ 200	+ 50	- 50	- 50	+6130	+128	+ 70	- 23	- 23
	6.0	1.7	+ 53	+ 60	+1900	- 50	-88	+4517	+12666	- 33	- 58	- 67	+ 377	+223	- 30	- 38	- 40
	5.5	1.7	- 88	- 92	- 94	- 96	-96	+3066	+ 1300	- 67	- 75	- 77	+ 15	- 37	- 54	- 70	- 70
	5.0	1.7	- 92	- 95	- 96	-96	-97	- 50	- 50	- 67	- 70	- 80	0	- 54	- 62	- 70	- 70
	4.5	1.7	- 94	- 95	- 95	- 97	-99	- 50	- 50	- 67	- 79	- 80	- 23	- 54	- 54	- 62	- 70
	4.0	1.7	- 94	- 95	- 95	- 98	-99	- 58	- 67	- 70	- 82	- 83	- 23	- 46	- 70	- 90	- 96

NOTE: x = control

Table 3- Average percentage increase or decrease over initial inoculum of thermophilic bacteria in dextrose tryptone broth containing increasing concentrations of NaCl.

Salt concentration	CULTURE # 1518 Incubation period in hours					CULTURE # 1503 Incubation period in hours					CULTURE # 1401 Incubation period in hours				
	6	12	24	48	96	6	12	24	48	96	6	12	24	48	96
No salt added															
x 0.0	+70000	+36000	- 15	-61.5	-61.5	+416570	+28170	-96.2	-96.4	-97	+28050	+23040	+2048	+1863	+1800
0.5	+84500	+ 800	+300	+ 77	+ 54	+ 161	+ 19	-92.2	-93.3	-95	+24340	+ 2800	+ 455	+ 85	- 65
1.5	+66000	+17200	+530	+ 52	- 71	- 66	- 72	-78.3	-80.7	-94	+ 2500	- 4	- 11	-33.3	- 60
2.5	- 39	-46.8	-52.3	-52.3	- 55	- 69	- 74	-79	-91	-91	0	- 22	-33.3	- 48	- 66
5.0	-46.8	-50	-52.3	-52.3	- 66	- 69	- 73	-89	-91.5	-92.2	-14.8	- 40	-60	- 63	-63
10.0	-42.7	-43.3	-49.9	-53	-73.8	-74.7	- 76.6	-90	-92	-93	-18.5	- 26	-63	-63	-63
15.0	-43.8	-42.7	-43.1	-44.6	-76.1	-74	- 76.6	-91	-92	-93	-28	-37	-63	-66	- 77
20.0	-46.1	-53.1	-53.8	-53.8	-67	-74	- 82	-90	-92	-93	-48	-55	-63	-65	-66
25.0	-46	-53	-54	-54	-67	-76.6	-82	-90	-92	-92	-44	-55	-63	-77	-79
x 0.0	+900	+ 566	-66			+975	+1080	-75.3	-78.5	-90					
0.5	+600230	+2760	-76			- 57	+4200	-56	-85	-86					
1.5	+19260	+24900	+566	+584	+66.6	+ 40		-83.8	-90.3	-91.4	-91.4				
2.5	+ 854	+ 813				- 75		-85	-91.4	-91.4	-91.4				
5.0						-83.8		-92.4	-92.4	-94.6	-94.6				
10.0						-92.4		-85	-92.0	-95	-96				
15.0						-93		-95.6	-94.6	-94.6	-96				
20.0						-93		-94.6	-95	-96	-97				
25.0						-94		-94	-95	-96	-98				
				CULTURE # 26					CULTURE # 1373						

NOTE: x = control

Table 4— Average percentage increase or decrease over original inoculum of thermophilic bacteria in dextrose tryptone broth containing increasing concentrations of sugars.

culture no	sugar concentration	in. inoc per ml X 10^4	DEXTROSE Incubation period in hours 6	12	24	48	96	FRUCTOSE Incubation period in hours 6	12	24	48	96	SUCROSE Incubation period in hours 6	12	24	48	96
	control																
x	0.5%	2.7	+66560	+33260	-80	-95	-96	+ 800	+1900	+ 50	+ 6	- 13	+3900h	+5000h	+ 35	- 30	- 50
	5 %	2.7	+49900	+89800	-81	-90	-96	+280h	+5540	+3000	+152	+108	+2751	+3280	+ 50	- 40	- 50
	10 %	2.7	- 87	-88	-85	-95	-97	+300	+ 200	+ 122	+ 80	+ 33	+2561	+5937	+ 40	- 75	- 90
26	20 %	2.7	- 89	-90	-92	-96	-98	- 60	- 70	- 75	-80	- 82	- 28	- 65	-65	- 68	- 75
	30 %	2.7	- 90	-90	-90	-96	-98	- 63	-78	- 80	-80	-84	- 70	- 55	-60	- 70	- 80
	40 %	2.7	-93	-94	-96	-96	-97	- 50	-60	-70	-82	-86	- 50	- 75	-78	- 70	- 75
	50 %	2.7	-93	-94	-94	-97	-97	- 37	-57	-68	-72	-84	- 65	- 75	-76	-78	- 78
x	cont	1.3	+1400	+1700	-20	-82	-94	+973h	+166h	+500	+116	+100	+1800h	+3360h	+145h	+112	+220
	0.5 %	1.3	-72	-82	-85	-98	-99	+1460h	+123h	+933	+666	+366	+1120h	+2690h	+38750	+2800	+2540
	10 %	1.3	-95	-95	-97	-98	-99	+630h	+430h	+1266	+633	-66	+3620	+1864	+1740	+2800	+150
	20%	1.3	-96	-98	-98	-98	-99	0	0	-33	-42	-66	- 80	- 96	- 96	- 96	- 97
1401	30 %	1.3	-97	-98	-98	-98	-99	0	-2	- 14	-66	-72	- 84	- 86	- 94	- 94	- 96
	40 %	1.3	-98	-98	-98	-98	-99	-17	-19	- 34	-60	-88	- 87	- 90	- 92	- 93	- 94
	50%	1.3	-98	-99	-99	-99	-99	-18	-30	- 38	-69	-70	- 90	- 90	- 96	- 96	- 98
x	con	1.6	+820	+320	-89	-96	-97										
	0.5%	1.6	+3000	+900	-40	-95	-96										
	5 %	1.6	- 92	-92	-93	-95	-96										
	10 %	1.6	- 92	-94	-95	-96	-98										
1373	20 %	1.6	- 92	-95	-94	-97	-98										
	30 %	1.6	- 92	-95	-95	-96	-98										
	40 %	1.6	- 93	-93	-95	-98	-98										
	50 %	1.6															

NOTE: x = control

Table 4-(Continued)- Average percentage increase or decrease over original inoculum of thermophilic bacteria in dextrose tryptone broth containing increasing concentrations of sugar.

Culture No	sugar concentration	An. inoc per ml X 10^4	DEXTROSE Incubation period in hrs. 6	12	24	48	96	FRUCTOSE Incubation period in hours 6	12	24	48	96	SUCROSE Incubation period in hours 6	12	24	48	96
x	cont 0.5%	1.7	+8140	+255	+150	-70	-70	+4408	+500	+18	-50	-80	+382h	+2630h	+10360	+263	+126
	5 %	1.7	+4	-66	-66	-70	-70	+808h	+500	-35	-70	-77	+2810h	+2000	+412	+120	-5
	10 %	1.7	-55	-66	-70	-72	-72	+725	+463	-38	-72	-80	+2570h	+1600	+125	+24	+19
1503	20 %	1.7	-55	-60	-60	-74	-74	-65	-75	-80	-85	-90	+25056	+390	+380	+150	+87
	30 %	1.7	-63	-66	-70	-80	-80	-70	-76	-84	-90	-92	-15	-38	-63	-75	-78
	40 %	1.7	-74	-52	-55	-77	-77	-74	-78	-79	-82	-94	-13	-63	-64	-68	-70
	50 %	1.7	-26	-50	-60	-77	-77	-84	-86	-86	-86	-86	-30	-63	-66	-82	-86
x	con. 0.5%	1.5	+18140	+115	-55	-85	-92	+40666	+4130	+400	-20	-50	+874h	+11875	+5525	+3600	+40
	5 %	1.5	+1600	+1000	-63	-91	-94	+79000	+33333	+5000	+1760	+1740	+1124h	+119h	+9800	+60	+56
1518	10 %	1.5	-78	-78	-85	-92	-97	+46	+5417	+50	-33	-60	+624	+6865	+4600	+1350	+870
	20 %	1.5	-80	-82	-85	-92	-92	-46	-52	-60	-62	-68	+105	+50	-80	-85	-87
	30 %	1.5	-81	-81	-82	-92	-93	-44	-58	-59	-60	-73	-30	-50	-70	-84	-84
	40 %	1.5	-78	-78	-78	-90	-96	-54	-56	-58	-65	-74	-20	-60	-80	-80	-87
	50 %	1.5	-78	-84	-85	-90	-92	-52	-53	-58	-61	-63	-10	-60	-80	-81	-83

Note- h = hundred
x = control

ANALYSIS OF RESULTS AND DISCUSSION

The growth of Thermophiles

From Table 1 and Fig. 1 it is seen that in all cases maximum growth was evidenced after 8- 14 hours of incubation. This was followed by a very rapid decrease in viable numbers which, in several cases, were much lower than the size of the initial inocula after 96 hours of incubation.

Reasons for the rapid growth and death rates of Thermophilic bacteria:

Gaughran[3], Imsenecki and Solnzeva[5] and Hansen[8] all noted that the growth of thermophilic bacteria begins almost immediately after inoculation of the culture medium, the initial stationary phase being either absent or is too short to be detectable. Both growth and death rates were found to be much more rapid than among the mesophiles, i.e. the generation time is much shorter and the logarithmic growth phase during which the rate of multiplication remains constant was not evident in population curves. Imsenecki and Solnzeva also noted that proteolysis, denitrification and hydrolysis of starch by thermophilic bacteria proceed at a rate seven to fourteen times that of cultures of mesophilic bacteria. Such rapid multiplication and death rates of cells have been attributed to;

1- The extremely high biochemical activity of thermophilic bacteria as a result of their rapid growth.

2- The inability of thermophilic bacteria to sporulate readily due to the very low oxygen tension in the liquid medium at such an elevated temperature[3].

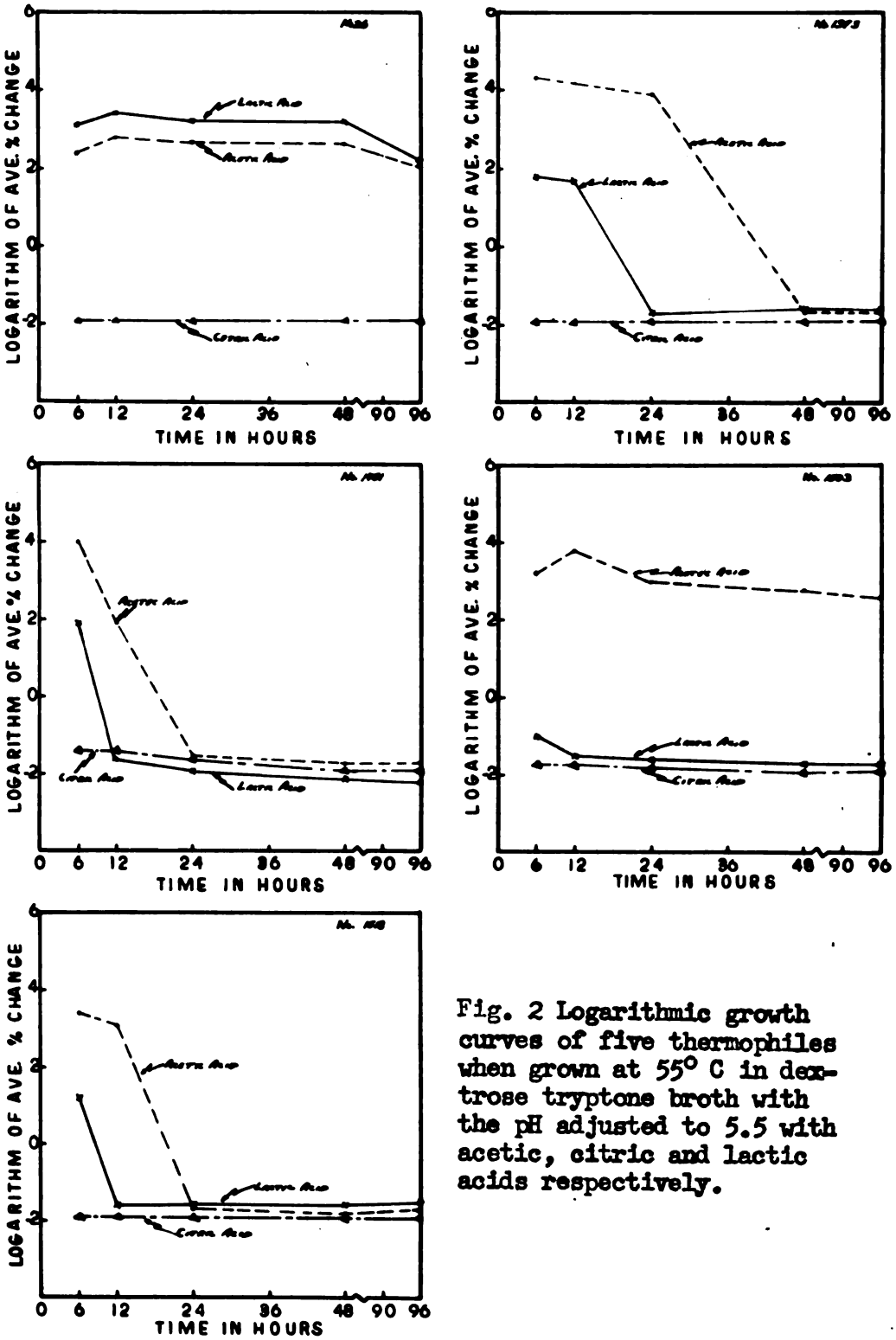

Fig. 2 Logarithmic growth curves of five thermophiles when grown at 55° C in dextrose tryptone broth with the pH adjusted to 5.5 with acetic, citric and lactic acids respectively.

3- The rapid formation and accumulation of acids through the decomposition of carbon compounds in the medium if such are not neutralized, since the fermentation capacity of thermophiles has been calculated to be perhaps thirty times as great as that of Streptococcus lactis at 20° C.

4- Cytolysis induced by autolysis with the resultant accumulation of toxic products in the medium have been suggested for observed decreases in the total cell count. Evidence of autolysis has been obtained by way of the detection of an accumulation of enzymes in media inoculated with thermophiles[5].

The effect of acids, salt and sugars:

The effect of increasing concentrations of acids, salts and sugars was determined in terms of per cent increase or decrease of cell numbers relative to the initial inoculum. Results are recorded in Tables 2, 3 and 4.

A- The effect of Acids:

From Table 2, and Fig. 2 it may be noted that at pH 5.5 and incubation time of 6 or 12 hours the germicidal powers of the acids used follow the order citric > acetic > lactic. In the case of citric acid an increase in acidity beyond pH 6.0 down to pH 5.5 resulted in an increase in cell destruction. Beyond pH 5.5, however, an increase in acidity resulted in no significant decrease in the number of cells. For acetic and lactic acids, multiplication occured at pH 5.5 and germicidal action started at pH 5.0. A decrease in pH below pH 5.0 germicidal action afforded no significant decrease in cell numbers.

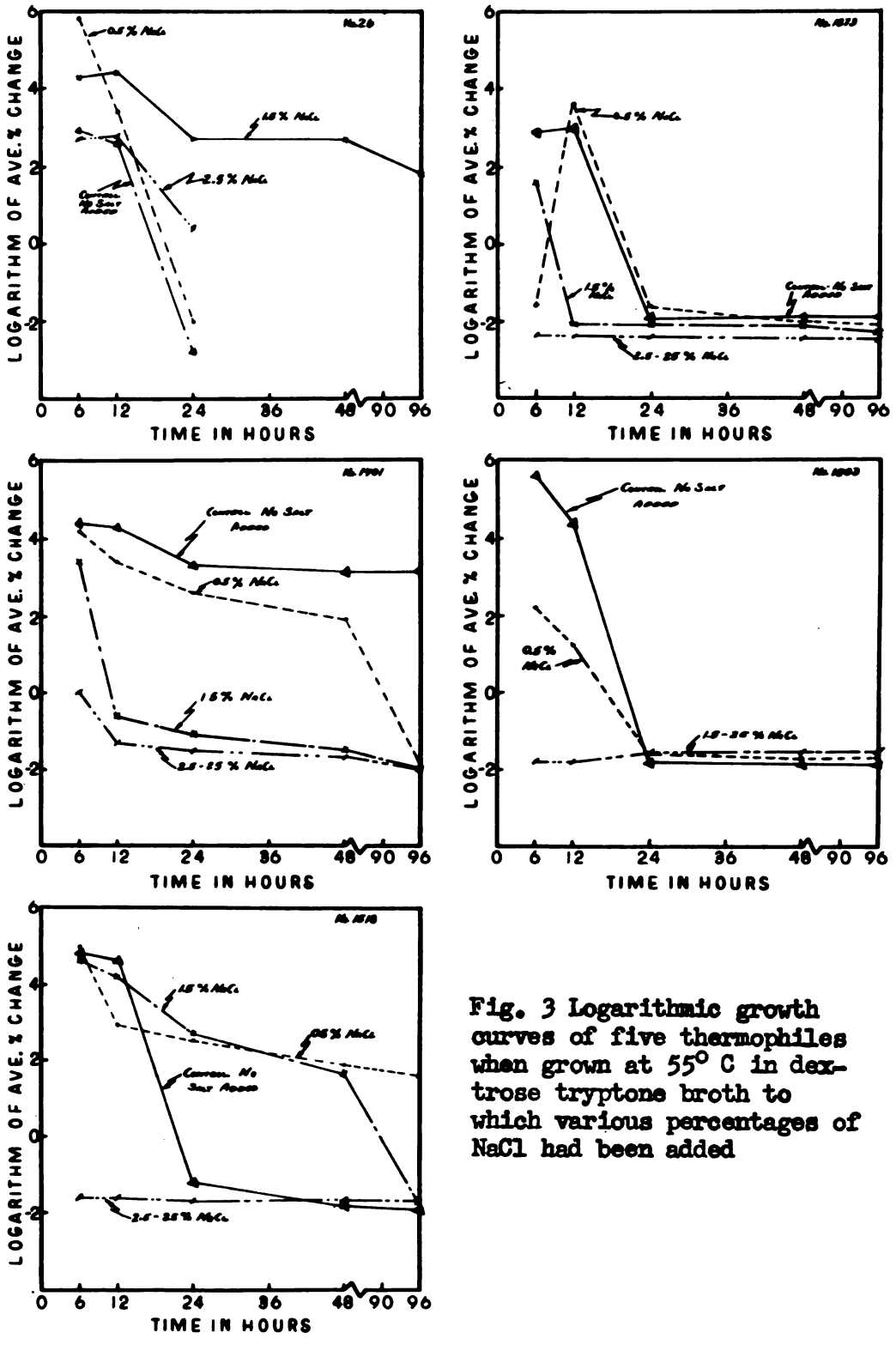

Fig. 3 Logarithmic growth curves of five thermophiles when grown at 55° C in dextrose tryptone broth to which various percentages of NaCl had been added

Explanations for the mode of action of organic acids:

What are the reasons for the order of the germicidal powers of the acids tested?

Several investigators have attributed the effect of added acids in suppressing the growth of bacteria to the change in the hydrogen ion concentration. However, Anderson et al [11] and Levine and Fellers [14] have pointed out that the toxicity of certain organic acids is due in part to the undissociated molecule and the anion in addition to the effect of the hydrogen ion.

Cohen and Clark [21] suggested that the action of acetic acid may be due to the free acetate radical exerting a synergistic effect upon the disinfecting power of the hydrogen ion.

Reid [22] believes that the anion and undissociated molecule may act independently in exerting their disinfectant action or as positive catalysers increasing the bactericidal power of the hydrogen ions.

The influence of acids on the surface tension of the medium and the size of the molecule have been mentioned by Bach [23] as possible reasons for observed germicidal action. He also stated that the hydrogen ions control the antiseptic effect but the undissociated part of lactic acid is the germicidal factor when the pH value is important.

Traube's rule [24] states that in a homologous series of fatty acids the lowering of surface tension is parallel to the increase in molecular weight of the fatty acid. This may explain why citric

acid is more germicidal than either acetic or lactic since of the three acids citric has the largest molecular weight

Acetic acid = m.w. = 60.0

Lactic acid m.w. = 90.6

Citric acid m.w. =192.0

The dissociation of the acids may also account for the order of germicidal action observed:

Dissociation constants of the acids used

	Acid	Dissociation constants(K.)
1-	Citric	8.7×10^{-4} ($K_2 = 1.8 \times 10^{-5}$)
2-	Acetic	1.752×10^{-5}
3-	Lactic	1.37×10^{-4}

The above order indicates that if dissociation was the prime factor in the germicidal action then the order should be citric > lactic > acetic. This again may explain why citric acid was more toxic than either acetic or lactic acid. Since acetic acid was found to be more germicidal than lactic acid the undissociated molecule may be toxic as suggested by Anderson and co-workers[11] and Levine and Fellers[14].

The effect of Salt:

From Table 3 and Fig. 3 it may be seen that up to 1.5 per cent sodium chloride was non-germicidal except in the case of organism #1503. For # 1503 germicidal action took place at 1.5 per cent but not at 0.5 per cent. Concentrations of 2.5 per cent or greater proved germicidal in all cases. With culture # 26 total destruction took

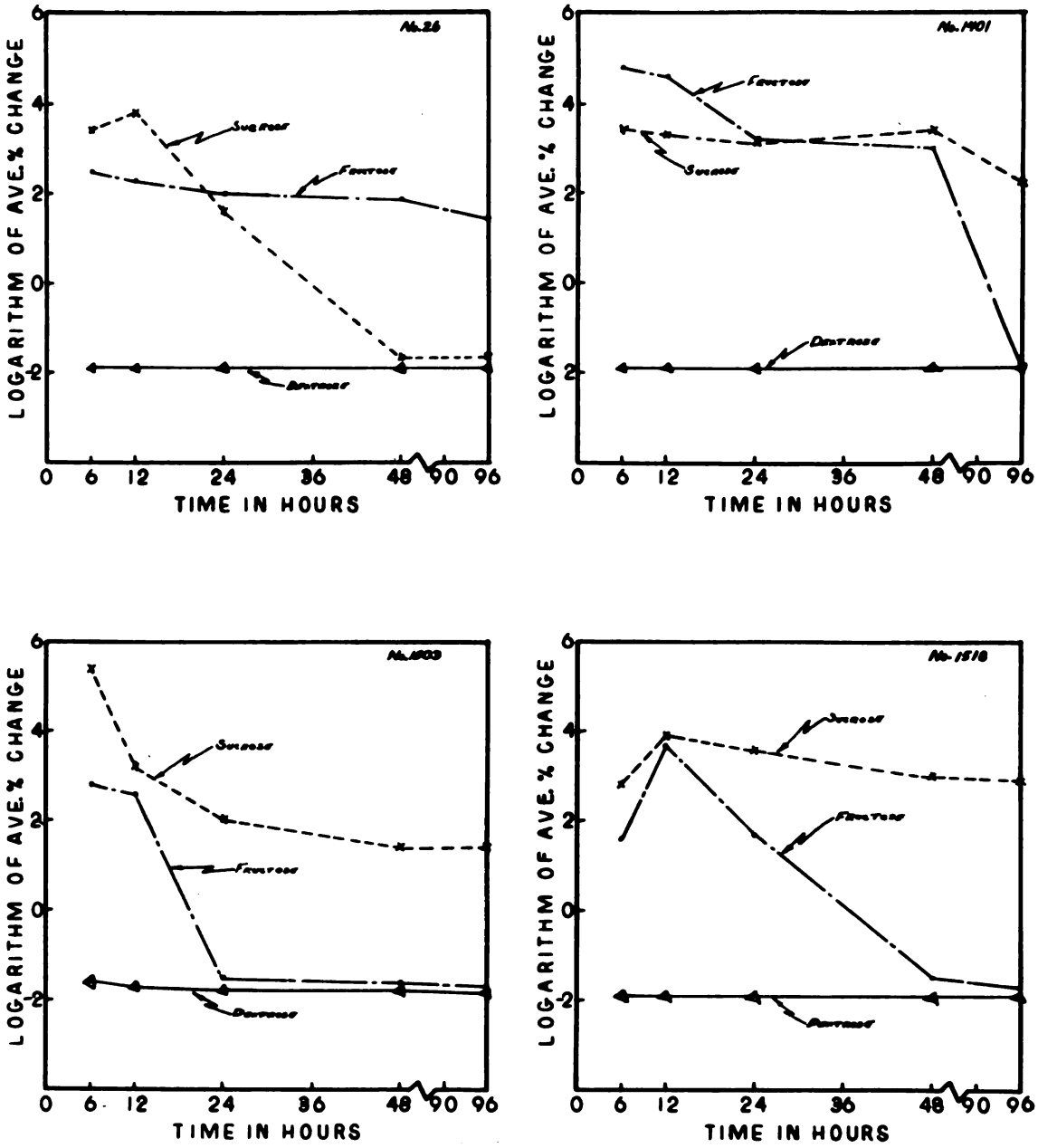

Fig. 4 Logarithmic growth curves of five thermophiles when grown at 55° C in tryptone broth to which different percentages of dextrose, fructose and sucrose respectively had been added.

place at 5.0 per cent and greater.

The reason for the action of salt in inhibiting the growth of bacteria or destroying the cells is not readily apparent.

Anderson er al [11] observed that there is obviously no toxic entity in salt. Moreover, as pre-mentioned, certain investigators including Viljoen[17] have attributed a protective effect from heat on some micro-organisms by certain concentrations of salt. Anderson and his colleagues think that, in as much as the addition of increasing amounts of salt to media is known to result in increased hydrogen ion concentrations, the lethal effect of salt may be due at least in part to the lowered pH of the substrate.

The effect of salt on bacteria is thought by many investigators to be mainly and simply plasmolytic. Plasmolysis, however, is believed to last for only a few hours and not all bacterial cells are affected.

Rockwell and Ebertz[25] suggested that at least five factors are involved in the detrimental action of salt on bacteria, namely;

1- The direct effect of the chloride ion.

2- Dehydration.

3- The removal of oxygen.

4- Sensitization against carbon dioxide.

5- Interference with the rapid action of proteolytic enzymes.

In addition to the above postulates it may be that, due to the dissociation of sodium chloride in solution, the sodium and chloride ions, though necessary for the growth of some organisms, and acceleratory in small amounts,

become toxic in large quantities i.e. as the concentration of salt is increased. This is an established fact in bacterial physiology. The law of diminishing returns seems evident as the concentration of salt gets beyond 2.5 per cent since even a concentration of 25.0 percent gave no significant increase in germicidal action.

The effect of sugars

From the average percentages of increase or decrease over the initial inocula in the presence of varying concentrations of dextrose, fructose and sucrose shown in Table 4 and Fig. 4 it may be seen that 5.0 per-cent dextrose did not suppress growth over the first 6-12 hours of incubation in the case of cultures 26, 1373 amd 1518. A decrease of 66 per cent occured after 12 hours in culture 1503. For culture 1401 a decrease of 72 per cent took place after 6 hours. For all organisms a germicidal action ensued after 6 hours in the presence of 10.0 per cent or greater.

During incubation periods of 6-12 hours multiplication took place in the presence of 10.0 per cent fructose. Concentrations of 20 per cent or greater proved germicidal in all cases.

Except for organisms #1401 sucrose in equivalent concentrations gave results in the same trend as fructose. Sucrose however, was less destructive beyond 20.0 per cent. The reverse of this holds true for organism 1401.

For all three sugars increasing the concentration beyond that needed for germicidal action endowed no increased lethality. The

effectiveness of the sugars in reducing the bacterial count may therefore be written as dextrose > fructose > sucrose.

Reasons for the action of the sugars and the order of germicidal action found

The reasons for the germicidal action of sugars against micro-organisms are not well established. Concentrations of 10-50 per cent of sucrose and dextrose are known to cause no appreciable change in the pH of culture media, hence no significant portion of the germicidal action of the sugars can be attributed to pH changes.

Fay[20] thinks that in both the protective and germicidal actions the influence of sugars on osmotic pressure determines their effectiveness. In his opinion the permeability of the individual cell apparently plays an important part in regulating the rate of death in water and in sugar suspensions. He hypothesises that resistance to the effects of sugars is limited to those cells which are highly sensitive to water at slightly increased temperatures.

Plasmolysis of the microbial cell may explain why dextrose exerted a germicidal effect at a lower concentration than either fructose or sucrose and why fructose exerted a greater germicidal action at the same concentration as sucrose. Osmotic pressure and hence plasmolysis depends on the number of particles in solution. Dextrose and fructose each with a molecular weight of 180 would therefore contain more molecules per unit of weight than would sucrose. The activity should therefore tend to decrease as the molecular weight increases.

On a chemical basis, dextrose, an aldehyde sugar, should be less reactive than fructose, a keto sugar, as evidenced by the fact that many chemical tests can be carried out on the latter in the cold while the former must be heated. This order of activity apparently does not hold biologically since dextrose was found to be more germicidal than fructose. The reason for this is not apparent.

SUMMARY AND CONCLUSIONS

The growth of thermophilic bacteria was found to be very rapid reaching a maximum within 8-14 hours followed by a rapid decline in cell numbers. The lag phase of the growth cycle is not very pronounced and after 96 hours there were less cells present than in the initial inocula.

The order of germicidal action of the acids studies is citric > acetic > lactic. An increase in acidity beyond pH 5.0 gave no increased germicidal action.

Concentrations of sodium chloride up to 1.5 percent were not germicidal except for organism # 1401 in which case a lethality took place at this concentration. Concentrations of 2.5 per cent and greater were germicidal in all cases. An increase in salt concentration beyond 2.5 per cent gave no added germicidal effect.

The order of effectiveness of the sugars as germicides was found to be dextrose > fructose > sucrose. In all cases concentrations beyond 20.0 per cent gave no significant increase in germicidal action.

From the growth curves and tables 1, 2, 3 and 4 it may be concluded that the deleterious action of various food ingredients on thermophilic bacteria occurs during the first 6 to 12 hours of incubation. This interval corresponds roughly to the phase of physiological growth and the early logarithmic phase of the bacterial growth curve.

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