SOME STUDIES ON FLAT SOUR TYPE MICROORGANISMS PRESENT IN BEET SUGAR

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I. INTRODUCTION

Spoilage of non-acid canned vegetables is often caused by the activity of bacteria or enzymes which they elaborate. Three groups of thermophilic spore forming bacteria of major importance have been definitely associated with this spoilage. These are: (a) The flat sour type, characterized by the production of acids without gas from carbohydrates. (b) The hard swell type, a thermophilic anaerobe characterized by the production of acid and gas (not hydrogen sulphide) from carbohydrates. (c) The anaerobic thermophiles, characterized by the production of hydrogen sulphide gas. (1).

Cameron and Williams (2) in 1926 reported on the bacteriological examinations of the raw materials used by a large canning company. They were able to isolate the above mentioned groups of thermophilic spoilage organisms from refined white sugar. In this work a definite relationship was established between the bacteriological condition of the sugar and the thermophilic spoilage of non-acid canned foods. This led to a desire on the part of the canners to buy a sugar that had been tested for suitability for canning

and started investigational work on the part of the sugar refining companies. This work was followed by an announcement by some of the companies that they were ready to supply a suitable canning sugar as judged by their own standards (3). This, in turn, created a need for a suitable bacterial standard for sugar to be met by all refiners. The National Canners Association introduced a bacterial standard for sugar in 1931 which they used in testing sugars for member canners. A revision of this standard was made in 1932, and at the present time in "Bacterial Standards For Sugar" (4) a method is given for the testing of sugar for the presence of these three types of spoilage organisms and a limit is set for their tolerance. Five samples from any one shipment of sugar are examined bacteriologically. The limit of tolerance for the flat sour type spoilage organisms ~~being~~ is a maximum of not more than 75 spores and an average of not more than 30 spores per ten grams of sugar.

While plating numerous samples of beet sugar to detect the presence of the spores of "flat sour" bacteria, as recommended by the National Canners Association, it was observed that colonies of acid producing organisms of the flat sour type developed

on plates incubated at 23° C and 30° C. It was primarily with the organisms isolated at the above temperatures that the experimental work to be discussed in this thesis was conducted. Flat sour types of organisms capable of growing at these temperatures are of particular interest because of their possible relationship to this type of spoilage of improperly processed canned goods held in storage.

II. EXPERIMENTAL

A. Isolation and Description of Organisms

Historical

The research laboratories of the National Canners Association (4) give the following method for the detection of the spores of the flat sour type bacteria. "Place 20 grams of sugar in a sterile 150 ml. Erlenmeyer flask marked to indicate a volume of 100 ml. Add sterile water to the 100 ml. mark. Bring rapidly to boiling and boil for five minutes. Replace evaporation with sterile water. Into each of five petri dishes pipette 2 ml. of the boiled sugar solution. Cover and mix the inoculum with Bacto dextrose tryptone agar. (This agar was developed by Difco Laboratories, Detroit, Michigan, and contains an indicator, bromcresol purple.) Incubate the plates at 55° C for from 36 to 48 hours. The combined counts for the five plates represent the number of spores in two grams of sugar. Multiply this by five in order to express the results in terms of number of spores per ten grams of sugar." The colony formed by flat sour bacteria is round, measures from two to five milimeters in diameter, presents a typical opaque central spot and by reason of acid production and

the presence of the indicator is usually surrounded by a yellow halo in a field of purple.

In 1926 Cameron and Esty (5) reported on studies carried out on organisms isolated from spoiled canned foods. They isolated 214 organisms and divided them into the following groups:

Group I--The aerobic spore formers.

Group II--The facultative thermophilic organisms.

Group III--The strict thermophilic organisms.

Members of Group I are not considered as causative agents in food spoilage and no further mention will be made of them. Organisms in Groups II and III are considered as typical in the production of flat sour type spoilage. The following table taken from this work gives the essential characteristics of these organisms in the form of index numbers.

Table II (Cameron and Esty)

Essential Characteristics

(Index numbers -- Soc. Am. Bact. Descriptive Chart)

Group II.	5221, 52220, 1222
	5221, 52230, 2223
Group III.	5212, 52120, 1222
	5212, 52220, 1232
	5212, 52120, 2232
	5212, 52220, 2232

Briefly the organisms in Group II were gram Positive rods producing terminal endospores,

facultative anaerobes and in nitrate broth produced nitrites with no gas. Acid without gas was formed in sucrose and dextrose broths. A description of this group is given because it was with organisms conforming more or less completely with this description that the following experimental work was conducted. Group III is not further considered for they are strict thermophiles.

Experimental

While plating sugar samples according to the previously mentioned method four sets of plates were made. One set each of these plates was incubated at the following temperatures: 23° C, 30° C, 37° C and 55° C. At the end of 48 hours incubation these plates were examined for the colonies of flat sour bacteria. Table I gives a count of the number of colonies developing at the various incubation temperatures as compared with the standard count at 55° C.

Table 1.

Spore Count of Flat Sour Type Bacteria in Beet Sugar

Sample No.	Incubation Temperature			
	23° C	30° C	37° C	55° C
206	5	5	340	400
207	10	20	215	320
270	5	20	205	300
72	10	40	270	420
22	0	0	0	10
139	170	505	625	750
78	10	210	395	480
46	25	40	470	645
288	55	160	---	230
231	5	35	120	295
226	15	85	100	160
279	0	25	---	175

Expressed as spores per 10 grams of sugar.

Acid producing colonies growing at 23° C and 30° C were chosen for this work. A transfer of

a well isolated colony was made on a plain agar slant and incubated at 30° C. In all 23 cultures were isolated from fifteen samples of refined beet sugar obtained from factories in Michigan, Ohio, and Illinois. Cultures numbers one to eight inclusive were isolated at 23° C, numbers 12 to 28 were isolated at 30° C. Inasmuch as comparative studies were to be made at 30° C, 37° C and 55° C, stock cultures of these organisms were carried in the laboratory at 30° C. Three additional cultures, numbers 29, 30, and 33; and two typical flat sour cultures, numbers 31 and 32, (the latter two obtained from the National Canners Association) were used for comparative purposes. All five cultures were isolated at 55° C. In the future these will be referred to only by number. As a check upon the purity of the cultures they were periodically plated and examined for contamination. Of the 28 cultures originally isolated, 21 were satisfactorily carried throughout this work, the experimental data being reported only on these.

The organisms isolated all conformed within reasonable limits to the previously described group II of the flat sour type of Cameron and Esty in that they were all gram positive rods

averaging about 1 by 4.0 microns in size, producing terminal endospores which sometimes caused a bulging of the rod. They were all facultative anaerobes and produced acid without gas in dextrose and sucrose broths. In addition to the above grouping they were divided into groups according to their ability to grow on potato slants. Those that showed no apparent growth on potato slants were designated as group A while those that showed an abundant yellowish brown butyrous growth were designated as Group B. Being interested in the ability of these organisms to produce acid from carbohydrates and the heat resistance of the spores of these organisms no further work was done on their classification.

Summary

Examination of Table I shows that refined beet sugar contains spore forming organisms that have the ability to produce acid at 30° C. This leads to the possibility that these organisms might prove to be a spoilage hazard in the canning industry. The organisms isolated conform very closely to Group II of the flat sour bacteria described by Cameron and Esty and due to their

ability to produce a nongaseous acid fermentation it is the opinion of the author that they should be considered as flat sour types. Their abilities to produce acid, to grow in concentrated sucrose solutions and to withstand high temperatures are points that will be considered in sections B and C of the experimental part.

B. Studies on Acid Production

Historical

The typical flat sour type spoilage is that of a nongaseous acid fermentation with slight if any odor and the pH value not markedly above 5.0. (6)

There is an apparent lack of information concerning acid production by flat sour type organisms isolated from sugar; however numerous papers have appeared concerning acid production of flat sour type organisms isolated from other sources. Wyant and Tweed (7) studied a group of these organisms in regard to their ability to produce the flat sour condition in canned foods. In this work they reported 5.6 as the lowest pH value that was obtained. Cameron and Esty (5) found that their group of facultative thermophilic organisms would produce acid in corn, peas, hominy and other non-acid canned vegetables reducing the pH value in all cases to below 5.0, and in most cases below 4.5.

Experimental

Acid production in dextrose broth--Aerobic

The ability of the organisms isolated in part A to ferment dextrose was determined as follows: Tubes containing 10 ml. of one per cent dextrose broth were inoculated with 0.3 ml. of a 24 hour broth culture of the organisms and along with uninoculated control tubes were incubated at 30° C, 37° C and 55° C. After a six day incubation period a five ml. sample from each of the inoculated tubes was diluted with five ml. of distilled water and titrated with N/20 NaOH using phenolphthalein indicator. The uninoculated control tubes were titrated in the same manner. The difference between the titration figures gives the total acidity produced by the microorganisms. These data are reported in Table II under AP (acid production). Group A being the organisms showing no growth on potato slants, group B the organisms showing growth on the potato slants.

Acid production in dextrose broth--Anaerobic

Tubes containing 10 ml. of one per cent dextrose broth were heated in flowing steam to expel the air,

cooled to about 40° C, inoculated with 0.3 ml. of a 24 hour broth culture of the organisms, sealed with two to three centimeters of sterile paraffin, and along with uninoculated control tubes were incubated at 30° C, 37° C, and 55° C. These were titrated at the end of a six day incubation period, the acid production being determined in the same manner as under aerobic conditions. The results of this work are reported in Table III under AP.

Acid production in pea juice.

Twelve organisms were studied to determine their ability to produce a flat sour condition in pea juice. This medium was obtained by pouring the juice from sound canned peas. Tubes containing 10 ml. of this juice were autoclaved at 15 pounds pressure for 20 minutes, cooled to about 40° C, inoculated with 0.3 ml. of a 24 hour broth culture of the organisms, sealed with about two centimeters of sterile melted paraffin and along with uninoculated control tubes incubated at 30° C and 55° C. After a six day incubation period the pH values of these tubes were determined electrometrically. Table IV gives the pH values of the inoculated tubes as compared with the uninoculated tubes.

Anaerobic conditions were employed in this work to simulate the conditions found in canned food products.

Acid production in and inhibitory action of sucrose.

Twelve cultures from Group A and five cultures from Group B of the organisms isolated under Part A were studied in regards to their ability to produce acid from sucrose and to determine the effect on acid production of various concentrations of sucrose. This work was carried out aerobically to simulate the conditions found in the canning factory when tanks of syrup have been held for a prolonged period. Tubes of broth containing 10 ml. of one per cent, 10 per cent, 20 per cent, and 40 per cent sucrose broth were prepared and inoculated with 0.3 ml. of a 24 hour culture of the organisms. One set each of these along with uninoculated control tubes were incubated at 30° C, 37° C, and 55° C. At the end of a six day incubation period a five ml. sample of each tube was diluted with five ml. of distilled water and titrated with N/20 NaOH using phenolphthalein indicator. These experimental data are reported in Table V, the acid production being calculated in the same manner as in Tables II and III.

TABLE II

Acid Production In Dextrose Broth Under Aerobic Conditions.

Incubation Temp.		30° C		37° C		55° C	
		AP*	DB**	AP	DB	AP	DB
Organism Number							
	1	.15	?	.10	?	.60	?
	2	.15	?	.25	?	.70	x
	3	.15	x	.25	x	.55	x
	4	.25	x	.20	x	.00	-
	6	.25	x	.30	x	.25	x
	8	.15	x	.40	x	.30	x
	15	.20	x	.15	x	.40	x
	17	.15	x	.10	x	.55	x
	21	.00	x	.35	x	.40	x
Group A	22	.05	x	.35	x	.40	x
	25	.05	x	.15	x	.60	x
	26	.05	x	.25	x	.20	-
	28	.30	x	.30	x	.55	x
	29	.00	-	.50	x	1.70	x
	30	.05	-	.45	x	1.90	x
	31	.00	-	.10	x	.70	x
	32	.00	-	.10	?	.40	x
	5	.55	x	.50	x	.10	-
	12	.45	x	.80	x	.45	x
	13	.75	x	.70	x	.50	x
	14	.65	x	.90	x	.65	x
Group B	18	.50	x	.85	x	.40	x
	19	.35	x	.65	x	.40	x
	20	.45	x	.50	x	.35	x
	23	.60	x	.65	x	.55	x
	24	.65	x	.85	x	.45	x
	33	.60	x	1.00	x	.45	x

*AP denotes increase in titratable acidity. This is the difference expressed in ml. between the titration figures for the experimental tubes and the control tubes when 5 ml. portions from these were titrated with N/20 NaOH using Phenolphthalein indicator.

**DB denotes growth in tubes as judged by turbidity.

x growth ? Questionable - No growth

TABLE III

Acid Production In Dextrose Broth Under Anaerobic Conditions.

	Incubation Temperature	30° C		37° C		55° C	
		AP*	DB**	AP	DB	AP	DB
Organism Number							
	1	.05	-	.05	-	.30	x
	2	.00	-	.05	-	.15	x
	3	.05	?	.10	x	.10	x
	4	.10	?	.05	?	.00	?
	6	.15	x	.05	x	.50	x
	8	.15	x	.05	?	.00	?
	15	.05	?	.25	?	.00	?
	17	.00	?	.40	?	.30	?
Group A	21	.00	?	.85		.05	?
	22	.00	?	.15	?	.35	x
	25	.00	?	.00	?	.30	x
	26	.00	?	.00	?	.00	?
	28	.30	x	.30	x	.05	-
	29	.00	-	.70	x	.85	x
	30	.00	-	.40	x	.85	x
	31	.00	-	.05	?	.30	x
	32	.00	-	.00	-	.45	x
	5	.15	x	.30	x	.05	?
	12	.70	x	.85	x	.20	?
	13	.65	x	.65	x	.45	x
	14	.55	x	.55	x	.25	x
Group B	18	.40	x	.80	x	.25	x
	19	.70	x	.70	x	.25	x
	20	.65	x	.85	x	.25	x
	23	.65	x	.70	x	.65	x
	24	.80	x	.65	x	.40	x
	33	.80	x	.85	x	.25	x

*AP denotes increase in titratable acidity. This is the difference expressed in ml. between the titration figures for the experimental tubes and the control tubes when 5 ml. portions from these were titrated with N/20 NaOH using phenolphthalein indicator.

**DB denotes growth as judged by turbidity.

x growth ? questionable -No growth

TABLE IV

Acid Production in Pea Juice

Incubation Temperature		30° C	55° C
Organism number		Final pH Value	
Group A	1	5.86*	5.89*
	2	5.94	5.94
	6	5.91	5.81
	28	5.41	5.75
	29	5.42	4.91
	31	5.95	5.05
	32	5.80	4.96
	12	5.18	5.62
	13	5.18	5.59
	14	5.35	5.40
	24	5.38	5.61
	33	5.37	4.85
Control (uninoculated)		6.10	6.05

*pH value determined electrometrically

TABLE V

Acid Production in Sucrose Broth

Incubation Temperature		30° C				37° C				35° C			
Per cent sucrose		1	10	20	40	1	10	20	40	1	10	20	40
Organism Number													
	1	.05*	.05	.00	.00	.05	.05	.00	.00	.00	.00	.00	.00
	2	.00	.05	.00	.00	.10	.05	.05	.00	.00	.00	.00	.00
	3	.00	.05	.00	.00	.10	.05	.05	.05	.05	.05	.00	.00
	4	.05	.05	.00	.00	.10	.10	.05	.05	.10	.00	.00	.00
	6	.05	.05	.05	.00	.20	.10	.05	.05	.10	.00	.05	.00
Group A	8	.05	.00	.00	.00	.00	.00	.00	.00	.35	.05	.05	.00
	25	.05	.05	.00	.00	.10	.05	.05	.00	.10	.05	.00	.05
	28	.10	.20	.10	.05	.20	.05	.05	.05	.10	.00	.00	.00
	29	.10	.10	.05	.00	.25	.15	.05	.00	1.40	1.15	.45	.00
	30	.10	.10	.00	.00	.30	.30	.05	.05	1.00	.85	.85	.00
	31	.00	.00	.00	.00	.00	.00	.00	.00	.20	.15	.05	.00
	32	.00	.00	.00	.00	.05	.00	.00	.00	.15	.15	.05	.00
	5	.15	.30	.20	.10	.35	.25	.05	.05	.05	.00	.00	.00
	12	.35	.30	.25	.20	.55	.35	.20	.15	.20	.20	.20	.10
Group B	13	.45	.45	.20	.15	.45	.35	.25	.10	.25	.30	.20	.15
	14	.60	.30	.20	.10	.35	.30	.20	.10	.15	.05	.05	.00
	33	.15	.10	.00	.00	.20	.20	.15	.05	1.25	1.00	.35	.00

*This is the increased acidity as determined by titrating 5 ml. sample from the experimental tubes and 5 ml. sample from the control tubes with N/20 NaOH using phenolphthalein indicator the results being expressed in ml. increased acidity, i. e. the difference between the titration figures for the experimental tubes and the control tubes.

Summary

Examination of Tables II and III shows that the organisms isolated at 23° C and 30° C produced acid in dextrose broth under both aerobic and anaerobic conditions at 30° C, 37° C and 55° C. Maximum acid production occurred at 30° C and 37° C with the exception of two organisms which preferred 55° C for maximum acid production. Since the greatest acid production, as judged by titratable acidity, was in the aerobic tubes, the author feels justified in considering these organisms as facultative anaerobes.

The hydrogen ion concentration of pea juice inoculated with these organisms and incubated anaerobically at 30° C was markedly increased. The final pH values ranged from 5.18 to 5.94 in juice with an initial pH of 6.10. In pea juice with an initial pH of 6.05, inoculated with the same organisms but incubated anaerobically at 55° C, the final pH values ranged from 4.85 to 5.94.

Acid production, aerobically, was noted in one per cent sucrose broth with decreasing acid production in tubes of increasing concentration of sucrose.

C. Heat Resistance.

Historical

The resistance of bacterial spores to heat has been studied in great detail. In 1920 Bigelow and Esty (8) made a comprehensive study of the heat resistance of thermophilic flat sour type spores. They stated that the initial concentration of the spores and the hydrogen ion concentration of the medium influenced greatly the thermal death point of the spores of resistant organisms.

The effect of the hydrogen ion concentration in regard to the thermal death point was determined by heating a definite number of spores in food juices of different pH values and it was concluded that, as the pH value of a medium is lowered the time required for the complete destruction of the spores is decreased. They studied the effect of the initial concentration of spores in corn juice with a pH value of 6.0 at temperatures of from 100° C to 130° C. The results of this work led to their conclusion that in a medium of known hydrogen ion concentration, at a given temperature, the larger the number of spores present the longer will be the time required to sterilize the medium. They report finding spores of thermophilic organisms that would survive a temperature of 100° C for 20 hours.

Wyant and Tweed (7) in 1923 made a study of the thermal death times of the spores of flat sour type organisms isolated from cold packed canned peas. In no case did they have an organism that would resist a temperature of 110° C for over 10 minutes.

In 1928, Cameron and Williams (2) made an interesting study on the thermal death time of the spores of flat sour thermophiles isolated from sugar. They found that the effective killing time for a spore suspension of 100,000 per ml. in phosphate solution of pH 6.97, at a temperature of 110° C, varied from 5 to 240 minutes.

Experimental

Preparation of Stock Spore Suspensions

Suspensions of the organisms containing between 90 and 100 per cent spores without resorting to a heat treatment to kill the vegetative cells, were prepared in the following manner: Dextrose agar slants were inoculated with the organisms and incubated for 48 hours at 30° C (organisms numbers 29 to 33 being incubated at 55° C). These slants were then placed in jars and incubated for a period of from 4 to 8 days under reduced pressure (approximately 22 inches of vacuum). Suspensions of these spores were made by carefully washing the slants with sterile saline (pH--6.9) and transferring to sterile tubes. These spore suspensions

were then examined microscopically to determine the percentage of spores and standardized by dilution with sterile saline until one ml. of the suspension contained approximately one million spores as determined by the standard plate count, allowances being made for the vegetative cells. This was considered as the stock spore suspension and held in the ice box for future use.

Heat Resistance Determinations.

By experimentation it was found that a temperature of 100° C could be controlled by immersing a 200 ml. Erlenmeyer flask containing saline in an oil bath held at between 105° C and 110° C.

To determine the thermal resistance of the spores, 200 ml. Erlenmeyer flasks containing 99 ml. of sterile saline were immersed in an oil bath held at between 105° C and 110° C until they started to boil. (Temperature--100° C). They were then inoculated with 1 ml. of the stock spore suspension and thoroughly mixed. (This mixing must be done by keeping the flasks immersed in the oil, otherwise there is a rapid drop in temperature.) At definite time intervals, enrichment tubes containing 5 ml. of dextrose tryptone broth were inoculated with one ml. of the spore suspension which was being held at 100° C.

The sterility of this spore suspension was determined by incubating the enrichment tubes for four days and determining the acid production as indicated by bromcresol purple indicator. The first tube showing no acid production ~~boiler~~ was considered sterile. Table VI shows the heat resistance of these spores as determined by the above method. As a check upon the efficiency of this method for determining the sterility of the spore suspensions, dextrose tryptone agar plates were made using one ml. samples from the first enrichment tubes which showed no acid production after four days incubation. Upon examination after a 48 hour incubation period all of these plates were found to be sterile.

Further heat resistance studies were made on 10 of the stock spore suspensions when subsequently suspended in pea juice pH 6.10. Erlenmeyer flasks containing 99 ml. of sterile pea juice were immersed in an oil bath, the temperature being regulated to keep the contents of the flask at 100° C. They were then inoculated with one ml. of the stock spore suspension, the thermal death time being determined by the previously described method. Table VII gives the thermal death times for these spores when suspended in pea juice pH 6.10 as compared to their thermal death times when suspended in saline (pH 6.9).

An attempt was made to determine quantitatively the thermal resistance of these spores. For this work

flasks containing 99 ml. of sterile saline were held at 100° C in an oil bath and inoculated with one ml. of the standard spore suspension and thoroughly mixed. At definite time intervals tubes containing nine ml. of sterile saline were inoculated with one ml. of the spore suspension from these flasks. Dilution plates were then made in an attempt to determine the number of viable spores present at the end of the various time intervals. It was determined that a rapid rate of killing was obtained during the first intervals of heating with a small percentage of heat resistant spores surviving. Inconsistent results were obtained when the number of viable spores was determined by plating methods. It was often noted that when few viable spores remained it was impossible to obtain a definite trend in the rate of killing. Qualitative methods gave results that indicated a definite and orderly trend in the rate of killing when the same method of heating and sampling was used.

TABLE VI

Thermal Death Time at 100° C

Spores suspended in saline (pH 6.9)

Organism	Number	Minutes required to destroy spores at 100° C* Inefficient	Efficient
	1	80	100
	2	80	100
	3	60	80
	4	10	20
	6	10	20
	8	50	60
	15	10	20
	17	20	30
Group A	21	5	10
	22	30	40
	25	10	20
	26	10	20
	28	10	20
	29	180	240
	30	180	240
	31	360	? **
	32	360	? **
	5	10	20
	12	50	60
	13	50	60
	14	60	70
Group B	18	5	10
	19	10	20
	20	20	30
	23	60	70
	24	5	10
	33	180	240

*Concentration of spores 10,000 per ml.

**Organisms 31 and 32 were the organisms received from the National Canners Association. They report that a concentration of 100,000 spores per ml. of these organisms, suspended in corn juice with a pH value of 6.1 would resist a temperature of 100° C for 17 hours.

TABLE VII

Thermal Death Time at 100° C

Minutes required to destroy spores at 100° C*

Organism Number	Spores in pea juice pH 6.10		Spores in saline pH 6.90	
	Inefficient	Efficient	Inefficient	Efficient
1	50	60	80	100
8	10	20	50	60
12	50	60	50	60
13	50	60	50	60
14	60	70	60	70
15	10	20	10	20
22	10	20	30	40
23	60	70	60	70
25	5	10	10	20
17	5	10	20	30

*Concentration of spores 10,000 per ml.

Summary

The thermal death time at 100° C for the spores of the organisms isolated at 25° C and 30° C, when suspended in saline pH 6.90, varied from 10 minutes to 100 minutes. Thirteen of the 22 spore suspensions included in this experiment were destroyed within 30 minutes. Four additional spore suspensions were destroyed within 60 minutes while the remaining five suspensions required between 70 and 100 minutes for complete destruction.

The thermal death time at 100° C for the organisms isolated at 55° C was 240 minutes.

There was a decrease in the thermal death time at 100° C for the spores of these organisms when suspended in pea juice pH 6.10 as compared to the thermal death time when suspended in saline pH 6.90. Five of the ten organisms included in this work were destroyed within 20 minutes when suspended in pea juice as compared to two when suspended in saline. Three additional organisms were destroyed within 60 minutes when suspended in pea juice as compared to five organisms when suspended in saline. The remaining two of the suspensions in pea juice were destroyed within 70 minutes. Two of the remaining three suspensions in saline were destroyed within 70 minutes, the third requiring between 70 and 100 minutes for complete destruction.

III GENERAL DISCUSSION

In this work it was pointed out that refined beet sugar contains spore forming organisms that will produce acid at 30° C, with the possibility that these organisms would prove to be a spoilage hazard in the canning industry.

Studies on these organisms showed that at 30° C they would produce a non-gaseous acid fermentation in sucrose and dextrose broths. Further studies on acid production showed a marked increase in the hydrogen ion concentration of pea juice when inoculated with cultures of these organisms and incubated anaerobically for six days at 30° C. The final pH value in some cases being 5.10 as compared to the original pH value of 6.10. It is very probable that with a longer incubation period the pH value would have reached 5.0 to 4.5 which is frequently attained in the flat sour type spoilage of non-acid canned foods. Since it was desired to simulate the conditions that might be found in canned foods no determinations were made aerobically.

In studying the heat resistance of the organisms it was found that for a spore concentration of 10,000 per ml. suspended in physiological salt solution (pH 6.90) at 100° C the effective killing time varied from 10 minutes to 100 minutes, for the same concentration of spores suspended in pea juice

(pH 6.10) at 100° C the effective killing time varied from 10 minutes to 70 minutes. These results conform with the work of Bigelow and Esty (8) who have previously shown that as the hydrogen ion concentration of a medium is increased the time required for the complete destruction of the spores is decreased.

In this work there was a definite relationship noted between the optimum temperature for acid production and the thermal death times of the organisms. The higher the optimum temperature for acid production the greater the heat resistance of the organisms. This relationship can be clearly shown by making a comparative study of Tables II, III and VI.

IV. GENERAL SUMMARY

1. Refined beet sugar contains facultative anaerobic spore forming organisms that have the ability to produce acid without gas from carbohydrates at 30° C.
2. Many of these organisms, under anaerobic conditions, will produce acid without gas in pea juice lowering the pH value of the medium to 5.18 which can be considered as a flat sour condition.
3. Acid production of these organisms was greatly inhibited by a sucrose concentration of between 20 and 40 per cent.
4. There was a definite relationship noted between the optimum temperature for acid production and the thermal death time for these organisms. The higher the optimum temperature for acid production, the greater was their thermal death time.
5. As the hydrogen ion concentration of a medium is increased, the time required for complete destruction of the spores is decreased.
6. The heat resistance of the spores of the organisms isolated at 23° C and 30° C is such that they should not be considered of significance in the present commercial canning processes. However, the heat resistance of these organisms is significantly high so that it may be a spoilage hazard in the home canning practices.

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