THE EFFECT OF DIFFERENT STORAGE TEMPERATURES ON THE BACTERIAL FLORA OF MASSECUITE

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F. W. Fabian
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THE EFFECT OF DIFFERENT STORAGE TEMPERATURES ON THE BACTERIAL FLORA OF MASSECUITE

By

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INTRODUCTION

Over 7,800,000 short tons of sugar are consumed annually in the United States. Of this amount 1,847,738 tons are beet and 5,952,262 tons are cane sugar. The United States produces only 513,260 tons of cane sugar. The balance of this large tonnage is shipped from such countries as Hawaii, Puerto Rico, Virgin Islands, Philippines, and Cuba. In shipping sugar from such far distances there has always been the problem of bacterial deterioration taking place between the raw sugar factory and the refinery in the United States. The problem dealing with the deterioration of sucrose in storage has been approached from the standpoint of both beet and cane sugar. The bacteria involved and their control in both instances are similar although there are distinct problems in both the beet and cane sugar refinery.

Browne (1) reported losses as high as $1,150,925 a year due to the deterioration of raw cane sugar in shipments from Cuba. Owen (21) confirmed the findings of Browne and estimated the seasonal losses of 1923-24 at $1,450,000. This condition has been improving throughout the years but still remains a problem. Cameron and Williams (7) considered raw sugar coming into the sugar refinery as one of the three major sources of contamination of the refined sugar. If the number of micro-organisms in raw sugar could be kept at a minimum, it would help control one of the initial foci of contamination. Browne (1) did not believe that all these organisms played such a major role in sugar

deterioration since he found that sugar held in storage for a period of two years would be practically sterile at the end of this time. While it is doubtless true that bacteria would die out in raw sugar held for a period of two years, however, most of the raw sugar is not held so long but is refined much sooner.

This study was concerned primarily with two things; first, the influence of the storage temperature of raw sugar on the mesophilic and thermophilic bacterial counts and, second, a comparison of the National Canners Association's method, hereafter known as the N.C.A. method, of determining thermophilic bacteria in sugar with the method proposed by Jones (17).

REVIEW OF LITERATURE

The literature concerning the presence of micro-organisms in sugar and their role in the deterioration of sugar and contamination of foods by sugar which require a sweeting agent is voluminous. One of the most recent literature reviews on the subject is that of Hucker and Pederson (14). No attempt will be made in this paper to cover all the literature but only that immediately applicable to the problem.

The work may be divided into two parts, first, the role of the mesophilic organisms found in sugar factories and, second, the microbial deterioration of sugar. The first recorded work in literature pertaining to bacteria in sugar was that of Kircher (18) in 1839 when he found micro-organisms in slimy beet sugar, and discovered that he

could produce this slimy condition when growing the organisms in a sugar solution.

In 1878 Cienkowski (10) working with the organism discovered by Kircher, which was commonly known in the sugar industry as "frog spawn", named it Ascococcus mesenteroides. Later Van Tieghem (26) in a study of this slime producing organism, changed its name to Leuconostoc mesenteroides by which it is still known.

Owen (21) was one of the first workers to recognize the importance of sporogenic group of bacteria as a cause of the deterioration of raw sugar in storage. His conclusion was that the destruction of sucrose was due to the production of levanase by a group of sporogenic bacteria, which resembled the potato bacillus. Van der Bijl (25), in studying the effect of temperature on this group of organisms concluded that they could withstand 100°C. (212°F.) for two hours or more. It was also found that this group of organisms could withstand a 1:50 solution of formalin or a one percent solution of sodium fluoride for 30 minutes.

The other group of mesophiles that were studied from the standpoint of their ability to deteriorate sugar were yeast and molds. Owen (21) (22) and Browne (1) showed the ability of yeasts and molds to invert sucrose, which they considered a fundamental cause in the breakdown of raw sugar. Owen (21) showed the ability of three species of molds to invert sucrose to levulose; Penicillium glaucum, Monilia nigra, and Torula communis.

Owen (22) suggested a method of preserving raw sugar by the addition or inoculation of sugar with Torulae. These yeasts were

capable of growth in the high tension film of molasses surrounding the crystal of raw sugar, but were incapable of fermenting sucrose. In the growth of this yeast on the sugar film, carbon dioxide was produced from the levulose fermentation. According to Owen the carbon dioxide surrounding the sugar crystal prevented the growth of other bacteria.

The second period may be considered one of the most important in some respects as it was during this period that the emphasis was placed on sugar as a source of micro-organisms when added to other food products. The workers during this period were more interested in the number and type of bacteria and their effect on foods that required a sweeting agent, than the effect of the micro-organism on the sugar itself.

Weinzirl (27) in working with the spoilage of candy, discovered that 85 percent of 33 samples tested contained anaerobes. This was the first record in literature that sugar was a source of bacterial contamination in candy.

The work of Barlow (2) in 1912 showed the presence of thermophilic bacteria in sugar, but it was not until 1928 when Cameron, Williams, and Thompson (8) traced the spoilage of canned corn to the sugar added to it, that their real significance was recognized. This work (8) led Cameron and Williams (7) and Cameron (3) (4) (5) to make a complete study of the sporogenic, thermophilic bacteria in sugar. They found three groups of bacteria in sugar of major importance to the canner which they classified as : (a) thermophilic anaerobes which produce acid but no gas and are commonly known as "flat sours," (b)

thermophilic anaerobes which form acid and gas (gas is CO_2 and H_2) and known as "gassy anaerobes" or "hard swells" and (c) thermophilic anaerobes which produce traces of acid and gas consisting chiefly of hydrogen sulphide and commonly known as "stinkers" or "H_2S producers." Of the three groups of bacteria group (a), or the flat sour type, is by far the most common and causes the greatest amount of trouble. It is likewise the most insidious of the three groups because it produces no gas - only acid - and therefore gives no warning of its presence in canned foods. The other two groups advertise their presence by the gas they produce and the effect it has on the concave can.

Cameron and Yesair (9), and Cameron and Biglow (6) made a thorough investigation of the previous work done and proved the high resistance of the thermophilic organisms found in sugar.

In a study on the thermophilic food spoilage organisms in beet sugar, Hall (11) (12) showed that there was a gradual decrease in the thermophilic flora of refined sugar after eight and 20 months storage. He found that the most rapid reduction occurred shortly after the sugar was manufactured. The sugar was packed in four mediums; paper, burlap, toweling, and glass. There was no noticeable effect in the type of container used and the survival of the micro-organisms present.

To eliminate the sporogenic group of bacteria from sugar, Hall and Keane (13) tried the incorporation of ultra violet light, (2537 $\mathring{A}$), into the refining process. It was found that the bacterial flora could be

reduced by 50 percent by the use of these lights, but even so it is not used by many of the refiners today.

It was found that by careful sanitary methods in the refining process that the sporogenic group of bacteria could be kept to a minimum. With this knowledge at hand the National Canners Association (19) has set up standards for "Canning Grade Suger."

PROCEDURE AND METHOD

In carrying out this study, samples of massecuite were received weekly from the Revere Sugar Refinery, Charlestown, Massachusetts, for a period of one month. Each sample was divided into four batches of five pounds each, and placed in one gallon cardboard Sealright Sanitary Containers. The raw sugar was stored at four different temperatures; 55°C. (131°F.), 21°C. (70°F.), 4.5°C. (40°F.), and -23.3°C. (-10°F.).

EXAMINATION FOR MICRO-ORGANISMS

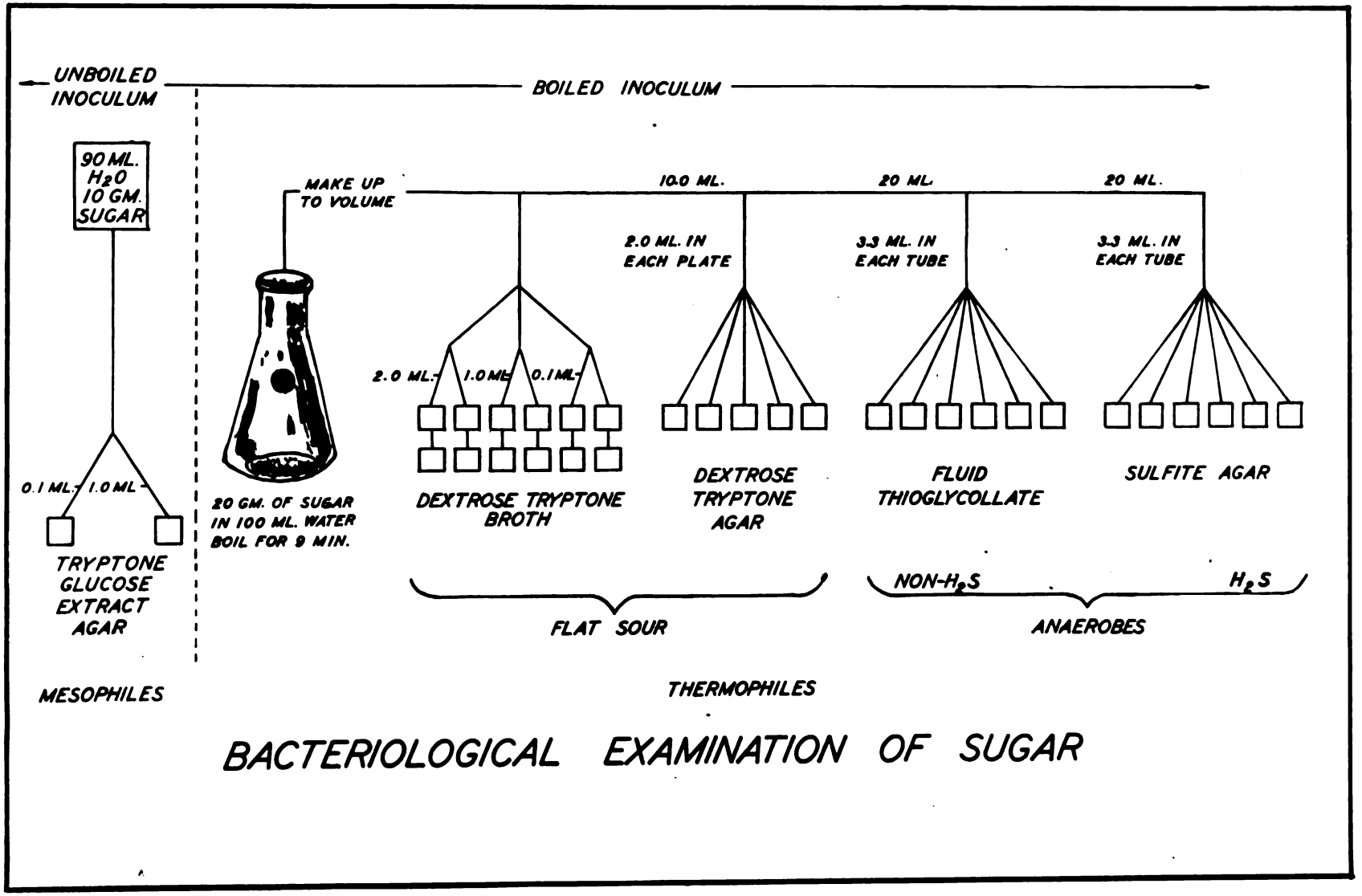
The massecuites held at the various temperatures were analyzed bacteriologically for both the mesophilic and thermophilic type of bacteria. The method of examination for thermophilic micro-organisms was the one established by the National Canners Association (19) and adopted as the official method by the Association of Official Agricultural Chemists (20). In addition to the National Canners Method of determining flat sour thermophiles, the method proposed by Jones (17) was also run to see if there was a correlation between the two methods. The

media used for determining the different thermophilic groups were Difco dextrose tryptone agar for flat sour bacteria of the Bacillus stearothermophilus type according to the National Canners Association method (19) and the same medium, made in the laboratory, without agar according to the Jones method (17). Jones (17) suggested substituting dextrose tryptone broth with brom cresol purple as an indicator, for the detection of flat sour organisms. Using pure cultures he showed that there was a direct correlation between the two methods. Each tube contained the Durham fermentation tube to show the presence of gas if other types of thermophiles were present. The typical flat sour organism is one that produces acid without gas from dextrose.

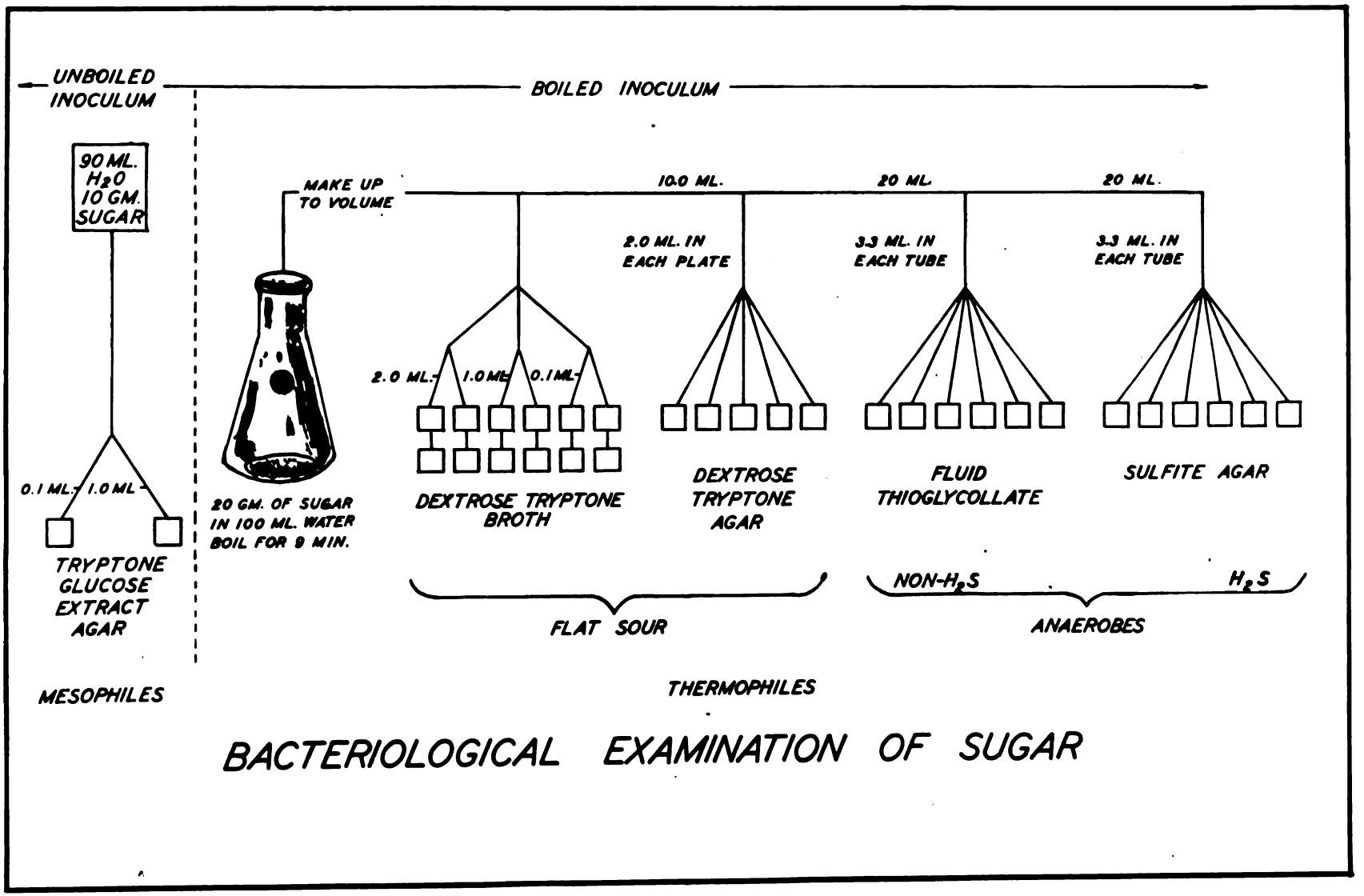
Modified sulfide agar was used for detecting thermophilic hydrogen sulfide bacteria of the Clostridium nigrificans type according to the National Canners Association method (19) and a modified thioglycollate medium for detecting gassy anaerobes of the Clostridium thermosaccharolyticium type. All plates were incubated at 55°C. for 48 hours.

The mesophiles were determined by plating on Difco tryptone glucose beef extract agar and incubated at 30°C. and counted at the end of 48 to 72 hours.

Fig. 1 illustrates diagramatically the schema used for determining the respective groups of bacteria present in the massecuite.



BACTERIOLOGICAL EXAMINATION OF SUGAR



BACTERIOLOGICAL EXAMINATION OF SUGAR

DISCUSSION

The most important group of thermophilic organisms from the standpoint of their ability to spoil canned non-acid foods, are the flat sour bacteria. In this study, the emphasis was placed on this group of organisms, as it is felt by most of the workers that they give the best overall index of contamination. If time and material allowed for only one test to be run, the test for flat sours would be the most important one.

Table 1. Showing relative viability of the flat sour bacteria at various storage temperatures over a six month period.

(National Canners Association method)

Sample	55°C.	21.1°C.	4.4°C.	-23.3°C.
	Percentage reduction of original count.			
A	-100.0	-56.6	- 7.6	- 3.3
B	-100.0	-76.4	-46.2	-34.8
C	- 96.6	-41.9	- 7.7	-10.0
D	- 88.4	-10.8	- 8.1	-30.3

(Jones' method)

Sample	55°C.	21.1°C.	4.4°C.	-23.3°C.
	Percentage reduction of original count			
A	-90.9	- 9.0	- 9.6	+ 1.5
B	-78.1	-10.4	-10.0	- 5.9
C	-95.5	-13.5	- 4.8	-15.9
D	-84.5	- 3.0	- 0.9	- 4.8

Two methods of analysis were used in determining the effect of different storage temperatures on this group of organisms. These were the N.C.A. methods and the method proposed by Jones.

The greatest reduction in the flat sour bacteria occurred at 55°C., in which case there was approximately 100 percent reduction in all four samples during 85 days storage period as shown in Table 3, Fig. 2a, b, c, and d, and Fig. 3a, b, c, and d. At 21.1°C. there was an average reduction of 46.4 percent over a six months period, Table 4, Fig. 4a, b, c, and d, and Fig. 5a, b, c, and d. At temperatures of 4.4°C. and -23.3°C. there was an average reduction of 17.4 and 19.6 percent respectively, which indicates at these temperatures the bacteria died very slowly.

The Jones' method for the analysis of flat sour bacteria is analogous to the dilution method used in the analysis of coliforn bacteria in water and milk. However, in the Jones' method, a positive tube for flat sours indicates the formation of acid without gas, while in the latter it indicates the fermentation of lactose with gas. The method for plotting the viability of the flat sour organisms, as determined by the Jones' method, was a comparison of the percent positive tubes, to the total number of tubes in all of three dilutions. For example, if there were nine positive tubes out of the 12 total, this would mean that 75 percent of the tubes had viabile bacteria present. This method of calculation was used throughout this paper. The trend line was determined by the method of least squares and plotted accordingly. Although it is impossible to calculate the coefficient of correlation between the two

methods, the respective graphs showed similar trends with respect to the number of viable bacteria in the massecuite when stored at different temperatures for the same period of time. Table 3, Fig. 2a, b, c, and d, Jones' method, and Table 3, Fig. 3a, b, c, and d, N.C.A. method, showed corresponding trends at 55°C. where there was a respective reduction of 87.2 and 100 percent. However, as the temperature decreased the correlation between the two methods was less marked because the Jones' method is more sensitive than the N.C.A. method since it picks up more positive flat sour bacteria. For example, at 21.1°C. (Table 4, Fig. 4a, b, c, and d) Jones' method and Table 4, Fig. 5a, b, c, and d, N.C.A. method there was a reduction of only 9.4 percent by the Jones' method and 46.4 percent by the N.C.A. method. At the lower temperatures of 4.4°C. Table 5, Fig. 6a, b, c, and d, Jones' method and Table 5, Fig. 7a, b, c, and d, N.C.A. method, there is a reduction of 6.4 and 17.4 percent respectively. At -23.3°C. Table 6, Fig. 8a, b, c, and d, Jones' method, and Table 6, Fig. 9a, b, c, and d, N.C.A. method, the respective reductions were 6.3 and 19.6 percent. This shows that there is a greater correlation at the two lower temperatures (4.4° and -23.3°C.) than at room temperature.

A study was also made of the influence of storage temperatures on the total aerobic, thermophilic and mesophilic bacterial plate counts. These data are given in detail in Table 3 to 6. The data in Table 2 were determined by calculating the trend line in each case by the method of least squares and then computing the increase or decrease of the actual counts in terms of this trend line. The data so obtained show that at

55°C. over an 85 day period there was a decrease in both the mesophilic and thermophilic counts in all samples. In fact the samples were practically sterile at the end of this time.

Table 2. Showing relative viability of the thermophilic and mesophilic group of bacteria at various storage temperatures over a six month period.

(thermophilic bacteria)

Sample	55°C.	21.1°C.	4.4°C.	-23.3°C.
	Percentage increase or reduction of original count.			
A	- 87.1	-11.6	+12.5	+71.1
B	-100.0	-40.2	+ 6.3	+40.2
C	- 83.1	+26.3	+39.4	+39.4
D	- 87.3	+20.3	+76.6	+ 5.6

(mesophilic bacteria)

Sample	55°C.	21.1°C.	4.4°C.	-23.3°C.
	Percentage increase or decrease of original count.			
A	-100.0	+33.5	-58.5	+35.9
B	- 76.6	-29.3	-62.4	-76.6
C	-100.0	-19.1	-63.3	-32.5
D	-100.0	- 1.5	-76.1	- 1.7

At 21.1°C. over a six month period the thermophilic count showed a decrease in half the samples and an increase in the other half while the mesophilic count showed a decrease in three out of four samples. At 4.4°C. there was no decrease in thermophiles but a slight increase while in the mesophiles there was a decrease in all samples.

This may have been due to the sporogenic nature of the two groups. At the still lower storage temperature, over the six month period, the thermophiles again showed no decrease but an increase while the mesophiles showed a decrease in all except one sample.

Two other groups of thermophilic bacteria were also determined to make the bacteriological picture complete; the anaerobic sulfide spores, "stinkers," and the anaerobic "hard swell" spores.

The "stinker" spores, which were first reported as the cause of spoilage of canned corn, were not predominate in any large numbers in three of the four samples tested. In sample "C" there was a large number of these spores present, which ran as high as 150 spores per 10 grams of raw sugar. Again it was noticed that the greatest reduction took place at 55°C. and then at 21.1°C. At the cooler temperatures of 4.4° and -23.3°C. there was no noticeable reduction over the six month period.

The anaerobic "hard swell" spores were predominate in all six tubes over the entire testing period with the exception of the samples stored at 55°C. At this temperature there was an approximate 90 to 100 percent reduction in the 85 day testing period. In massecuite this test is not sensitive enough, due to the large inoculum and the number of spores present.

Critical Temperature Studies

At the completion of the first study, it was felt that there was a more critical temperature for the reduction of the thermophilic spore

count that would fall between the ranges of 30° to 55°C. An additional 20 pounds of massecuite was obtained from the Revere Sugar Refinery and divided into four batches of five pounds each. These were stored at 30°, 37°, 45°, and 55°C., and examined bacteriologically each week up to a period of 46 days.

Table 8. Influence of temperatures between 30° to 55° C. on the thermophilic and mesophilic counts over a 46 day period.

Temperatures	Flat sours N.C.A. method	Flat sours Jones' method	Total thermophiles	Total mesophiles
	Percentage increase or decrease in count.			
30°C.	-30.0	-15.6	- 45.2	+12.3
37°C.	-43.1	-20.0	- 28.4	+20.0
45°C.	-19.6	-17.3	- 30.8	-73.7
55°C.	-96.5	-46.5	-100.0	-89.8

It was found that the greatest reduction in the flat sour spore count again occurred at 55°C. with a 96.5 percent reduction in the 46 days. On the whole the next most favorable temperature for the reduction of organisms was 45°C. where there was a decrease in most cases. In general as the temperature was lowered there was less decrease in the number of bacteria. This was especially true in the case of the mesophiles where there was a slight increase at 37° and 30°C.

Table 3. Thermophilic and mesophilic bacterial plate counts of massecuite held at 55°C. for 85 days.

SAMPLE A (55°C.)

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
1-14	155	0.0	100	130	+ + + +	+ + + +	+ - - -	500
1-23	60	0.0	84	40	+ + + +	+ + + +	- - - -	150
1-30	75	0.0	100	55	+ + + +	+ + - -	+ - - -	350
2-4	50	0.0	84	20	+ + + +	+ - - -	+ - - -	200
2-18	45	0.0	50	10	+ + + +	+ + - -	- - - -	150
2-25	40	0.0	67	5	+ + - -	- - - -	- - - -	200
4-6	20	0.0	17	0	- - - -	- - - -	- - - -	0

SAMPLE B (55°C.)

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
1-14	285	0.0	100	185	+ + + +	+ + + +	+ - - -	350
1-23	120	0.0	84	85	+ + + +	+ + + +	- - - -	400
1-30	125	0.0	84	125	+ + + +	+ + + +	+ + - -	200
2-4	90	0.0	84	70	+ + + +	+ + + +	- - - -	100
2-18	60	0.0	67	50	+ + + +	+ + + +	+ - - -	200
2-25	55	0.0	50	25	+ + + +	+ + - -	- - - -	200
4-6	0	0.0	0	0	- - - -	- - - -	- - - -	100

* H.S. = Hard swells * D.T. = Dextrose tryptone

Table 3. (Cont'd.)

SAMPLE C (55°C.)

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
1-14	130	0.0	100	95	+ +	+ +	+ +	
					+ +	+ +	+ -	650
1-23	65	2.5	100	40	+ +	+ +	- -	
					+ +	+ +	- -	600
1-30	85	0.0	84	70	+ +	+ +	+ -	
					+ +	+ -	- -	100
2-4	85	0.0	100	40	+ +	+ +	+ -	
					+ +	- -	- -	200
2-18	85	0.0	50	35	+ +	+ -	- -	
					- -	- -	- -	200
2-25	20	0.0	67	5	+ +	- -	- -	
					+ -	- -	- -	200
4-6	15	0.0	34	5	- -	- -	- -	
					- -	- -	- -	100

SAMPLE D (55°C.)

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
1-23	290	0.0	100	145	+ +	+ +	+ -	
					+ +	+ +	- -	400
1-30	160	0.0	84	90	+ +	+ +	- -	
					+ +	+ +	- -	350
2-4	125	0.0	84	70	+ +	+ +	- -	
					+ +	+ -	- -	200
2-18	50	0.0	50	30	+ +	+ +	- -	
					+ +	- -	- -	200
2-25	25	0.0	17	10	+ +	- -	- -	
					+ -	- -	- -	100
4-6	0	0.0	0	0	- -	- -	- -	
					- -	- -	- -	0

* H.S. = Hard swells * D.T. = Dextrose tryptone

Table 4. Thermophilic and mesophilic bacteria plate count of massecuite held at 21.1°C. for six months.

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
1-4	980	7.5	100	700	+ + + +	+ + + +	+ + + +	800
1-11	830	2.5	100	430	+ + + +	+ + + +	+ + + -	1000
1-21	650	7.5	100	545	+ + + +	+ + + +	+ + + -	950
1-28	790	0.0	100	560	+ + + +	+ + + +	+ + + -	1200
2-6	835	2.5	100	690	+ + + +	+ + + +	+ + + -	800
2-13	850	2.5	100	630	+ + + +	+ + + +	+ + + -	1150
2-20	810	5.0	100	525	+ + + +	+ + + +	+ + + -	1400
2-28	710	0.0	100	395	+ + + +	+ + + +	+ + - -	1400
3-5	865	2.5	100	320	+ + + +	+ + + +	+ + - -	1300
3-21	850	0.0	100	380	+ + + +	+ + + +	+ + + -	600
3-29	870	0.0	100	405	+ + + +	+ + + +	+ + + -	500
4-6	815	0.0	100	330	+ + + +	+ + + +	+ + + -	500
4-13	860	0.0	100	380	+ + + +	+ + + +	+ + - -	600
4-20	780	5.0	100	370	+ + + +	+ + + +	+ + - -	750
5-9	710	0.0	100	400	+ + + +	+ + + +	+ + + -	800
5-20	875	0.0	100	350	+ + + +	+ + + +	+ + + -	1100
6-22	725	0.0	100	295	+ + + +	+ + + +	+ + + -	700
6-29	800	0.0	100	270	+ + + +	+ + + +	+ + - -	1300
7-9	730	0.0	100	295	+ + + +	+ + + +	+ + - -	1700
7-15	675	0.0	100	310	+ + + +	+ + + +	+ + + -	1700

* H.S. = Hard swells * D.T. = Dextrose tryptone

Table 4. (Cont'd.)

SAMPLE B (21.1°C.)

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
1-4	395	15.0	100	210	+ +	+ +	+ +	950
					+ +	+ +	+ -	
1-11	500	5.0	100	240	+ +	+ +	+ +	800
					+ +	+ +	- -	
1-21	835	10.0	100	720	+ +	+ +	+ +	750
					+ +	+ +	+ -	
1-28	760	2.5	100	610	+ +	+ +	+ +	600
					+ +	+ +	+ -	
2-6	660	5.0	100	490	+ +	+ +	+ +	300
					+ +	+ +	- -	
2-13	425	5.0	100	345	+ +	+ +	+ +	600
					+ +	+ +	- -	
2-20	340	2.5	100	280	+ +	+ +	+ +	700
					+ +	+ +	- -	
2-28	575	5.0	100	275	+ +	+ +	+ +	1000
					+ +	+ +	- -	
3-5	375	5.0	100	200	+ +	+ +	+ +	800
					+ +	+ +	- -	
3-21	475	10.0	100	170	+ +	+ +	+ -	600
					+ +	+ +	- -	
3-29	825	27.5	100	175	+ +	+ +	+ -	300
					+ +	+ +	- -	
4-6	490	10.0	100	185	+ +	+ +	+ -	500
					+ +	+ +	- -	
4-13	440	2.5	100	190	+ +	+ +	+ -	450
					+ +	+ +	- -	
4-20	395	2.5	100	230	+ +	+ +	+ +	400
					+ +	+ +	+ -	
5-9	405	0.0	100	190	+ +	+ +	+ +	400
					+ +	+ +	- -	
5-20	310	0.0	100	210	+ +	+ +	+ +	400
					+ +	+ +	- -	
6-22	355	0.0	100	150	+ +	+ +	+ +	500
					+ +	+ +	+ -	
6-29	340	7.5	100	125	+ +	+ +	+ +	700
					+ +	+ +	- -	
7-9	315	0.0	100	125	+ +	+ +	+ -	750
					+ +	+ +	- -	
7-15	300	2.5	100	145	+ +	+ +	+ -	550
					+ +	+ +	- -	

* H.S. = Hard Swells * D.T. = Dextrose tryptone

Table 4. (Cont'd.)

SAMPLE C (21.1°C.)

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
1-4	345	180.0	100	175	+ + + +	+ + + +	+ + + -	800
1-11	1310	55.0	100	590	+ + + +	+ + + +	+ + + -	650
1-21	395	22.5	100	280	+ + + +	+ + + +	+ + + +	700
1-28	420	50.0	100	300	+ + + +	+ + + +	+ + + -	800
2-6	380	47.5	100	325	+ + + +	+ + + +	+ + - -	450
2-13	540	142.5	100	310	+ + + +	+ + + +	+ + - -	500
2-20	650	180.0	100	310	+ + + +	+ + + +	+ + - -	1100
2-28	475	60.0	100	200	+ + + +	+ + + +	+ + + -	800
3-5	580	92.5	100	305	+ + + +	+ + + +	+ + - -	850
3-21	620	296.0	100	310	+ + + +	+ + + +	+ + - -	600
3-29	915	82.5	100	330	+ + + +	+ + + +	+ + - -	600
4-6	580	37.5	100	220	+ + + +	+ + + +	+ + - -	550
4-13	630	122.5	100	310	+ + + +	+ + + +	+ + + -	600
4-20	580	97.5	100	300	+ + + +	+ + + +	+ + + -	550
5-9	860	50.0	100	355	+ + + +	+ + + +	+ + + -	490
5-20	850	30.0	100	250	+ + + +	+ + + +	+ + + -	500
6-22	460	125.0	100	155	+ + + +	+ + + +	+ + - -	600
6-29	735	80.0	100	175	+ + + +	+ + + +	+ + - -	650
7-9	600	30.0	100	175	+ + + +	+ + + +	+ + - -	650
7-15	690	32.5	100	225	+ + + +	+ + + +	+ + - -	700

* H.S. = Hard Swells * D.T. = Dextrose tryptone

Table 4. (Cont'd.)

SAMPLE D (21.1°C.)

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
					+ +	+ +	+ +	
1-21	280	25.0	100	235	+ +	+ +	- -	400
					+ +	+ +	+ +	
1-28	375	2.5	100	200	+ +	+ +	- -	450
					+ +	+ +	+ +	
2-6	405	10.0	100	280	+ +	+ +	- -	550
					+ +	+ +	+ +	
2-13	415	22.5	100	300	+ +	+ +	- -	600
					+ +	+ +	+ +	
2-20	455	2.5	100	210	+ +	+ +	+ -	600
					+ +	+ +	+ -	
2-28	350	0.0	100	180	+ +	+ +	- -	700
					+ +	+ +	+ +	
3-5	480	15.0	100	250	+ +	+ +	- -	600
					+ +	+ +	+ +	
3-21	460	7.5	100	180	+ +	+ +	- -	500
					+ +	+ +	+ -	
3-29	645	7.5	100	175	+ +	+ +	- -	300
					+ +	+ +	+ +	
4-6	480	5.0	100	180	+ +	+ +	- -	350
					+ +	+ +	+ +	
4-13	525	7.5	100	250	+ +	+ +	- -	300
					+ +	+ +	+ +	
4-20	510	0.0	100	220	+ +	+ +	- -	300
					+ +	+ +	+ +	
5-9	430	5.0	100	200	+ +	+ +	- -	350
					+ +	+ +	+ +	
5-20	480	2.5	100	275	+ +	+ +	- -	600
					+ +	+ +	+ +	
6-22	390	0.0	100	170	+ +	+ +	- -	300
					+ +	+ +	+ +	
6-29	450	5.0	100	200	+ +	+ +	- -	600
					+ +	+ +	+ +	
7-9	475	5.0	100	220	+ +	+ +	- -	600
					+ +	+ +	+ -	
7-15	485	7.5	100	205	+ +	+ +	- -	650

* H.S. = Hard Swells * D.T. = Dextrose tryptone

Table 5. Thermophilic and mesophilic bacteria plate count of massecuite held at 4.4°C. for six months.

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
1-17	330	2.5	100	205	+ +	+ +	+ +	2600
					+ +	+ +	- -	
1-31	745	5.0	100	500	+ +	+ +	+ +	2450
					+ +	+ +	+ -	
2-7	745	0.0	100	605	+ +	+ +	+ +	1200
					+ +	+ +	+ -	
2-14	730	0.0	100	405	+ +	+ +	+ +	1500
					+ +	+ +	+ -	
2-21	1135	2.5	100	490	+ +	+ +	+ +	1600
					+ +	+ +	+ -	
3-1	675	0.0	100	305	+ +	+ +	+ +	1300
					+ +	+ +	- -	
3-7	705	0.0	100	275	+ +	+ +	+ +	1300
					+ +	+ +	- -	
3-17	745	7.5	100	400	+ +	+ +	+ +	1050
					+ +	+ +	+ -	
3-30	800	0.0	100	275	+ +	+ +	+ +	900
					+ +	+ +	- -	
4-7	800	2.5	100	275	+ +	+ +	+ +	1100
					+ +	+ +	- -	
4-14	820	2.5	100	480	+ +	+ +	+ +	1250
					+ +	+ +	- -	
4-24	780	2.5	100	385	+ +	+ +	+ +	1200
					+ +	+ +	+ -	
5-14	755	0.0	100	400	+ +	+ +	+ +	850
					+ +	+ +	+ -	
5-21	720	0.0	100	350	+ +	+ +	+ +	750
					+ +	+ +	+ -	
6-21	760	0.0	100	355	+ +	+ +	+ +	1000
					+ +	+ +	- -	
7-1	800	7.5	100	375	+ +	+ +	+ +	1100
					+ +	+ +	- -	
7-13	770	7.5	100	385	+ +	+ +	+ +	1000
					+ +	+ +	- -	
7-20	750	2.5	100	375	+ +	+ +	+ -	1150
					+ +	+ +	- -	

* H.S. = Hard Swells * D.T. = Dextrose tryptone

Table 5. (Cont'd.)

SAMPLE B (4.4°C.)

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
1-17	500	7.5	100	480	+ + + +	+ + + +	+ + + -	2100
1-31	435	10.0	100	380	+ + + +	+ + + +	+ + - -	500
2-7	375	40.0	100	285	+ + + +	+ + + +	+ - - -	400
2-14	425	7.5	100	325	+ + + +	+ + + +	+ - - -	950
2-21	380	5.0	100	225	+ + + +	+ + + +	+ - - -	550
3-1	365	7.5	100	200	+ + + +	+ + + +	+ - - -	1450
3-7	315	0.0	100	185	+ + + +	+ + + +	+ + - -	800
3-17	550	5.0	100	225	+ + + +	+ + + +	+ - - -	600
3-30	525	7.5	100	195	+ + + +	+ + + +	+ + - -	550
4-7	475	12.5	100	165	+ + + +	+ + + +	+ - - -	700
4-14	325	5.0	100	170	+ + + +	+ + + +	+ - - -	750
4-24	560	7.5	100	260	+ + + +	+ + + +	+ - - -	800
5-14	420	22.5	100	185	+ + + +	+ + + +	+ - - -	600
5-21	405	15.0	100	195	+ + + +	+ + + +	+ + - -	650
6-21	425	12.5	100	200	+ + + +	+ + + +	- - - -	500
7-1	455	20.0	100	185	+ + + +	+ + + +	+ - - -	550
7-13	475	17.5	100	210	+ + + +	+ + + +	+ - - -	450
7-20	440	12.5	100	230	+ + + +	+ + + +	+ + - -	400

* H.S. = Hard Swells * D.T. = Dextrose tryptone

Table 5. (Cont'd.)

SAMPLE C (4.4°C.)

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
1-17	225	125.0	100	195	+ +	+ +	+ +	
					+ +	+ +	+ -	1200
1-31	535	135.0	100	275	+ +	+ +	+ +	
					+ +	+ +	+ -	1500
2-7	520	57.5	100	265	+ +	+ +	+ +	
					+ +	+ +	+ -	8050
2-14	630	152.5	100	385	+ +	+ +	+ +	
					+ +	+ +	+ +	11700
2-21	705	27.5	100	440	+ +	+ +	+ +	
					+ +	+ +	- -	9200
3-1	650	47.5	100	350	+ +	+ +	+ +	
					+ +	+ +	+ -	8750
3-7	485	80.0	100	150	+ +	+ +	+ +	
					+ +	+ +	+ -	13300
3-17	475	75.0	100	125	+ +	+ +	+ +	
					+ +	+ +	- -	4500
3-30	485	70.0	100	125	+ +	+ +	+ +	
					+ +	+ +	- -	3700
4-7	540	67.5	100	150	+ +	+ +	+ +	
					+ +	+ +	- -	2400
4-14	770	65.0	100	400	+ +	+ +	+ +	
					+ +	+ +	+ -	1600
4-24	555	52.5	100	200	+ +	+ +	+ +	
					+ +	+ +	+ -	4700
5-14	590	72.5	100	250	+ +	+ +	+ +	
					+ +	+ +	+ -	4300
5-21	495	105.0	100	240	+ +	+ +	+ +	
					+ +	+ +	+ -	4100
6-21	690	130.0	100	275	+ +	+ +	+ +	
					+ +	+ +	+ -	3800
7-1	705	135.0	100	260	+ +	+ +	+ +	
					+ +	+ +	+ -	3150
7-13	775	135.0	100	270	+ +	+ +	+ +	
					+ +	+ +	- -	3900
7-20	790	125.0	100	255	+ +	+ +	+ +	
					+ +	+ +	- -	3850

* H.S. = Hard Swells * D.T. = Dextrose tryptone

Table 5. (Cont'd.)

SAMPLE D (4.4°C.)

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
1-17	195	10.0	100	130	+ +	+ +	+ +	900
					+ +	+ +	- -	
1-31	370	2.5	100	230	+ +	+ +	+ +	1050
					+ +	+ +	- -	
2-7	455	10.0	100	340	+ +	+ +	+ -	1100
					+ +	+ +	- -	
2-14	410	7.5	100	325	+ +	+ +	+ +	1050
					+ +	+ +	- -	
2-21	380	22.5	100	210	+ +	+ +	+ -	1100
					+ +	+ +	- -	
3-1	415	0.0	100	270	+ +	+ +	+ +	1000
					+ +	+ +	- -	
3-7	290	7.5	100	150	+ +	+ +	+ -	1700
					+ +	+ +	- -	
3-17	255	7.5	100	115	+ +	+ +	- -	800
					+ +	+ +	- -	
3-30	405	7.5	100	140	+ +	+ +	+ -	900
					+ +	+ +	- -	
4-7	875	10.0	100	205	+ +	+ +	+ +	700
					+ +	+ +	+ -	
4-14	525	7.5	100	255	+ +	+ +	+ +	600
					+ +	+ +	- -	
4-24	750	7.5	100	235	+ +	+ +	+ +	550
					+ +	+ +	- -	
5-14	780	2.5	100	185	+ +	+ +	+ +	250
					+ +	+ +	- -	
5-21	495	2.5	100	230	+ +	+ +	+ +	250
					+ +	+ +	- -	
6-21	505	2.5	100	230	+ +	+ +	- -	350
					+ +	+ +	- -	
7-1	525	0.0	100	250	+ +	+ +	+ +	350
					+ +	+ +	- -	
7-13	475	7.5	100	250	+ +	+ +	+ +	400
					+ +	+ +	- -	
7-20	545	10.0	100	245	+ +	+ +	+ +	300
					+ +	+ +	- -	

* H.S. = Hard Swells * D.T. = Dextrose tryptone

Table 6. Thermophilic and mesophilic bacterial plate counts of massecuite held at -23.3°C. for six months.

SAMPLE A (-23.3°C.)

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
1-19	335	0.0	100	215	+ + + +	+ + + +	+ + - -	3700
1-26	280	10.0	100	175	+ + + +	+ + + +	+ + - -	900
2-2	360	0.0	100	260	+ + + +	+ + + +	+ + - -	700
2-9	610	2.5	100	580	+ + + +	+ + + +	+ + + -	700
2-16	595	0.0	100	305	+ + + +	+ + + +	+ - - -	900
2-23	605	2.5	100	305	+ + + +	+ + + +	- - - -	1000
2-29	575	0.0	100	305	+ + + +	+ + + +	+ - - -	1300
3-18	690	2.5	100	350	+ + + +	+ + + +	+ - - -	1300
4-2	835	2.5	100	335	+ + + +	+ + + +	+ - - -	2700
4-10	835	0.0	100	375	+ + + +	+ + + +	+ - - -	3600
4-17	920	0.0	100	455	+ + + +	+ + + +	+ + - -	2000
5-1	810	5.0	100	355	+ + + +	+ + + +	+ + - -	1950
5-15	780	0.0	100	385	+ + + +	+ + + +	+ + - -	2300
5-22	760	0.0	100	345	+ + + +	+ + + +	+ + - -	1600
6-26	670	0.0	100	230	+ + + +	+ + + +	+ + - -	1400
7-7	600	2.5	100	245	+ + + +	+ + + +	+ + - -	2200
7-14	710	0.0	100	300	+ + + +	+ + + +	+ + - -	1800
7-21	760	0.0	100	325	+ + + +	+ + + +	+ - - -	1900

* H.S. = Hard Swells * D.T. = Dextrose tryptone

Table 6. (Cont'd.)

SAMPLE B (-23.3°C.)

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
1-19	535	0.0	100	485	+ + + +	+ + + +	+ + + +	600
1-26	230	0.0	100	180	+ + + +	+ + + +	+ - - -	2400
2-2	235	5.0	100	165	+ + + +	+ + + +	+ - - -	600
2-9	325	7.5	100	260	+ + + +	+ + + +	+ - - -	600
2-16	260	7.5	100	220	+ + + +	+ + + +	+ - - -	1200
2-23	230	0.0	100	145	+ + + +	+ + + +	+ - - -	500
2-29	170	7.5	100	155	+ + + +	+ + + +	+ - - -	600
3-18	175	0.0	100	165	+ + + +	+ + + +	+ - - -	500
4-2	500	5.0	100	185	+ + + +	+ + + +	+ - - -	600
4-10	305	0.0	100	200	+ + + +	+ + + +	+ - - -	500
4-17	400	2.5	100	225	+ + + +	+ + + +	+ - - -	450
5-1	350	2.5	100	160	+ + + +	+ + + +	+ - - -	500
5-15	300	0.0	100	120	+ + + +	+ + + +	+ + - -	600
5-22	340	0.0	100	150	+ + + +	+ + + +	+ + - -	450
6-26	475	0.0	100	165	+ + + +	+ + + +	+ + - -	350
7-7	330	2.5	100	195	+ + + +	+ + + +	+ - - -	400
7-14	350	0.0	100	175	+ + + +	+ + + +	+ + - -	300
7-21	425	0.0	100	190	+ + + +	+ + + +	- - - -	350

* H.S. = Hard Swells * D.T. = Dextrose tryptone

Table 6. (Cont'd.)

SAMPLE C (-23.3°).

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
1-19	265	62.5	100	205	+ + + +	+ + + +	+ + + +	1600
1-26	290	67.5	100	225	+ + + +	+ + + +	+ + + -	1500
2-2	670	60.0	100	445	+ + + +	+ + + +	+ + + +	2300
2-9	590	90.0	100	290	+ + + +	+ + + +	+ + + +	3050
2-16	640	280.0	100	230	+ + + +	+ + + +	+ + + +	4800
2-23	620	182.5	100	170	+ + + +	+ + + +	+ + + +	2400
2-29	435	182.5	100	165	+ + + +	+ + + +	+ + + -	5200
3-18	450	105.0	100	225	+ + + +	+ + + +	+ + - -	2400
4-2	595	187.5	100	205	+ + + +	+ + + +	+ + - -	2400
4-10	620	102.5	100	300	+ + + +	+ + + +	+ + - -	1800
4-17	750	65.0	100	295	+ + + +	+ + + +	+ + + -	1900
5-1	780	70.0	100	290	+ + + +	+ + + +	+ + + -	1700
5-15	795	50.0	100	220	+ + + +	+ + + +	+ + + -	2400
5-22	825	62.5	100	230	+ + + +	+ + + +	+ + + -	1200
6-26	700	42.5	100	265	+ + + +	+ + + +	+ + - -	2400
7-7	500	120.0	100	200	+ + + +	+ + + +	+ + - -	2200
7-14	620	125.0	100	230	+ + + +	+ + + +	+ + - -	2000
7-21	600	127.5	100	250	+ + + +	+ + + +	+ + - -	1900

* H.S. = Hard Swells * D.T. = Dextrose tryptone

Table 6. (Cont'd.)

SAMPLE D (23.3°C.)

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
1-26	235	0.0	100	205	+ + + +	+ + + +	+ + - -	300
2-2	555	0.0	100	400	+ + + +	+ + + +	+ + - -	1200
2-9	425	0.0	100	240	+ + + +	+ + + +	+ + - -	1000
2-16	455	7.5	100	275	+ + + +	+ + + +	+ + - -	900
2-23	470	10.0	100	275	+ + + +	+ + + +	+ - - -	3400
2-29	390	0.0	100	215	+ + + +	+ + + +	+ - - -	1300
3-18	375	20.0	100	225	+ + + +	+ + + +	+ - - -	1500
4-2	380	2.5	100	230	+ + + +	+ + + +	+ - - -	1500
4-10	525	2.5	100	220	+ + + +	+ + + +	+ - - -	1400
4-17	520	5.0	100	260	+ + + +	+ + + +	+ + + -	1200
5-1	395	5.0	100	215	+ + + +	+ + + +	+ + - -	1600
5-15	420	10.0	100	200	+ + + +	+ + + +	+ + - -	2900
5-22	445	7.5	100	190	+ + + +	+ + + +	+ - - -	900
6-26	555	15.0	100	225	+ + + +	+ + + +	+ + - -	600
7-7	350	10.0	100	205	+ + + +	+ + + +	+ - - -	1000
7-14	450	7.5	100	195	+ + + +	+ + + +	+ - - -	950
7-21	495	10.0	100	200	+ + + +	+ + + +	+ - - -	1100

* H.S. = Hard Swells * D.T. = Dextrose tryptone

Table 7. Thermophilic and mesophilic bacterial plate counts of massecuite held at temperatures of 30, 37, 45 and 55° C. for a period of six weeks.

SAMPLE A (30°C.)

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
					+ +	+ +	+ -	
7-16	555	0.0	100	175	+ +	+ +	- -	800
					+ +	+ +	+ -	
7-23	530	2.5	100	180	+ +	+ +	- -	750
					+ +	+ +	+ -	
7-30	480	5.0	100	150	+ +	+ +	- -	900
					+ +	+ +	+ +	
8-5	435	5.0	100	145	+ +	+ +	- -	700
					+ +	+ +	+ +	
8-15	350	2.5	100	155	+ +	+ +	- -	900
					+ +	+ +	+ -	
8-22	380	5.0	100	135	+ +	+ +	- -	800
					+ +	+ +	+ -	
8-30	305	2.5	100	120	+ +	+ +	- -	900

SAMPLE B (37°C.)

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
					+ +	+ +	- -	
7-16	565	2.5	100	145	+ +	- -	- -	600
					+ +	+ +	- -	
7-23	610	0.0	100	155	+ +	+ +	- -	700
					+ +	+ +	- -	
7-30	575	2.5	100	140	+ +	+ +	- -	750
					+ +	+ +	+ -	
8-5	305	5.0	100	125	+ +	+ +	- -	700
					+ +	+ +	+ +	
8-15	650	0.0	100	390	+ +	+ +	- -	800
					+ +	+ +	+ -	
8-22	615	2.5	100	245	+ +	+ +	- -	900
					+ +	+ +	+ -	
8-30	255	2.5	100	125	+ +	+ +	- -	650

* H.S. = Hard Swells * D.T. = Dextrose tryptone

Table 7. (Cont'd.)

SAMPLE C (45°C.)

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
7-16	490	5.0	100	135	+ + + +	+ + + +	+ - - -	1200
7-23	500	5.0	100	185	+ + + +	+ + + +	+ + - -	900
7-30	540	2.5	100	140	+ + + +	+ + + +	- - + -	850
8-5	385	7.5	100	125	+ + + +	+ + + +	- - + +	800
8-15	570	5.0	92	185	+ + + +	+ + + +	- - - -	600
8-22	450	2.5	100	145	+ + + +	+ + + +	- - - -	300
8-30	250	0.0	92	95	+ + + +	+ + + +	- - - -	400

SAMPLE D (55°C.)

Date 1948	THERMOPHILIC BACTERIA N.C.A. METHOD Total count per 10 gm.	Sulfide spores per 10 gm.	H. S.* percent tubes growth	Flat sour per 10 gm.	JONES' METHOD Flat sours D.T. broth* 2.0	1.0	0.1	MESOPHILIC BACTERIA Total count per 1.0 gm.
7-16	475	2.5	100	150	+ + + +	+ + + +	+ + - -	400
7-23	495	0.0	100	155	+ + + +	+ + + +	+ + - -	350
7-30	290	0.0	84	170	+ + + +	+ + + +	- - - -	200
8-5	265	0.0	50	180	+ + + +	+ + + +	- - - -	200
8-15	65	0.0	17	25	+ + + +	+ + + -	- - - -	100
8-22	55	0.0	17	15	+ + + +	+ + - -	- - - -	150
8-30	30	0.0	0	10	+ + + +	- - - -	- - - -	50

* H.S. = Hard Swells * D.T. = Dextrose tryptone

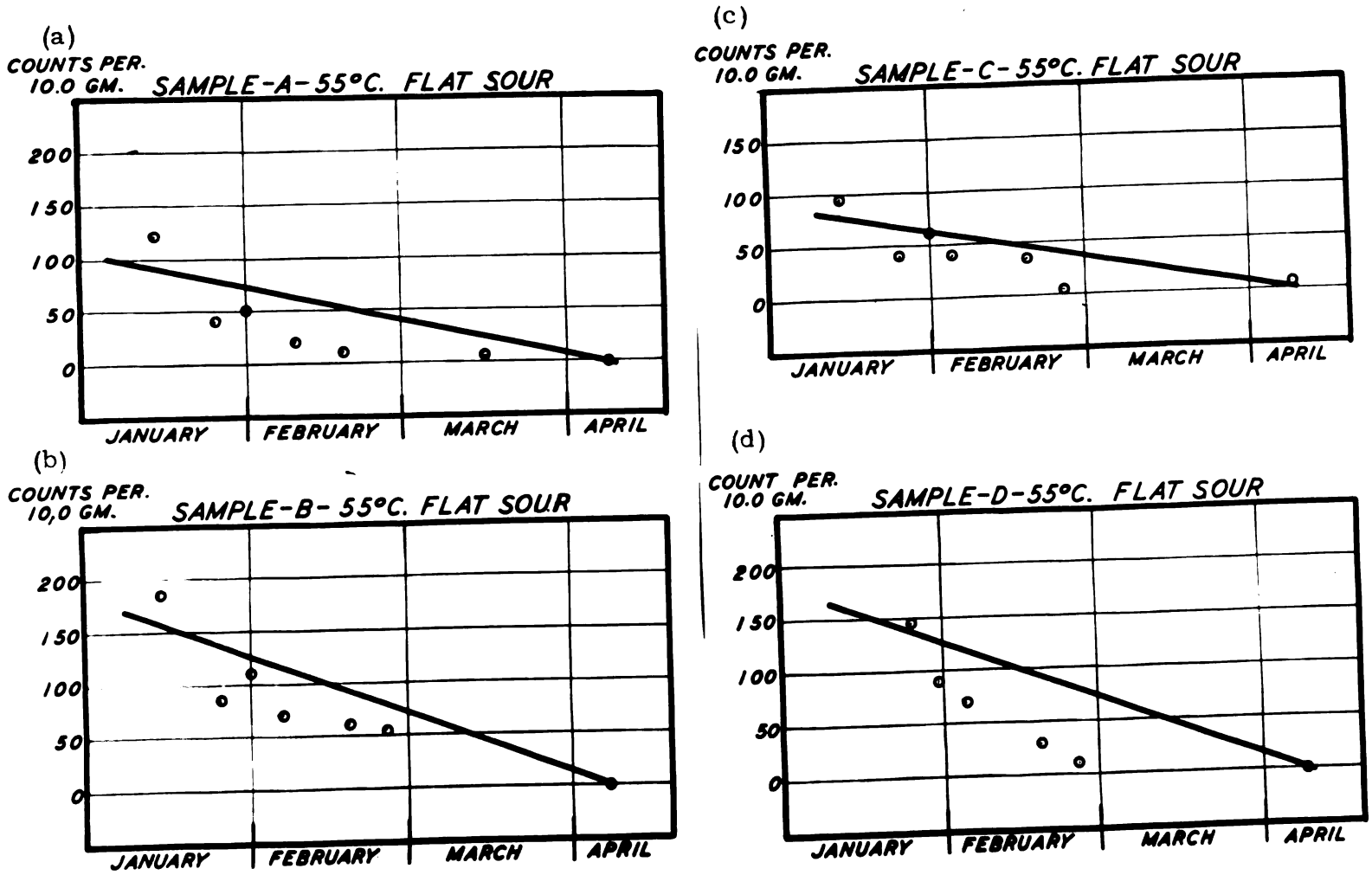


Fig. 3a, b, c, and d, N.C.A.'s method

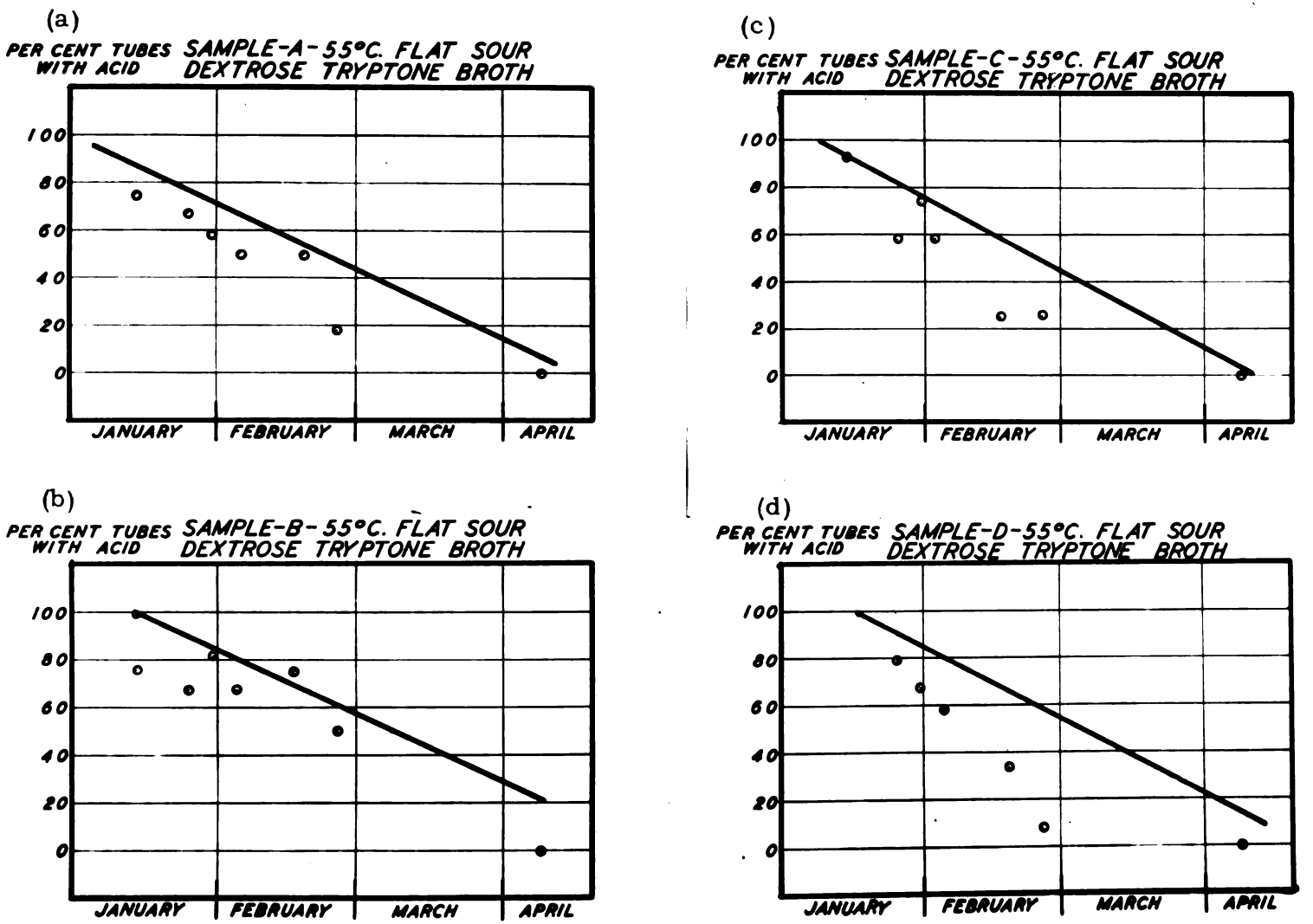
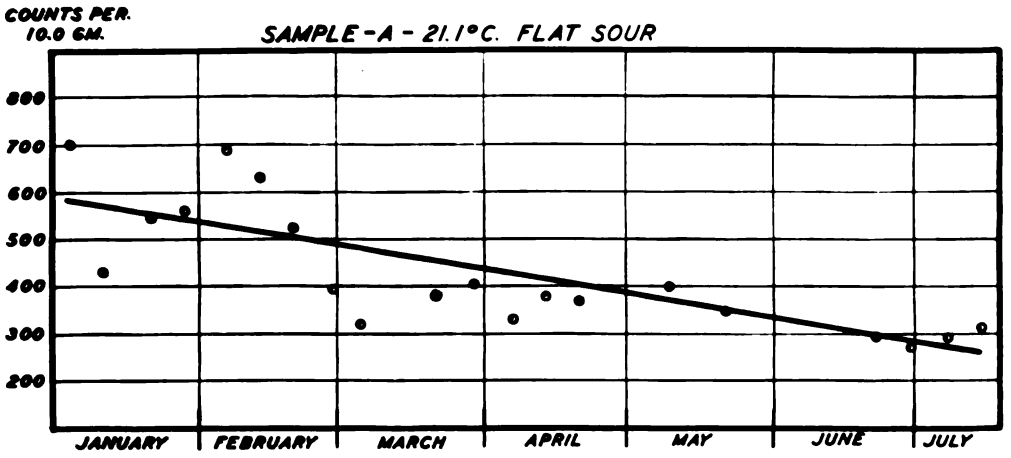


Fig. 2a, b, c, and d, Jones' method

(a)



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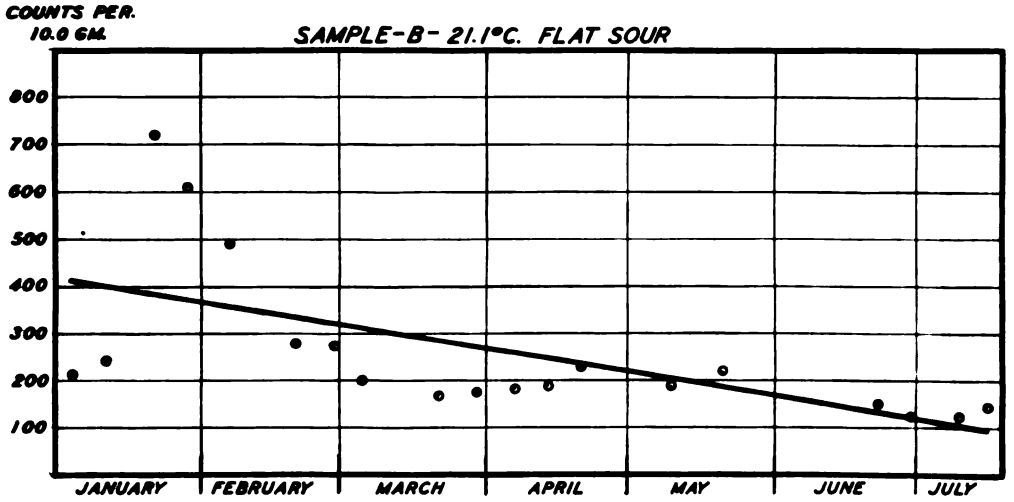

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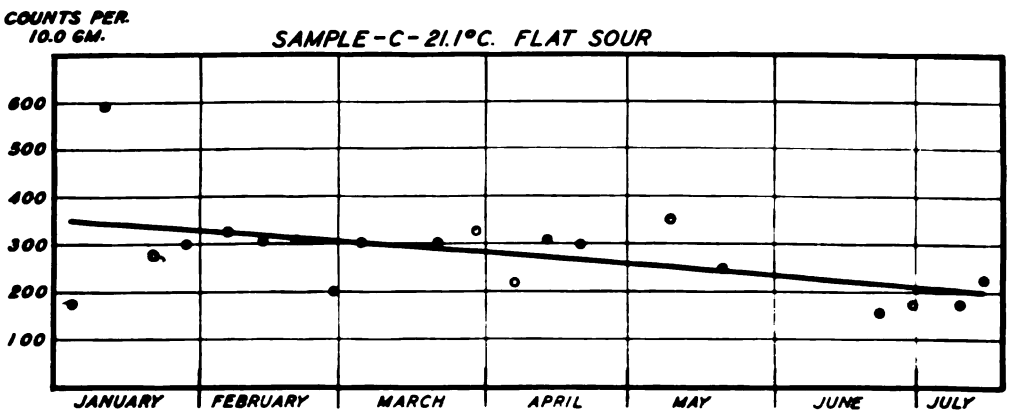
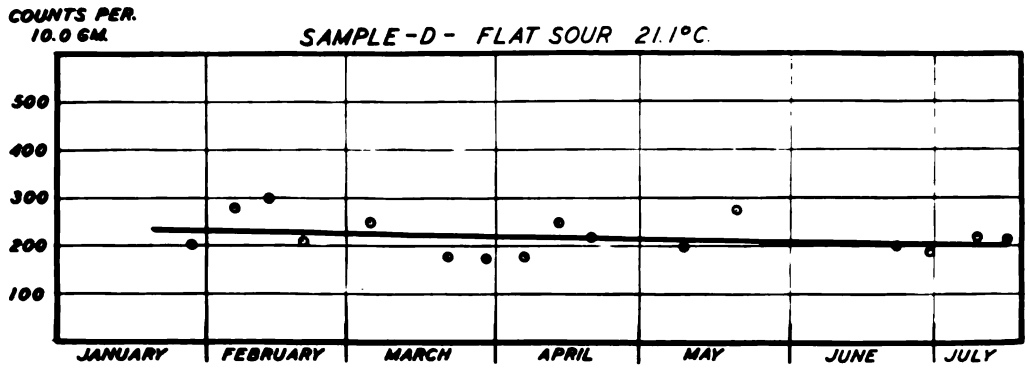
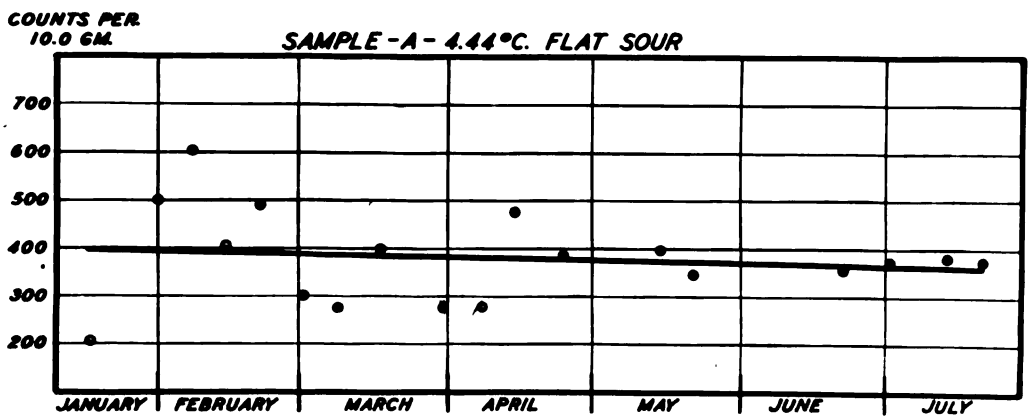


Fig. 5 a, b, and c, N.C.A. method

(d)



(a)



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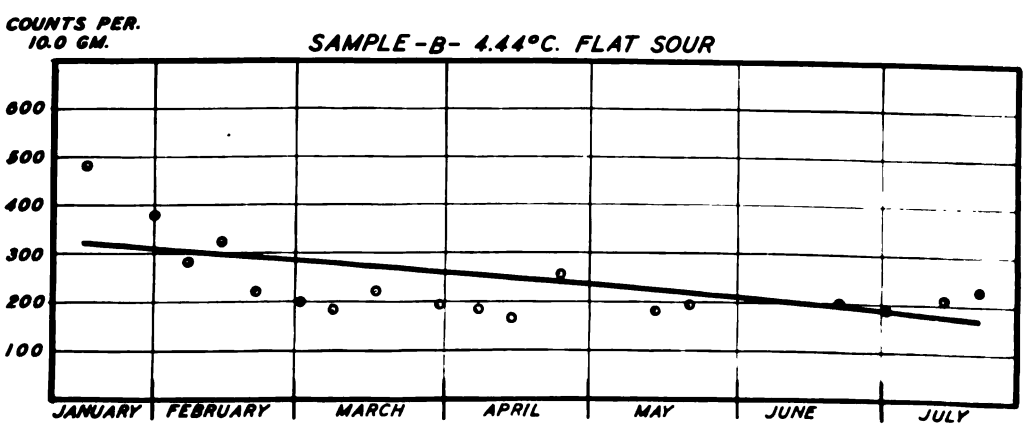
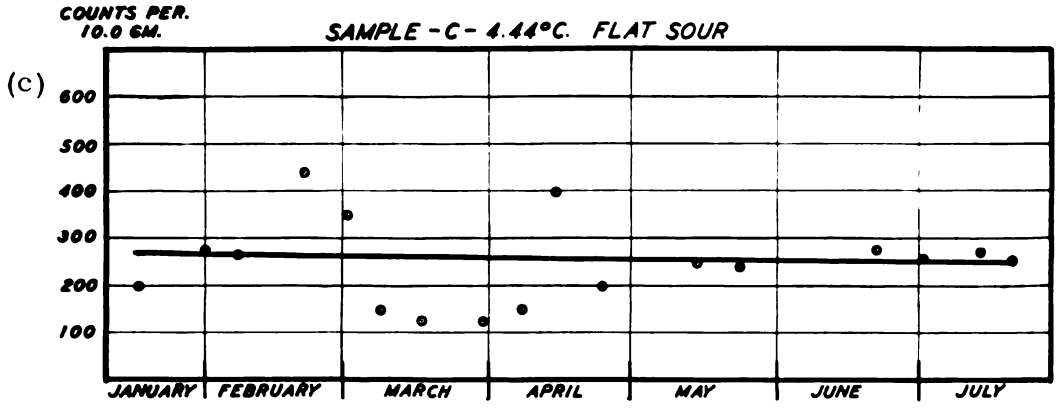
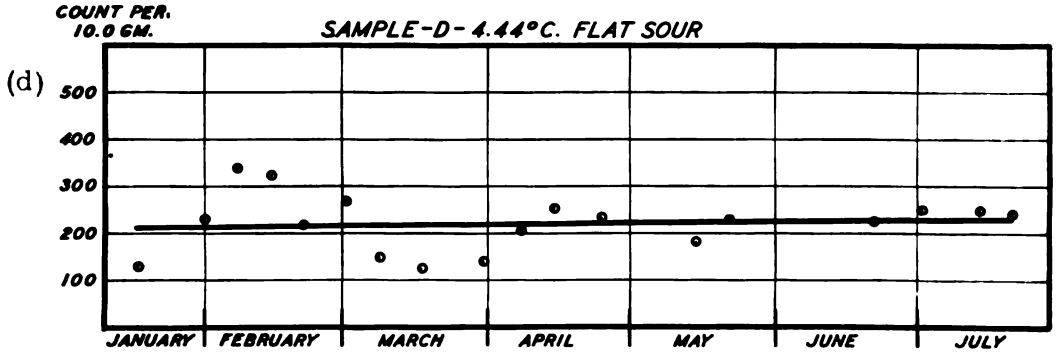


Fig. 5 d, Fig. 6 a and b, N.C.A.'s method





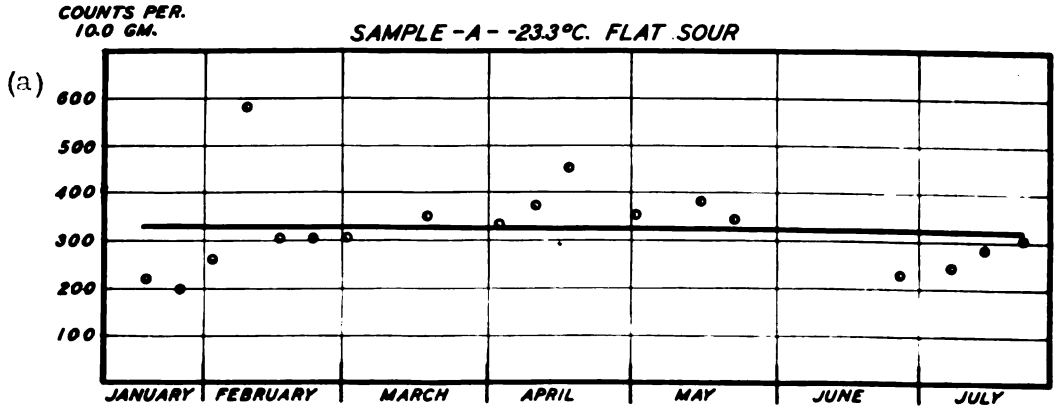
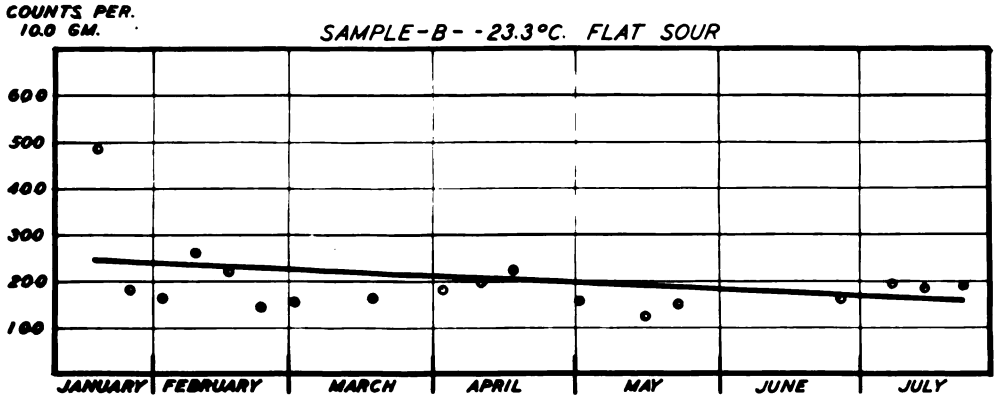
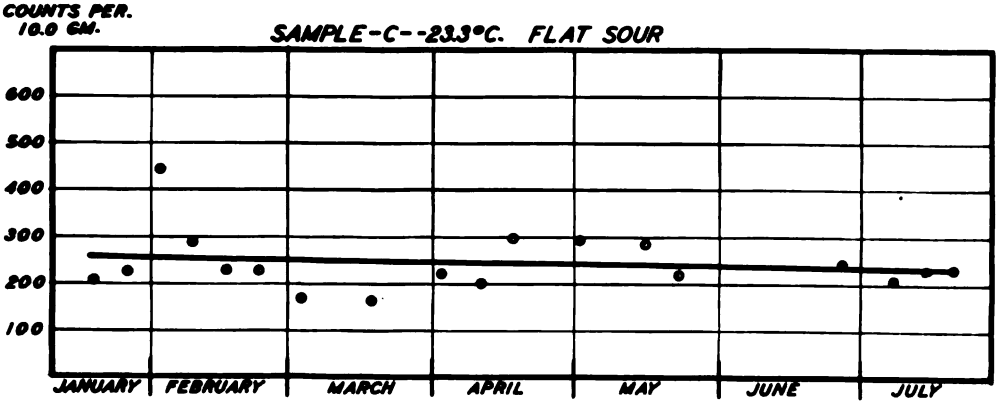


Fig. 6 c and d, Fig. 7 a, N.C.A.'s method

(b)



(c)



(d)

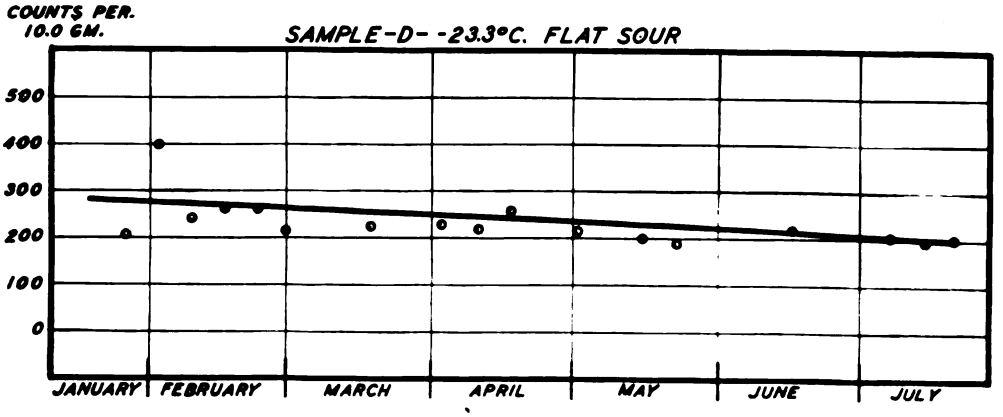
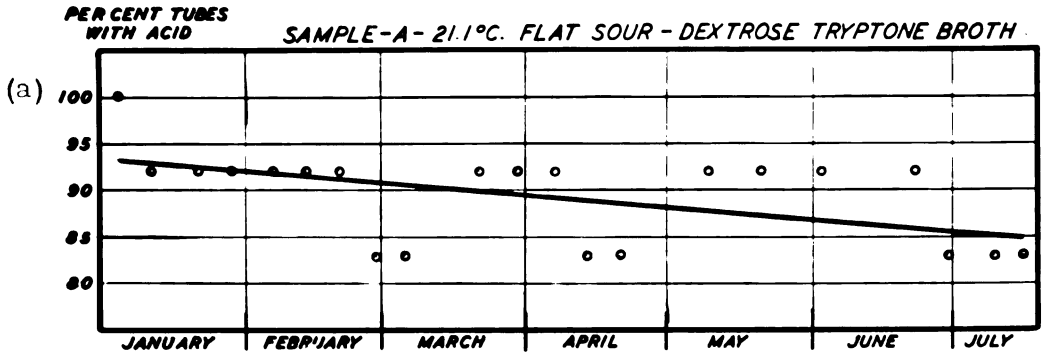
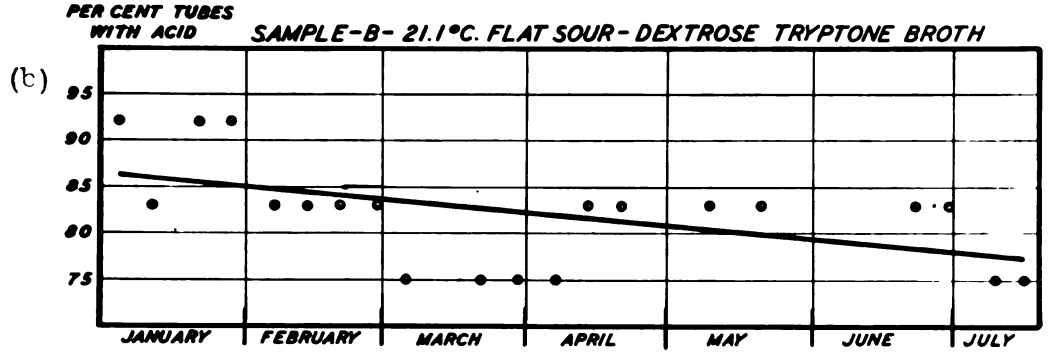


Fig. 7 b, c and d, N.C.A.'s method





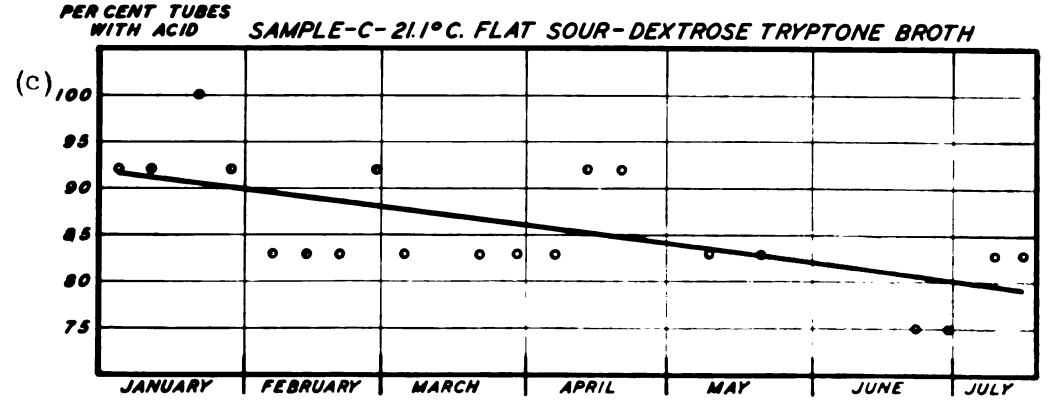
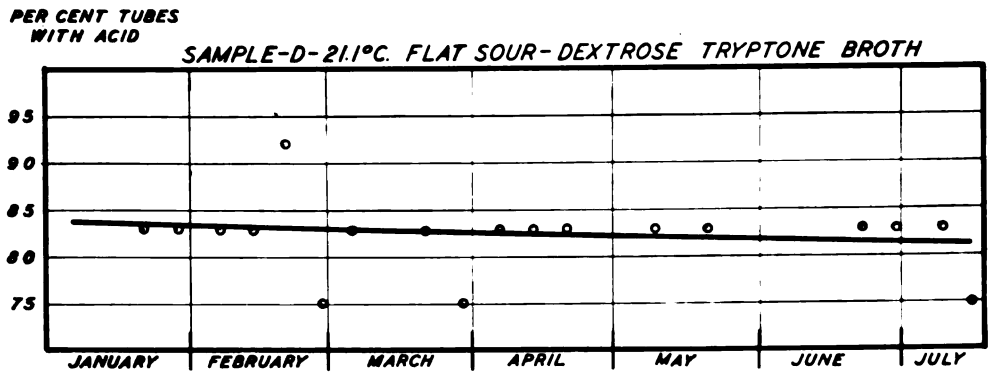


Fig. 8 a, b and c, Jones' method

(d)



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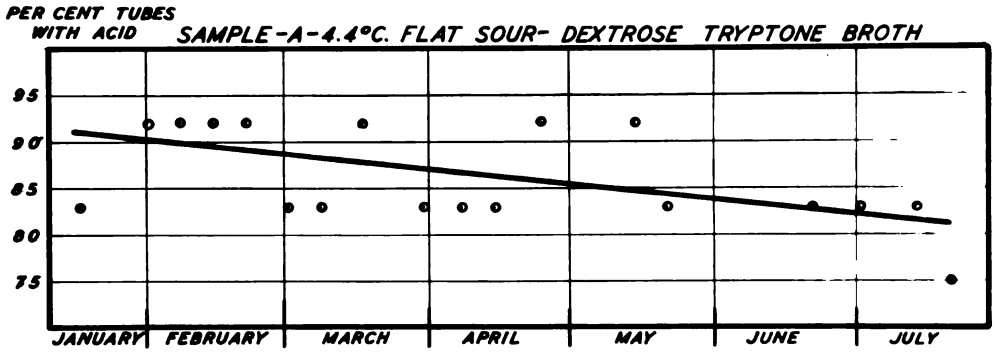

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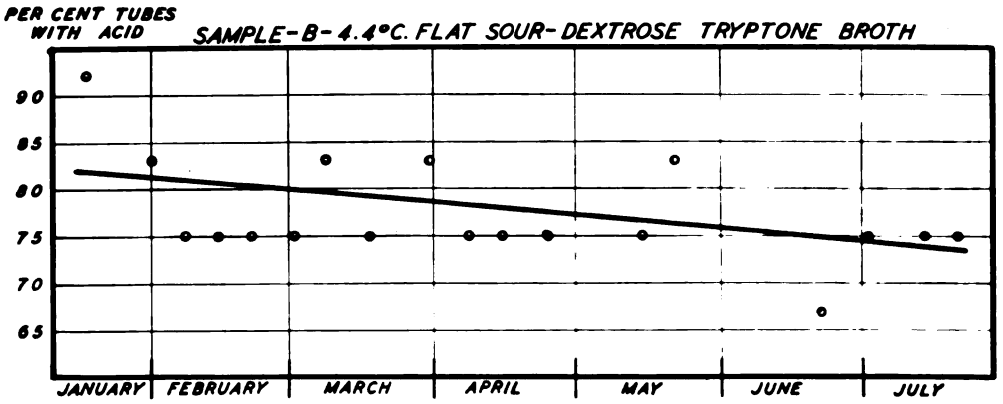
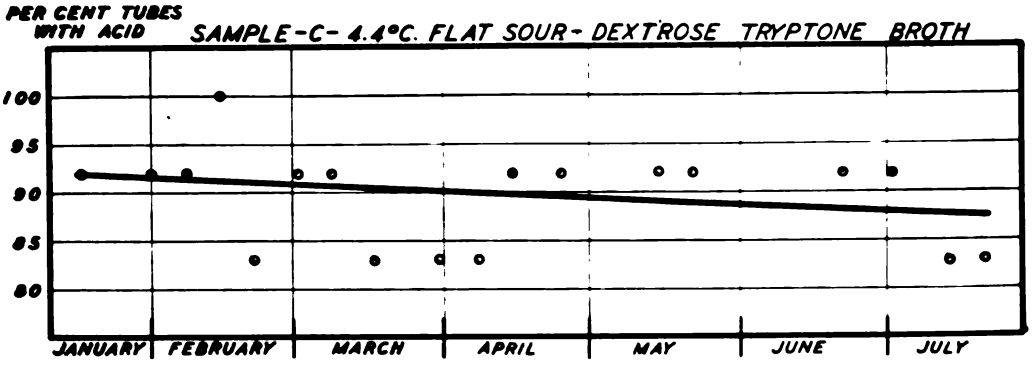


Fig. 8 d, Fig. 9 a and b, Jones' method

(c)



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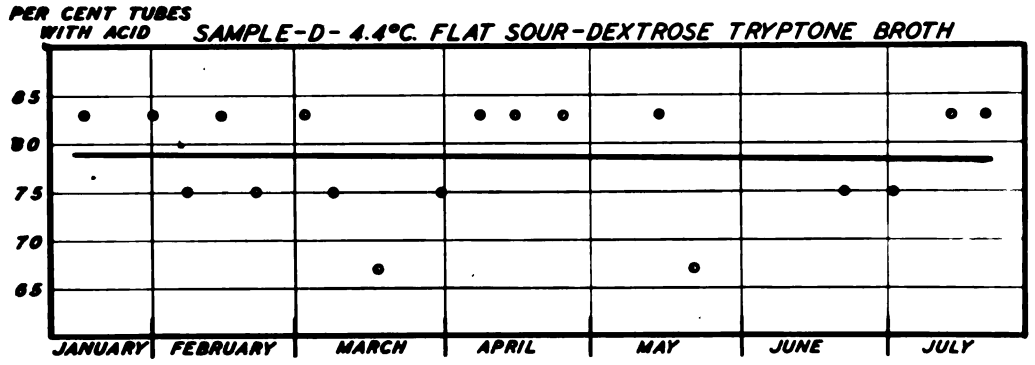


(a)

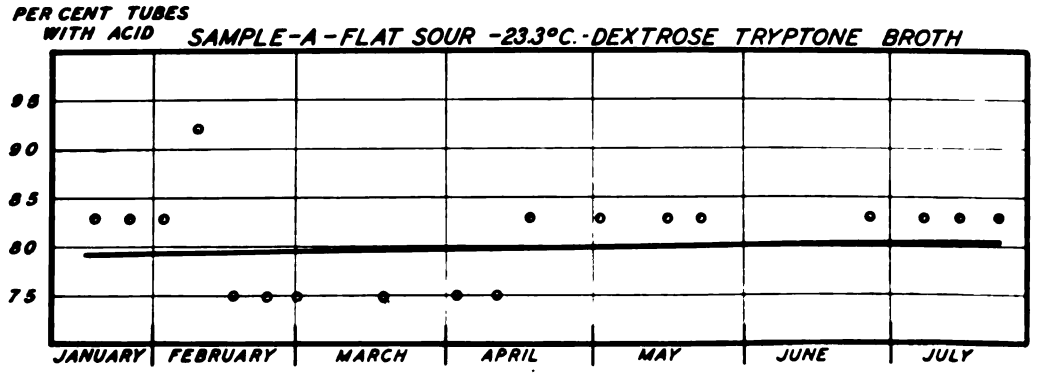
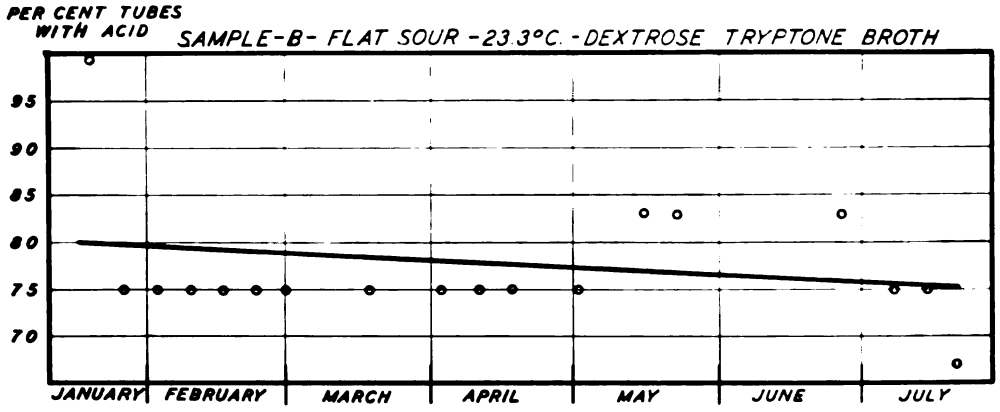


Fig. 9 c and d, Fig. 10 a, Jones' method

(b)



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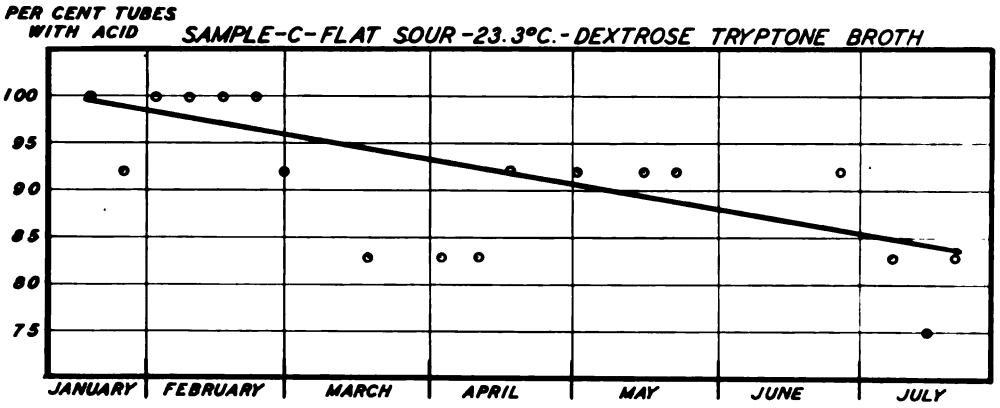

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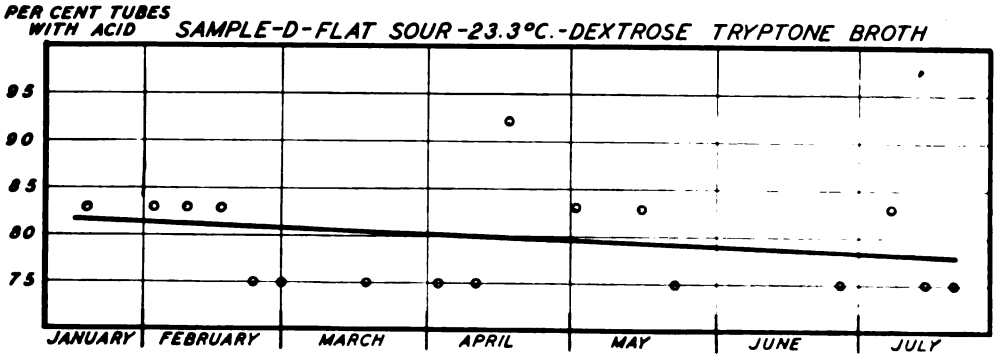


Fig. 10 b, c and d, Jones' method

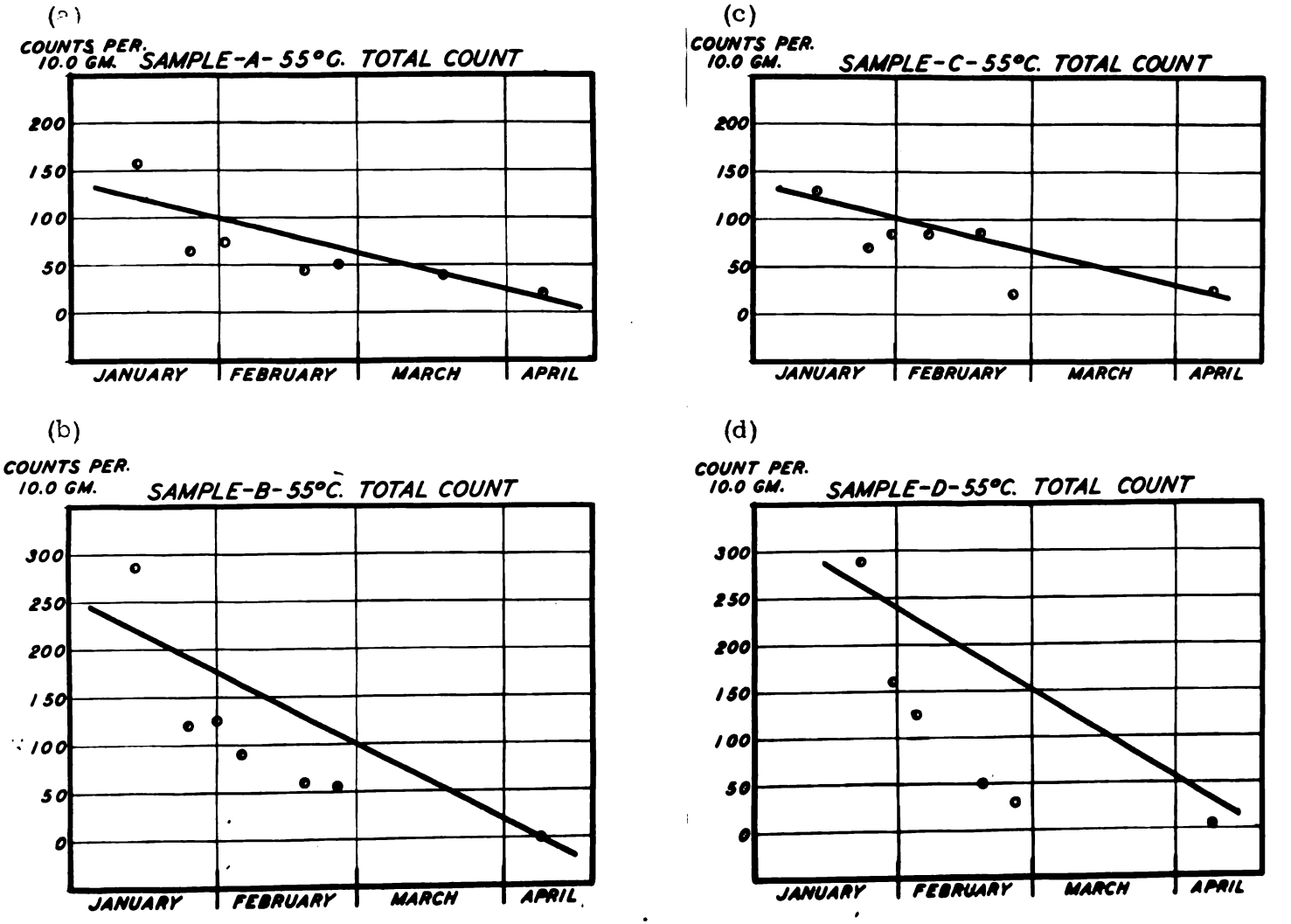
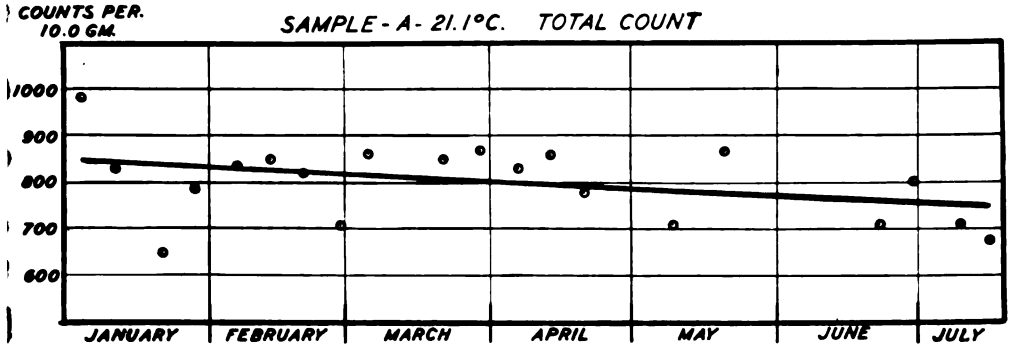
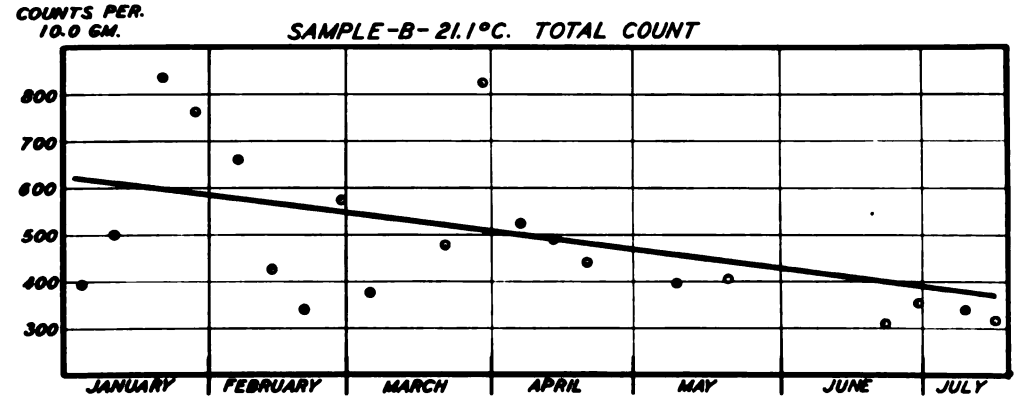


Fig. 11 a, b, c and d

(a)



(b)



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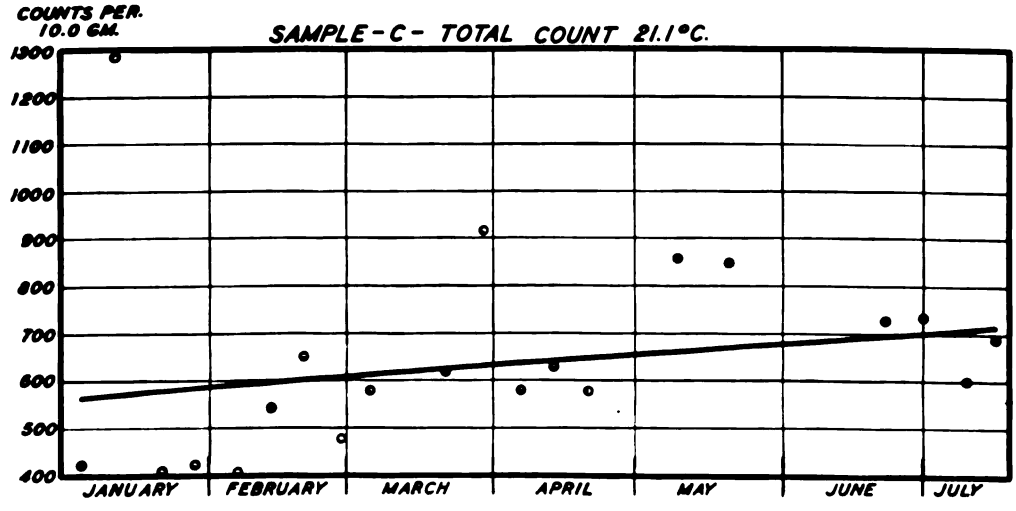
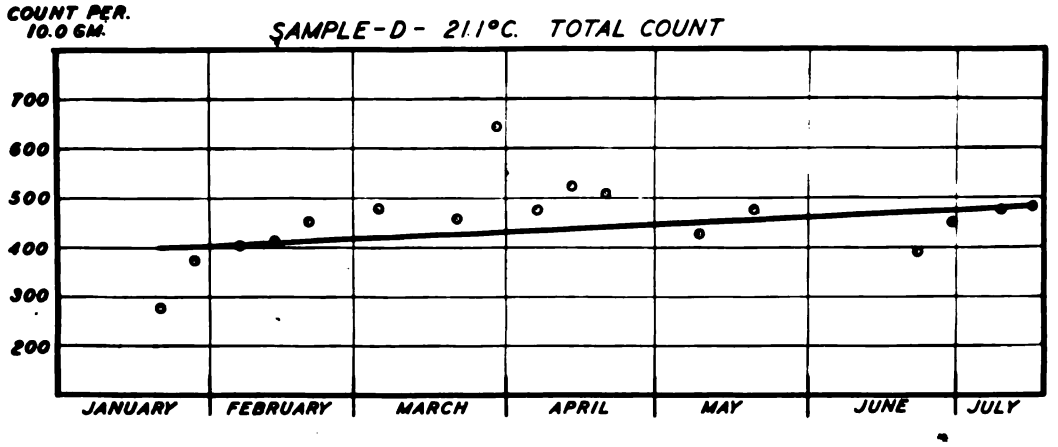


Fig. 12 a, b and c

(d)



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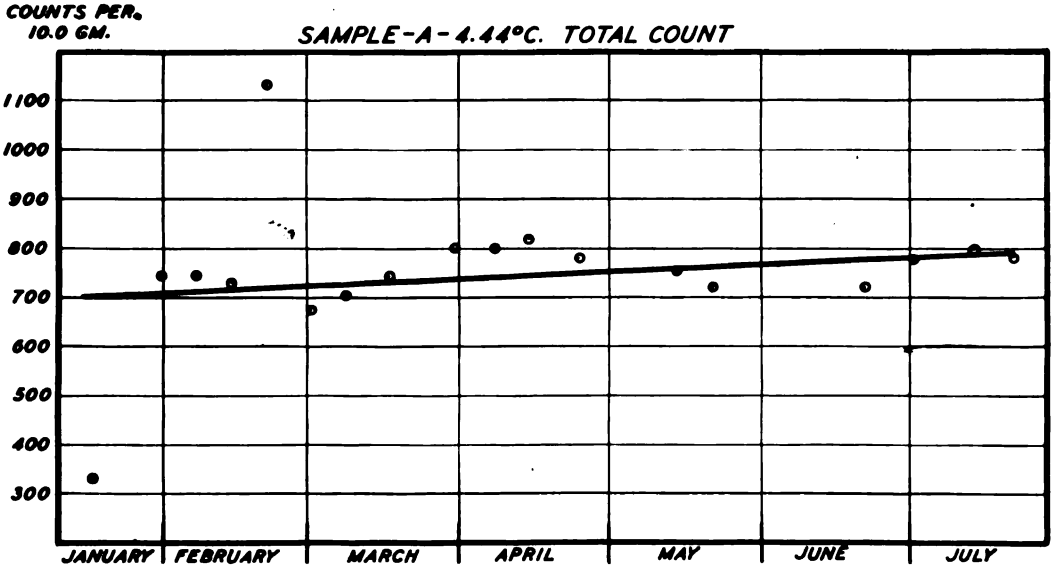

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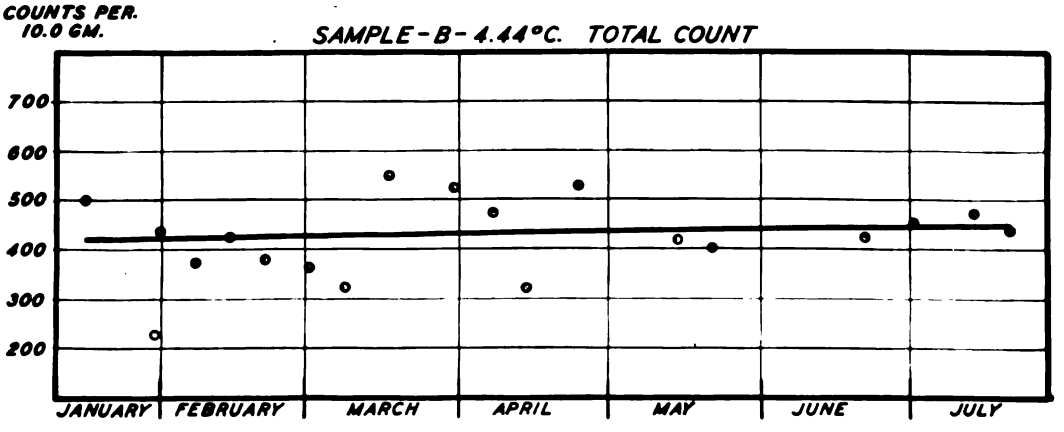
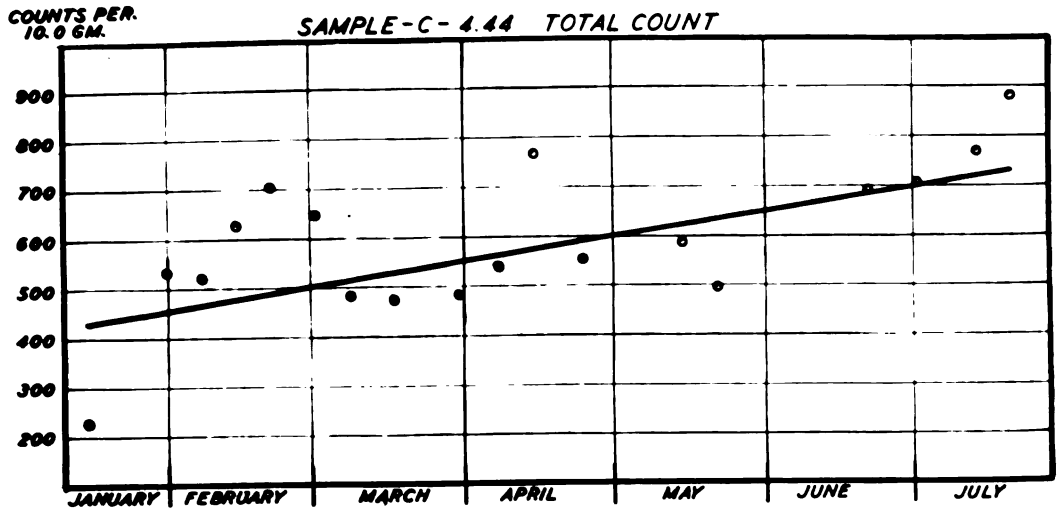


Fig. 12 d, Fig. 13 a and b

(c)



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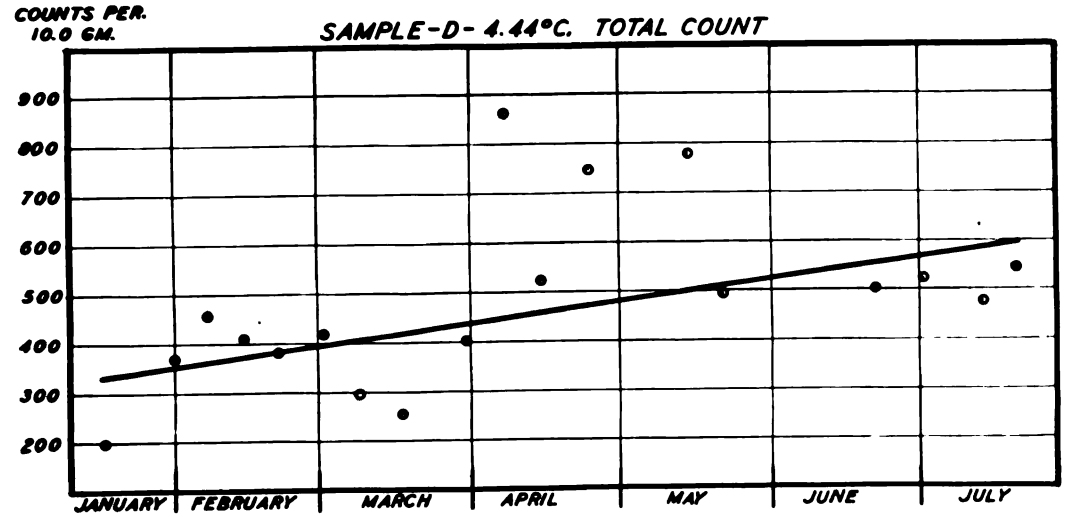

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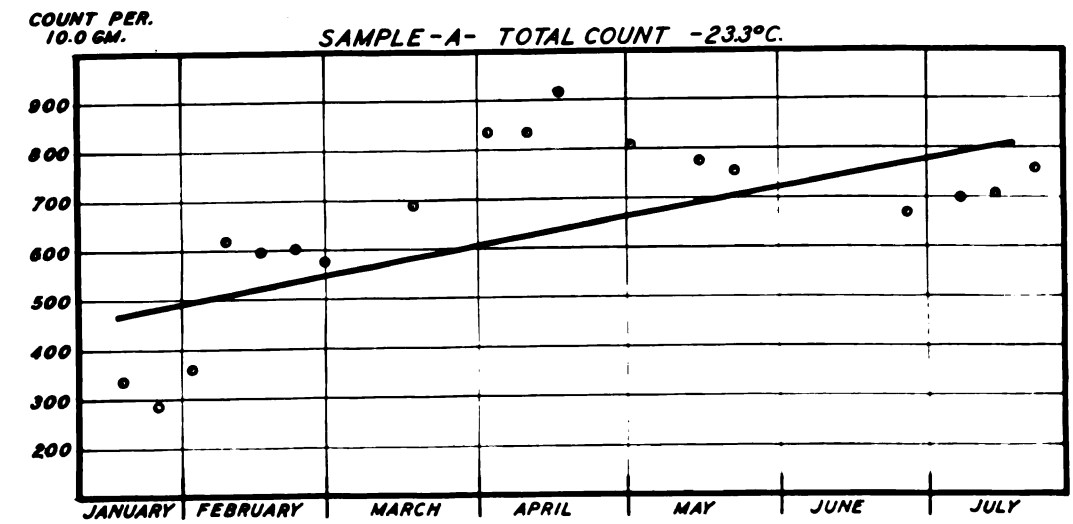
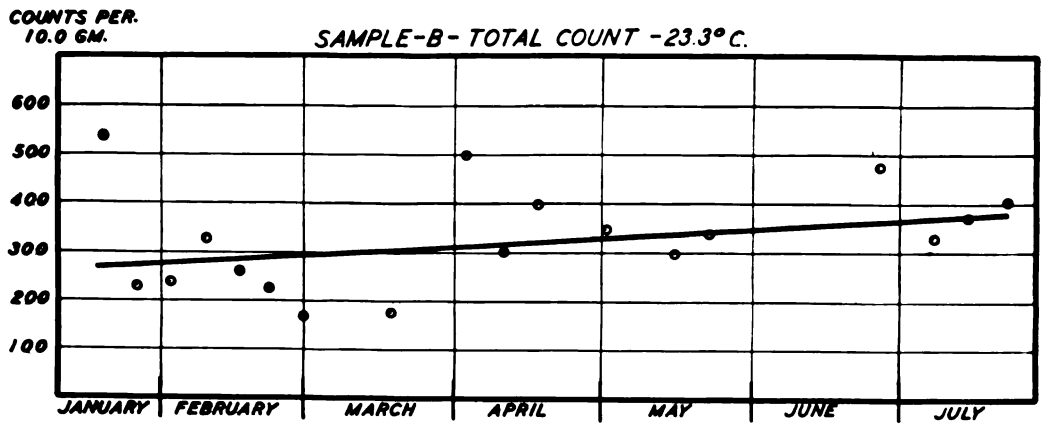


Fig. 13 c and d, Fig. 14 a

(b)



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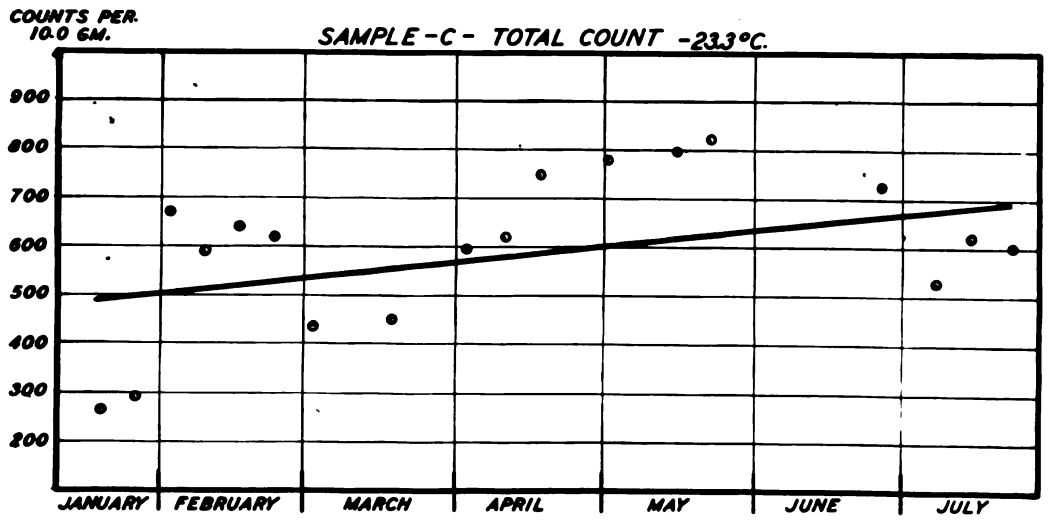

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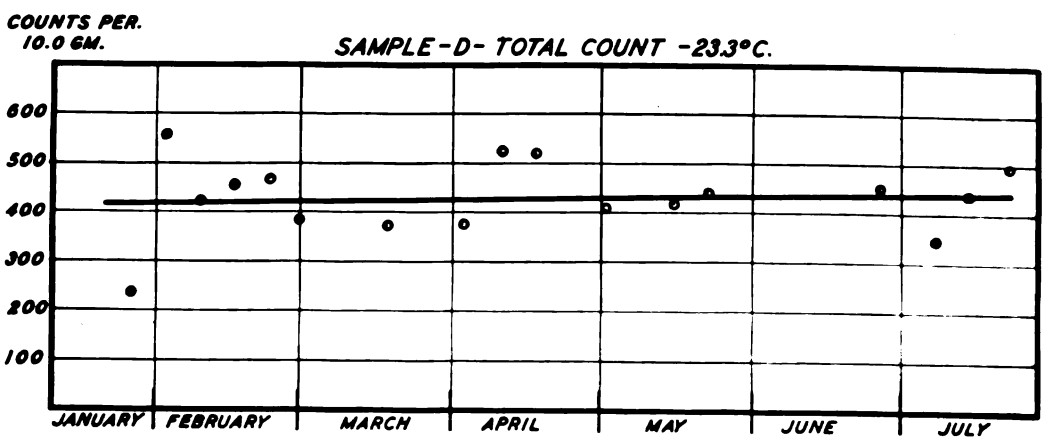

Fig. 14 b, c and d

SUMMARY

Under the conditions of the experiment there was the greatest reduction in both the mesophilic and thermophilic bacterial plate count at 55°C. The next greatest reduction was at about room temperature 21.1°C. (70°F.). As the temperature was decreased, there was less reduction in both the mesophilic and thermophilic counts.

A comparison of the N.C.A. with the method proposed by Jones indicates that the Jones' method is more sensitive in picking out the flat sour type of bacteria. It is less expensive and easier to read than the N.C.A. method.

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