COMPARISON OF ESCHERICHIA COLI AND STREPTOCOCCUS FAECALIS AS A TEST ORGANISM TO DETERMINE THE SANITARY QUALITY OF FOOD

PART II*

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Part II

Six strains of E. coli and two strains of Strept. faecalis were seeded into 12 different foods having a pH range of 2.8 to 6.7. Viability tests were run at different time intervals to study the viability of these organisms in the different foods under normal conditions. The results showed that the growth curve of E. coli in the foods depended upon the food and that the height of the curve was determined by the initial inoculation. E. coli var. communis was more viable in the foods than were the other strains. Strept. faecalis remained viable longer in some of the acid foods such as orange juice with a pH of 3.5 and mayonnaise with a pH of 3.7 than did any of the strains of E. coli. There appeared to be little difference between the viability of the two organisms in the less acid foods within the time limits studied.

Experimental Work

The first set of canned foods studied were those commonly used in the household which had a wide pH range. The cans were opened aseptically and foods of large particle size were ground in a sterile Waring blender. Approximately 75 ml portions were placed into dilution bottles and autoclaved for 15 minutes at 121°C.

The pH was determined with the Cenco pH meter using a glass and calomel electrode combination. A 1.0-ml sample plate check was made to determine the presence of mesophilic and thermophilic bacteria using tryptone glucose extract agar and nutrient agar. The autoclaved food was then inoculated with an actively growing 24-hour culture of *E. coli* strain *communior*. The food was plated in serial dilutions to obtain the initial inoculum of bacteria per ml. Distilled water blanks and tryptone glucose extract agar were utilized for this procedure.

The semi-solid food and the first 99-ml dilution blank to which one ml of sample was introduced were shaken with a mechanical shaker oscillating 180 times per minute. The best mixing was obtained by having the bottles in a horizontal position with the long axis of the bottle in line with the direction of shaking. All subsequent decimal dilutions were shaken manually.

Five agar plates were prepared for each dilution. Three plates out of the set of 5 were prepared by placing a thin layer of TGE agar upon the bottom of the sterile Petri plate and allowing it to harden before the dilutions were added to the plates. The dilution water containing the food was then added, and another thin layer of agar was poured into the plates. The two liquid elements were thoroughly mixed. The other two plates were made in the usual manner without a base layer of agar. They were shaken like the first set of three plates. It was found that there was closer correlation between the counts in those plates to which a thin base layer of agar had been added first, since they gave more uniform counts than plates made in the usual manner.

Colonies were counted on the Quebec colony counter after a three-day incubation period at 30° C. The graphic results are given in Figures 1, 2, 3, and 4.

The second set of foods was run in a slightly different manner. Foods were placed aseptically in sterile dilution and wide-mouth bottles. These foods were not autoclaved, but controls were run with each food using lauryl sulfate tryptose broth and lactose broth. Each type of food was then inoculated with 7 strains of *E. coli* using 0.2 ml of the 24-hour culture prepared as described previously. They were incubated at 30°C, and each day for 7 consecutive days a 0.1- to 1.0-ml sample of liquid food and 0.2- to 1.0-gm sample of dry solid food were inoculated into lauryl sulfate tryptose broth and lactose broth in which inserts were placed. The minimum transfer was used at the beginning of the seven day test, and when it was seen that the percentage of gas at 12 hours was decreasing, a larger inoculum was used. More inoculum was used for the dryer foods and a lesser amount for foods with liquor present. The liquor from semi-solid foods seeded with *E. coli* was transferred to the broth medium by pipetting. Examples of this type of food are peaches with syrup, tomatoes with juice, apricots with syrup, apple-sauce with hominy. Solid foods such as beans, meat, and sauerkraut were weighed to determine the relative amounts in 1.0- and 0.1-gm samples respectively, and approximate amounts used for inoculum.

Prior to transfer of the inoculated foods into the two broths, all samples were shaken for 10 minutes at 180 oscillations per minute, After 16 and 36 hours incubation, the percentage of gas present in the insert vials of the tubes were read. Positive tubes were confirmed by using the confirmatory test which is used for water samples. This test was initiated on the first, third, and seventh days. All brilliant green bile broth tubes, which were inoculated with three standard (4 mm) loops of lauryl sulfate tryptose broth, yielded confirmatory tests.

A duplicate set of the same twelve foods was inoculated with *Strept. faecalis* and transfers of the foods were made daily for seven consecutive days into dextrose azide broth. Turbidity was read at 16 and 36 hours at the beginning, but since the 36-hour reading gave the best results, the 16-hour reading was discontinued. A Gram stain of the broth was made at three days and studied.

Results

The growth curve of *E. coli* var. *communior* was influenced by the medium in which it grew. This is illustrated in the graphs shown in Figures 1, 2, 3, and 4.

Hominy with a pH of 7.2 supported rapid growth of *E. coli* reaching a count of about one billion at 24 hours which was the

*The Part I section was published in this Journal, July '54 issue, page 204.

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*The "bacterial food factor" was calculated from data in 12 tables not given here since these data were too voluminous but may be obtained from the original thesis.

greatest number in any of the foods tested. Figure 1 shows that this organism remained viable for a long time since there were four hundred thousand organisms per ml still present at the end of 19 days.

Figure 1 also shows how *E. coli* grew in canned peas at a pH of 6.0. With an initial seeding of one hundred thousand organisms, they had increased to 96,000,000 in twelve hours. They reached their maximum numbers in 48 hours when the count was 240,000,000 per ml. They died off rapidly, reaching 42,000 in six days when mold contamination caused discontinuance of the experiment.

Corn, with a pH of 6.2 (Fig. 2), fostered quick growth of *E. coli* which increased from an initial number of 100,000 at two hours to 400,000 at 24 hours. The logarithmic decrease was gradual for the 15 days of the test at the end of which time a count of 400,000 organisms per ml still persisted.

Chicken soup with a pH of 6.4 (Fig. 2), showed increase of *E. coli* from the initial amount of 660,000 to 51,000,000 in six hours. At 15 hours the count had increased to 290,000,000. The number of bacteria remaining showed but slight variation from the first until the 14th day when there was a gradual decrease. At the 7th day, 10,000,000 *E. coli* were present, but by the 14th day, the count had decreased to 140 organisms per ml.

E. coli was seeded into two samples of beef gravy (Fig. 3). One sample was inoculated with 75,000 organisms and the other with 750,000. The bacteria grew rapidly in both samples for the first 12 hours. Their numbers leveled off after reaching a peak of 40,000,000 and 77,000,000 respectively at 36 hours; 1,250,000 organisms remained viable in food determination No. 1 after 32 days, and 18,000,000 remained viable in determination No. 2 at 20 days. Organisms were present in large numbers up to 46 and 28 days when plating was discontinued. These data indicate that the amount of the original inoculum influences the number of organisms which are subsequently present.

E. coli inoculated into tomato soup having a pH of 4.6 (Fig. 4), did not multiply to any great extent. The initial amount of one million for the first set and about 2,000,000 per ml for the second set did not rise above 3,000,000 organisms per ml upon incubation and were in a state of sharp logarithmic decrease at 12 hours. There was a general leveling off of this decrease after five days, and less than 100 and 300 coliform organisms per ml were present after 30 and 35 days when determinations in this food were terminated.

Inoculum in excess of 100,000 organisms per ml reached the limit of their increases in from 24 to 48 hours. Of the foods used within the pH range of 4.6 to 7.2, all contained more than 10,000 organisms per ml at the end of 7 days incubation at 30° C.

Foods seeded with coliform organisms were inoculated into lauryl tryptose broth and lactose broth to determine the most promising common broth medium for rapid detection of coliforms. Lauryl tryptose broth gave 706 positive tubes to 630 for lactose broth. These results are the sum of 12- and 36-hours gas positive tubes. The food in which *E. coli* produced the most gas at 12 hours and remained viable for the longest period of time in one ml quantities were beef, hominy, beans, peaches, applesauce, and tomato with juice. The order was determined by calculating both lauryl tryptose broth and lactose broth fermentation with the production of gas at 12 and 36 hours.

Apricots showed a slowing of fermentation at 12 hours of incubation the third day after the food had been inoculated. Although the amount of food placed into the broth tubes was increased after the third day, the strain of *E. coli*, w-52950, isolated from a contaminated water sample, and strain 0-111 died out on the third and fifth day respectively. The fourth day after seeding the food, the coliform organisms were less viable since in the first 12 hours little gas was formed in the broths by any of the strains.

In orange juice with a pH of 3.5, the gas formation in the fermentation tubes lessened in quantity after it had been incubated one day. Gas appeared in lauryl tryptose broth from one to three days after the organisms failed to ferment the lactose broth. *E. coli* var. *communis* evidently was the hardiest strain of all since it was the only strain surviving to show continued fermentation after five days.

In potato salad with a pH of 4.8, 24 hours after food inoculation, all strains of *E. coli* fermented both lauryl tryptose broth and lactose broth at the 12-hour interval. By the second day after food inoculation, it was necessary to incubate the broth tubes 36 hours to get gas formation and the third day, after mixing coliform bacteria with the food, only five of the seven strains gave positive results; on the fourth day only three of the seven. On the sixth and seventh days, a heavier inoculum of potato salad in the broth tubes yielded positive results in lauryl tryptose broth for the human strain HS-04.

In sauerkraut with a pH of 3.8, two strains of *E. coli* gave negative tests at one day, and five of the seven strains gave negative tests at two days. Only *E. coli* var. *communis* remained viable to the fourth day with a positive test in lauryl tryptose broth after 36 hours of incubation.

Mayonnaise with a pH of 3.7, proved to be very bactericidal, yielding 6 out of 7 positive coli tests at 1 day, and only 1 positive coli test at 2 days after inoculation in the food. There were no positive tests after 48 hours even though the amount of food inoculated into the broth was increased.

Cranberry sauce with a pH of 2.8, showed positive tests after the first day and then in only 5 of the 7 strains. There were no positive tests the second day with 1 gram samples, and although 1 gram of the material was used as inoculum into the broths the third day, all sampling remained negative.

The foods used fell into three general groups in regard to the viability of *E. coli* in them and are arranged accordingly.

Group I presents the best possibility for using tests for *E. coli* to determine the sanitary quality of food. In this pH range of 6.7 to 4.6, bacterial growth is favored while the inhibitive action of the organic acids is the least. Organisms in this group of foods remained viable for the 7 days of the test. Gas was discernible within 16 hours in the test broths. In Group II foods with pH ranges of 3.5 to

TABLE 1—FOOD FACTORS OF VARIOUS FOODS GROUPED ACCORDING TO THE MAGNITUDE OF THE FACTOR.

Food	pH	Total titratable acidity	Lauryl tryptose factor
		Group I	
Beef	5.9	0.074N	93
Peach	4.2	0.0498	93
Hominy	6.7	0.0125	92
Bean, navy	6.2	0.0664	92
Applesauce	3.6	0.0581	88
Tomato	4.6	0.0664	88
			Lactose factor
Beef	5.9	0.074N	97
Hominy	6.7	0.0125	94
Beans	6.2	0.0664	89
Peaches	4.2	0.0498	86
Applesauce	3.6	0.0581	83
Tomato	4.6	0.0664	78
			Total lauryl tryptose + Lactose factor
Beef	5.9	0.074N	190
Hominy	6.7	0.0125	189
Beans	6.2	0.0664	181
Peaches	4.2	0.0498	179
Applesauce	3.6	0.0581	171
Tomato	4.6	0.0664	166
		Group II	
Food	*pH*	*Total titratable acidity*	*Lauryl tryptose factor*
Apricots	3.8	0.0872N	52
Orange juice	3.5	0.166	40
Potato salad	4.8	0.0498	35
			Lactose broth factor
Apricots	3.8	0.0872	41
Orange juice	3.5	0.166	30
Potato salad	4.8	0.0498	19
			Total lauryl tryptose + Lactose factor
Apricots	3.8	0.872N	93
Orange juice	3.5	0.166	70
Potato salad	4.8	0.0498	54
		Group III	
Food	*pH*	*Total titratable acidity*	*Lauryl tryptose factor*
Sauerkraut	3.8	0.0913	12
Mayonnaise	3.7	0.1577	6
Cranberry sauce	2.8	0.166	4
			Lactose broth factor
Sauerkraut	3.9	0.00913	4
Mayonnaise	3.7	0.1577	2.5
Cranberry sauce	2.8	0.166	2
			Total lauryl tryptose + Lactose factor
Sauerkraut	3.9	0.0913	16
Mayonnaise	3.7	0.1577	9
Cranberry sauce	28	0.166	6

4.8, *E. coli* organisms did not grow as well as they did in Group I foods. Organisms in Group III foods with pH's of 3.8 to 2.8 showed little gas production at 16 hours and yielded gas positive reactions for only a day or at the most, 2 days.

Strept. faecalis, as seen in Tables 2 and 3 remained viable for a longer period of time in mayonnaise and orange juice than did *E. coli*. Turbidity should not be used as a criterion for the presence or absence of streptococci. Non-turbid-appearing tubes showed the presence of streptococcus when observed by Gram's stain. Applesauce and apricots appeared turbid, but Gram's stain revealed the presence of a large gram-positive bacillus. Aside from mayonnaise and orange juice neither the *E. coli* nor the streptococcus method showed any advantage over the other in determining sensitivity of the test, or longevity of organisms in the foods used.

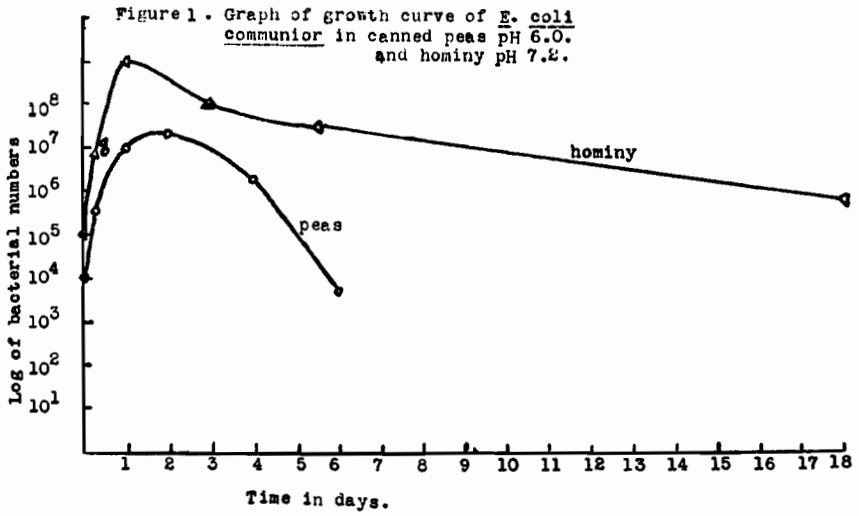
DISCUSSION OF RESULTS

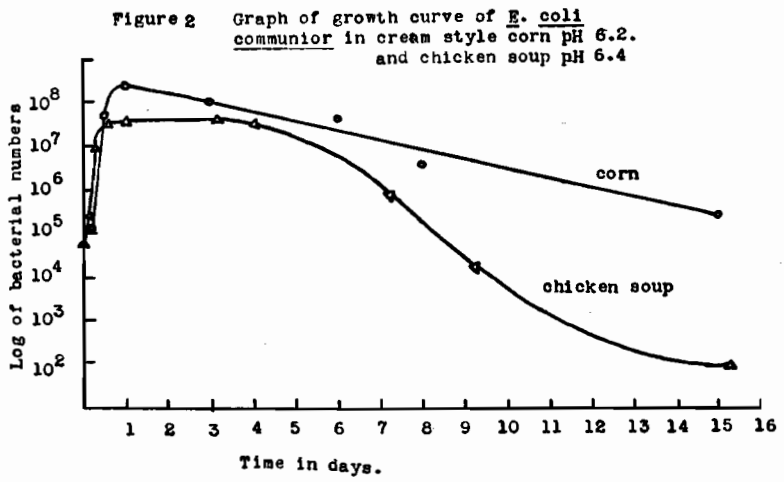
Plate counts of *E. coli* inoculated into foods having a wide range of pH values showed that this organism remained viable in them for long periods of time. During this time the food would show chemical changes or odor. It was found that in the foods with a pH of 6.2 to 3.8, the total plate count of organisms did not exceed one billion bacteria per ml. The growth curve of the organism used varied from food to food. Obviously no one curve could be said to be the representative growth curve for *E. coli* when considering their relationship to the entire field of food products.

Positive tests for the presence of *E. coli* in food were obtained with reasonable rapidity using methods of coliform detection developed for water analysis. The lauryl tryptose broth and lactose broth used as a presumptive test for *E. coli* proved to be more practicable in regards to the ease of observation, the use of a minimum of equipment, and quicker results than the present dextrose azide method and Gram's staining for the detection of streptococci. Lauryl tryptose broth proved to be superior to lactose broth in the more acid foods. Lauryl tryptose broth fostered a greater number of positive gas tubes when the coliforms were attenuated and

TABLE 2—STREPTOCOCCUS FACTOR FOR VARIOUS FOODS

Food	*pH*	*Titratable acidity*	*Streptococcus factor*
Hominy	6.7	0.0125	14
Beans	6.2	0.0664	14
Beef	5.9	0.074	14
Peaches	4.2	0.0498	14
Mayonnaise	3.7	0.01577	14
Orange juice	3.5	0.166	13
Tomato	4.6	0.0664	12
Apricot	3.8	0.0872	9
Applesauce	3.6	0.0581	8
Potato salad	4.8	0.0498	7
Sauerkraut	3.9	0.0813	7
Cranberry sauce	2.8	0.166	3





showed a higher percentage of gas positive insert tubes at 12 hours than lactose broth.

The *E. coli* detection was limited to the presumptive and confirmatory test used in finished water analysis.

Strept. faecalis showed no advantage in viability in the different foods except in the more acid foods especially orange juice and mayonnaise. However, its presence was more costly to determine since it necessitated the use of a microscope, glass slides, staining materials, and time to make and examine the stains after incubation. At times, better turbidity and subsequently better slides of gram-positive streptococci were obtained by using dextrose azide broth after the incubation time had been extended to three days.* Compared with this, the coliform presumptive test took 12 to 36 hours, and the brilliant green bile broth confirmatory test an additional 12 to 24 hours. Minimal time is important in detection of contamination of consumable and perishable products.

BACTERIAL FOOD FACTOR

An attempt was made to calculate the bacterial food factor on fermentation with gas production to see if pH or total titratable acidity could be used to indicate the longevity of coliform bacteria in foods.*

If a bubble of gas or more were present in the insert tube at 12 hours, a value of two points was given to that tube. If gas was present only at 36 hours, one point was assigned to that particular tube. If no gas was produced, the score was zero. The value thus determined for each tube was individually totaled at the end of seven days. Each total was added for each of seven strains. The reaction total was the grand total of the seven strains for seven days in one broth. This yielded an individual number for lauryl tryptose broth and one for lactose broth for one food.

The reaction total of lauryl tryptose broth, using all seven strains of *E. coli* for the seven days was calculated for each food. The reaction total of lauryl tryptose broth and lactose broth was added to get the total number depicting bacterial action for each food. This is called "bacterial food factor".

By separating the foods where the food factor takes the greatest proportionate jump, we find we have a naturally occurring set of three groups of foods.

Since there is no correlation

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*The "bacterial food factor" was calculated from data in 12 tables not given here since these data were too voluminous but may be obtained from the original thesis.

TABLE 3—LONGEVITY OF TWO STRAINS OF *Strept. Faecalis* IN FOODS OF VARIOUS pH'S AS DETERMINED "PRESUMABLY" BY TURBIDITY AND CONFIRMED BY GRAM'S STAIN.

Food	pH		ATCC 1325 Days 0	1	2	3	4	5	6	7	ATCC 6057 Days 0	1	2	3	4	5	6	7
Hominy	6.7	Turbidity at 36 hrs.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
		Gram smear of strept.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Beans	6.2	Turbidity at 36 hrs.	+	+	+	+	+	+	+	+	+	+	+	−	−	+	−	−
		Gram smear of strept.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Beef	5.9	Turbidity at 36 hrs.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
		Gram smear of strept.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Potato Salad	4.8	Turbidity at 36 hrs.	+	+	+	+	−	−	−	−	+	+	+	−	−	−	−	−
		Gram smear of strept.	+	+	+	−	−	−	−	−	+	+	+	+	+	−	−	−
Tomato	4.6	Turbidity at 36 hrs.	+	+	+	+	−	−	−	−	+	+	+	+	+	−	−	−
		Gram smear of strept.	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+
Peach	4.2	Turbidity at 36 hrs.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Gram smear of strept.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Apricots	3.8	Turbidity at 36 hrs.	+	+	+	−	−	−	−	−	−	−	+	+	+	−	−	−
		Gram smear of strept.	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+
Sauerkraut	3.8	Turbidity at 36 hrs.	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
		Gram smear of strept.	+	+	−	−	−	−	−	−	+	+	+	+	−	−	−	−
Mayonnaise	3.7	Turbidity at 36 hrs.	+	+	+	+	+	−	−	−	+	+	+	+	+	+	−	−
		Gram smear of strept.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Orange Juice	3.5	Turbidity at 36 hrs.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
		Gram smear of strept.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Cranberry Sauce	2.8	Turbidity at 36 hrs.	+	−	−	−	−	−	−	−	+	−	−	+	+	−	−	−
		Gram smear of strept.	−	−	−	−	−	−	−	−	+	−	−	+	+	−	−	−
Applesauce	3.6	Turbidity at 36 hrs.	+	−	+	−	−	−	−	−	+	+	−	+	+	−	−	−
			+	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+

between pH and titratable acidity and since these two factors or a combination of them is doubtful (the chief factors responsible for the longevity of not only *E. coli* but any other bacteria in foods) it is apparent that *E. coli* is inadequate to serve as a basis to determine the longevity of coliform bacteria in all kinds of food. However, they do serve to make a general grouping of foods with respect to the longevity of bacteria of which *E. coli* is representative. Furthermore, it demonstrates the possibility of the transmission of infectious disease by the enteric group of bacteria of which *E. coli* is representative, in certain types of foods such as in Group I, and under certain conditions in Group II and the improbability of enteric disease occurring in persons eating food classified in Group III.

Group I, because of high pH and correspondingly low organic acid content, readily permitted *E. coli* detection since these bacteria grow readily in these foods. The transitional group of foods is not as ideal a medium of growth as is Group I, consequently small initial contamination in foods listed in Group II might not be detected. Finally Group III seemed to be still less suitable for the growth of the coli-

form organisms. In fact these foods were bactericidal to these strains of coliform bacteria. Gas positive tubes in Group III foods would indicate recent or an extra large *E. coli* contamination.

The data obtained with *E. coli* in mayonnaise were in agreement with that obtained by Wethington and Fabian[3] who found that enterotoxigenic strains of food-poisoning staphylococci remained viable in commercial mayonnaise having a pH of 3.8 for 96 hours. Species of *Salmonella* survived one hour or less in samples of mayonnaise. It is therefore evident that these foods are not the ideal habitat of intestinal organisms of this type.

A totaling of the number of gram-positive streptococcus tubes for the seven day test yields a different sequence for the foods from that of the *E. coli* scheme.

There is no correlation between the viability and food factors of *Strept. faecalis* with pH or total titratable acidity.

Additional experimental work is needed to secure a more solid basis for defining degree of sanitary significance of positive reactions within a general grouping. Field samples should be taken and tests run for both *Strept. faecalis* and *E. coli* in order to establish a standard method and to interpret better the results. Each type of food to be indexed should be individually studied in the laboratory; carbon dioxide, oxygen, and surface tension studies should be in relationship to the biochemistry of the organisms; and the shelf life of food types should be ascertained so as to permit limitations of work.

SUMMARY

Studies of the growth curve of *E. coli* in a variety of foods indicated that the curve was dependent upon the food in which the organisms were grown and that the height of the curve was determined by the amount of initial inoculum.

More positive coliform tests were obtained using lauryl tryptose broth than with lactose broth. Lauryl tryptose broth gave more positive tests at 16 hours than did lactose broth.

E. coli var. *communis* showed slightly greater viability in the foods than did other strains of this organism. Strain 0-111, credited with causing infant diarrhea, was the least viable.

By arbitrarily establishing a bacterial food factor based upon the fermentation of lactose or lauryl tryptose broths, all foods studied could be divided into three groups. This general grouping of foods reflects the ability of coliform bacteria to survive in the respective groups and indirectly the possibility of these respective groups of food causing bacterial disease.

Strept. faecalis remained viable longer in orange juice with a pH of 3.5 and mayonnaise with a pH of 3.7 than did any of the strains of *E. coli.*

There appears to be little difference between the viability of *E. coli* and *Strept. faecalis* in the less acid foods within the time limits studied. However, the latter organism remained viable longer than *E. coli* in the more acid foods especially orange juice and mayonnaise.

Figure 3. Growth curve of duplicate determinations of E. coli strain communior in canned beef gravy.

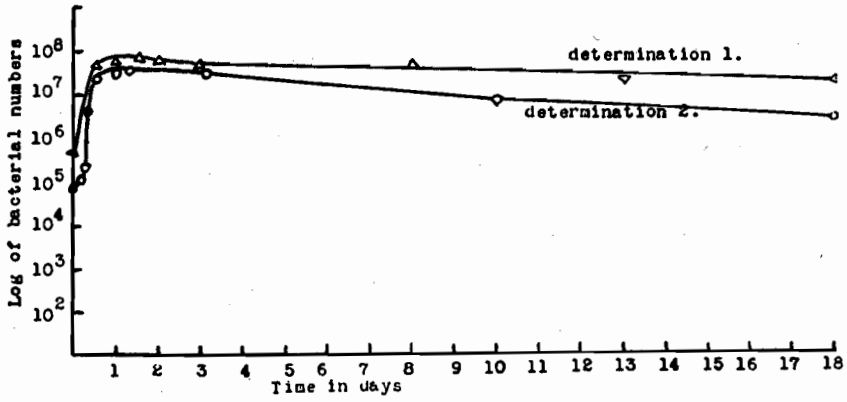
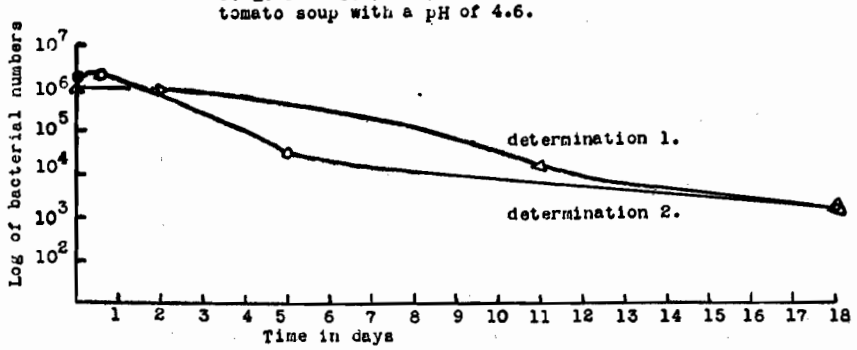


Figure 4. Growth curve of duplicate determinations of E. coli strain communior in canned tomato soup with a pH of 4.6.



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MISSING "LINKS"

Our office file of the proceedings and papers that have been presented at the annual meetings of our Association is not complete. This writer's personal set began with the 1928 volume. Dr. J. A. Gamble generously presented us with the volumes from the beginning of the Association in 1912 through the year 1919 inclusive.

The years 1920 to 1927 inclusive are missing.

If there are any "old-timers" who would like to help us close the gap, we should appreciate any of these volumes that they could furnish.

—J. H. Shrader