

# FATE OF COLIFORMS IN YOGURT, BUTTERMILK, SOUR CREAM, AND COTTAGE CHEESE DURING REFRIGERATED STORAGE<sup>1</sup>

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## ABSTRACT

*Aerobacter (Enterobacter) aerogenes* and *Escherichia coli* were inoculated separately into commercially produced samples of yogurt, buttermilk, sour cream, and cottage cheese. Inoculated products were stored at 7.2 C and were tested daily for up to 10 days to determine changes in numbers of coliforms and in pH values. The number of viable coliforms in yogurt declined dramatically and was markedly different from the initial value after only 24 hr of storage. Usually, survival of coliforms in yogurt did not exceed 3 days of holding. In buttermilk, most often a marked decline in numbers of coliforms was evident after 24 hr of storage. A substantial reduction in numbers (>50% of organisms present initially) of *A. aerogenes* B199 occurred in sour cream during the first 24 hr of storage, but a similar decline in numbers of *E. coli* and *A. aerogenes* FD was not evident until after 3 days of storage. Changes in numbers of *E. coli* and *A. aerogenes* in cottage cheese generally were not as rapid as in other products during the first days of storage. A few cottage cheese samples, however, did support rapid increases in test culture numbers. Because of the rapid decline in numbers of coliforms in yogurt, buttermilk, and sour cream, the provision in *Standard Methods for the Examination of Dairy Products* that permits examination of some of these products for up to 48 hr after manufacture seems inadvisable.

*Standard Methods for the Examination of Dairy Products (1)* specifies that coliform tests on cottage cheese be done within 24 hr after manufacture and that cultured milks be tested for coliforms within 48 hr after pasteurization. This requirement has made it difficult for receiving communities to do meaningful testing on these products when shipping times of more than 24 or 48 hr are involved.

Although it is generally believed that coliforms will be inactivated in acid products such as yogurt, cultured buttermilk, and sour cream, and conversely, will multiply in cottage cheese, definitive data to indicate the rate of death or multiplication are lacking. The present study was undertaken to characterize

the growth or destruction of some coliforms when suspended in these foods and held at a refrigeration temperature.

## MATERIALS AND METHODS

### Cultures

Two cultures each of *Escherichia coli* and *Aerobacter (Enterobacter) aerogenes* were used throughout this study (*E. coli* H52 and FD, *A. aerogenes* NRRL-B199 and FD). The FD strains were recent isolates from dairy products. Inocula for *E. coli* H52 and *A. aerogenes* B199 were prepared from skim milk cultures. The FD strain inocula were prepared by harvesting growth from 24-hr-32 C-trypticase soy agar slants and then washing it 3 times in distilled water before inoculating the dairy products under test.

### Products: Selection, inoculation, and examination

Dairy products used were obtained from grocery stores or the university dairy plant, except some cottage cheese samples which were obtained from processing plants. All samples were pretested for coliforms and only those that had initial concentrations of <10/g were used in this study.

Products other than buttermilk were inoculated and then stirred manually until the inoculum was dispersed homogeneously throughout the food. With buttermilk, the inoculum was mixed into the product by shaking. After inoculation, each sample of product was divided into 2 portions and each portion placed into a sterile beaker covered with aluminum foil. Samples were held at 7.2 C and were tested daily for a 10-day period to determine pH values and numbers of coliforms. Coliform counts were carried out as described in *Standard Methods for the Examination of Dairy Products (1)*. Violet red bile agar was used as the coliform medium throughout this study. Data in tables represent averages of results from tests on the 2 subsamples prepared from each product.

## RESULTS AND DISCUSSION

### Yogurt

Data on the behavior of *A. aerogenes* and *E. coli* in yogurt are summarized in Tables 1 and 2. It is evident that all test cultures were rapidly inactivated in this product; in many instances coliforms were essentially undetectable after 24 hr of storage. In fact, inactivation of coliforms in yogurt was so rapid

<sup>1</sup>Published with the approval of the Director of the Research Division of the College of Agricultural and Life Sciences, University of Wisconsin.

TABLE 1. FATE OF *Aerobacter aerogenes* IN YOGURT DURING REFRIGERATED STORAGE.

Sample	No. <i>A. aerogenes</i> /gram after days					pH values of yogurt after days				
	0	1	2	3	4	0	1	2	3	4
<i>A. aerogenes</i> B199 <sup>1</sup>										
1	330	35	<1	—	—	4.10	4.10	3.95	—	—
2 <sup>2</sup>	700	420	170	40	7	4.40	4.40	4.25	4.20	4.18
3	65	<1	—	—	—	4.00	4.05	—	—	—
4	<10	<1	—	—	—	4.10	4.05	—	—	—
5	<10	<1	—	—	—	4.00	3.90	—	—	—
<i>A. aerogenes</i> FD										
1	660	<1	<1	3	<1	3.74	3.84	3.71	3.69	3.68
2	950	20	<1	<1	<1	3.95	3.68	3.85	3.87	3.80
3	1000	<1	<1	<1	<1	3.88	3.81	3.80	3.85	3.77
4	740	<1	<1	<1	<1	4.00	3.90	3.98	3.85	3.95
5	770	<1	<1	<1	<1	3.65	3.65	3.72	—	—

<sup>1</sup>A similar inoculum was used for all samples. Inactivation of *A. aerogenes* in the product was so rapid that only a fraction of the organisms added to some samples could be recovered initially.  
<sup>2</sup>Peach yogurt.

TABLE 2. FATE OF *Escherichia coli* IN YOGURT DURING REFRIGERATED STORAGE.

Sample	No. <i>E. coli</i> /gram after days					pH value of yogurt after days				
	0	1	2	3	4	0	1	2	3	4
<i>E. coli</i> H52 <sup>1</sup>										
1	1100	260	23	<10	<1	4.20	4.05	4.05	4.05	4.05
2	110	1	<1	—	—	4.35	4.19	4.19	—	—
3	30	<1	—	—	—	4.04	4.00	—	—	—
4	28	<1	—	—	—	4.10	4.05	—	—	—
5	410	13	5	1	<1	4.20	4.10	4.10	4.00	4.00
<i>E. coli</i> FD										
1	110	<1	<1	1	<1	3.75	3.75	3.67	3.70	—
2	600	130	120	38	17	3.95	3.95	3.95	3.84	3.90
3	430	60	30	16	<1	3.91	3.88	3.88	3.77	3.79
4	490	110	20	31	15	3.90	3.90	3.95	3.85	3.92
5	150	<1	<1	<1	<1	3.65	3.65	3.65	3.62	3.78

<sup>1</sup>A similar inoculum was used for all samples. Inactivation of *E. coli* in the product was so rapid that only a fraction of the organisms added to some samples could be recovered initially.

TABLE 3. FATE OF *Aerobacter aerogenes* IN CULTURED BUTTERMILK DURING REFRIGERATED STORAGE.

Sample	No. <i>A. aerogenes</i> /gram after days											pH values of buttermilk after days				
	0	1	2	3	4	5	6	7	8	9	10	0	1	3	5	10
<i>A. aerogenes</i> B199																
1	890	640	580	420	350	310	280	220	160	65	35	4.90	4.70	4.45	4.40	4.40
2	850	670	600	440	420	340	270	250	170	100	33	4.85	4.65	4.45	4.45	4.40
3	880	640	520	460	450	345	260	210	170	140	70	4.85	4.80	4.60	4.60	4.60
4	750	680	470	380	370	290	170	100	40	18	13	4.80	4.70	4.45	4.45	4.45
5	840	690	510	410	380	340	280	200	160	110	130	4.70	4.60	4.40	4.40	4.40
<i>A. aerogenes</i> FD																
1	720	330	150	120	50	25	20	<1	<1	<1	<1	4.15	4.15	—	4.25	4.25
2	690	210	65	10	<1	<1	<1	<1	—	—	—	4.12	4.10	—	4.18	4.15
3	650	180	75	30	15	<1	<1	—	<1	<1	<1	4.15	4.10	—	4.15	4.18
4	740	320	220	170	160	80	60	25	10	—	—	4.30	4.30	—	4.40	4.35
5	720	440	280	180	220	200	100	80	20	35	<1	4.35	4.35	—	4.42	4.58

TABLE 4. FATE OF *Escherichia coli* IN CULTURED BUTTERMILK DURING REFRIGERATED STORAGE.

Sample	No. <i>E. coli</i> /gram after days											pH values of buttermilk after days				
	0	1	2	3	4	5	6	7	8	9	10	0	1	3	5	10
<i>E. coli</i> H52																
1	1300	1100	950	980	780	570	350	260	120	35	17	4.30	4.30	4.30	4.20	4.20
2	1600	880	450	220	65	28	18	10	13	8	<1	4.80	4.60	4.55	4.55	4.50
3	1500	1300	1100	700	380	170	23	20	10	<1	<1	4.80	4.65	4.60	4.60	4.55
4	1400	940	750	380	310	100	28	28	25	13	7	4.70	4.50	4.45	4.45	4.45
5	1400	960	650	330	220	110	20	13	15	<1	<1	4.50	4.35	4.35	4.35	4.35
<i>E. coli</i> FD																
1	920	600	450	310	260	110	70	30	20	<1	<1	4.15	4.15	—	4.28	4.22
2	730	640	370	260	80	110	35	40	20	10	<1	4.12	4.10	—	4.20	4.15
3	520	380	280	200	170	110	65	210	20	10	<1	4.15	4.10	—	4.39	4.35
4	670	460	470	360	280	300	180	260	180	95	85	4.30	4.30	—	4.39	4.35
5	740	440	450	440	300	170	320	200	230	130	70	4.35	4.35	—	4.45	4.55

TABLE 5. FATE OF *Aerobacter aerogenes* IN SOUR CREAM DURING REFRIGERATED STORAGE.

Sample	No. <i>A. aerogenes</i> /gram after days										pH values of sour cream after days					
	0	1	2	3	4	5	6	7	8	9	0	1	3	5	7	9
<i>A. aerogenes</i> B199																
1	860	350	210	130	65	55	23	23	3	<1	4.50	4.40	4.30	4.30	4.30	4.30
2	870	550	230	140	50	30	10	5	<1	—	4.70	4.60	4.55	4.55	4.60	—
3	780	340	110	13	<1	—	—	—	—	—	4.50	4.40	4.35	—	—	—
4	780	210	50	<1	—	—	—	—	—	—	4.60	4.50	4.40	—	—	—
5	860	330	140	63	30	8	3	<1	—	—	4.65	4.55	4.50	4.0	4.00	—
<i>A. aerogenes</i> FD																
1	1000	520	180	30	15	25	2	<1	<1	<1	4.18	4.05	4.12	4.17	4.17	4.20
2	1400	1300	640	680	170	160	130	35	20	3	4.20	4.21	4.15	4.19	4.17	4.22
3	1000	720	490	280	160	40	5	<1	<1	1	4.25	4.25	4.19	4.20	4.25	4.35
4	1400	860	720	400	200	270	160	140	80	20	4.50	4.42	4.40	4.40	4.47	4.60
5	770	220	10	<1	1	<1	<1	<1	<1	—	4.35	4.25	4.10	4.30	4.25	—

TABLE 6. FATE OF *Escherichia coli* IN SOUR CREAM DURING REFRIGERATED STORAGE.

Sample	No. <i>E. coli</i> /gram after days										pH value of sour cream after days						
	0	1	2	3	4	5	6	7	8	9	10	0	1	3	5	7	10
<i>E. coli</i> H52																	
1	1700	1300	930	500	370	370	300	400	300	180	200	4.70	4.70	4.61	4.60	4.60	4.60
2	1700	930	590	260	73	110	85	100	63	85	18	4.60	4.60	4.57	4.50	4.50	4.50
3	1700	400	240	95	70	30	23	50	4	<1	—	4.60	4.60	4.55	4.50	4.50	—
4	1500	1400	1200	1100	1000	1000	1000	1100	1000	880	830	4.69	4.65	4.65	4.70	4.70	4.70
5	940	800	680	560	570	370	200	190	110	38	8	4.40	4.40	4.33	4.20	4.20	4.20
<i>E. coli</i> FD																	
1	1000	520	360	30	30	6	13	<1	<1	<1	4.18	4.21	4.14	4.15	4.15	—	
2	1300	1600	740	380	260	160	90	25	5	<1	<1	4.20	4.25	4.10	4.10	4.15	4.15
3	980	820	550	340	150	170	70	90	50	<1	33	4.25	4.25	4.15	4.20	4.25	4.40
4	1400	1000	740	420	600	280	200	200	100	140	200	4.50	4.50	4.40	4.45	4.55	4.80
5	680	350	60	10	10	4	2	2	1	1	<1	4.20	4.20	4.15	4.25	4.25	4.25

with *A. aerogenes* B199 that sometimes an initial number could not be detected and at other times only a limited number of those organisms added were recovered a short time (< 30 min) later. It is noteworthy that 6 samples of yogurt permitted somewhat longer survival of coliforms, but even here the numbers declined markedly during the first 24 hr of

storage. The pH value of products in which coliforms survived longer tended to be somewhat higher initially but then dropped during storage. This may have contributed to the demise of the coliforms.

#### Cultured buttermilk

The behavior of *A. aerogenes* and *E. coli* in butter-

TABLE 7. FATE OF *Aerobacter aerogenes* IN COTTAGE CHEESE DURING REFRIGERATED STORAGE.

Sample	No. <i>A. aerogenes</i> /gram after days										pH value of cottage cheese after days						
	0	1	2	3	4	5	6	7	8	9	10	0	1	3	5	7	10
<i>A. aerogenes</i> B199																	
1	1000	1100	980	700	700	800	550	590	500	410	410	4.93	4.93	5.00	4.88	4.76	4.90
2	1100	1300	950	750	640	690	620	350	250	180	75	4.94	4.95	5.00	4.79	4.74	4.69
3	1100	5900	20000	16000	15000	15000	13000	8200	7300	7300	3300	4.94	4.90	5.01	4.83	4.94	4.87
4	1100	960	920	850	800	770	680	590	620	400	370	4.94	4.95	4.93	4.83	4.81	4.78
5	1100	900	880	600	600	560	470	340	280	170	130	4.94	4.95	5.03	4.90	4.81	4.59
<i>A. aerogenes</i> FD																	
1	140	130	140	110	120	140	120	120	160	200	340	4.80	4.80	4.80	4.80	4.70	4.75
2	140	170	160	130	210	330	540	1200	1900	3200	7100	4.85	4.80	4.85	4.70	4.75	4.83
3	100	120	260	670	2600	4000	3200	7x10 <sup>4</sup>	3x10 <sup>5</sup>	7x10 <sup>5</sup>	5x10 <sup>6</sup>	5.00	5.05	4.98	4.95	4.90	4.85
4	110	130	340	440	1400	990	1800	1300	2600	2x10 <sup>4</sup>	2x10 <sup>4</sup>	4.95	4.95	4.78	4.75	4.70	4.70

TABLE 8. FATE OF *Escherichia coli* IN COTTAGE CHEESE DURING REFRIGERATED STORAGE.

Sample	No. <i>E. coli</i> /gram after days										pH value of cottage cheese after days						
	0	1	2	3	4	5	6	7	8	9	10	0	1	3	5	7	10
<i>E. coli</i> H52																	
1	2200	2200	2000	1900	2100	2000	1700	1600	1500	870	500	5.10	5.00	4.84	4.76	4.73	4.61
2	2100	2200	2200	2100	2000	2100	1900	1900	1700	1800	1800	5.10	5.02	4.99	5.03	5.18	5.10
3	6400	7900	8800	3x10 <sup>4</sup>	5x10 <sup>4</sup>	8x10 <sup>4</sup>	1x10 <sup>5</sup>	1x10 <sup>6</sup>	1x10 <sup>6</sup>	2x10 <sup>6</sup>	5x10 <sup>5</sup>	5.10	5.03	5.05	5.08	5.13	4.99
4	890	830	860	780	730	790	760	720	640	690	610	5.06	5.01	4.93	4.75	4.72	4.67
5	680	780	750	755	830	790	670	670	690	680	650	5.07	5.07	5.09	5.08	5.05	5.02
<i>E. coli</i> FD																	
1	140	190	180	160	150	190	150	190	260	340	360	4.80	4.80	4.85	4.80	4.70	4.75
2	140	125	180	170	160	170	140	160	160	180	120	4.85	4.80	4.78	4.70	4.80	4.85
3	110	85	140	120	150	130	120	140	160	170	120	5.05	5.02	4.85	4.95	4.85	4.80
4	140	115	140	175	200	150	160	170	140	220	150	4.95	4.92	4.85	4.80	4.80	4.75

milk is detailed by data presented in Tables 3 and 4. Four of the 20 samples produced greater than 50% reduction of the test organism during the first 24 hr of storage. Additional decline in numbers occurred during further storage so that, after 4 days, the population nearly always declined to less than 50% of the number initially present. In general, *E. coli* appeared to be somewhat more sensitive than *A. aerogenes* to the destructive effects of buttermilk.

#### Sour cream

The decline in numbers of *A. aerogenes* and *E. coli* during storage of inoculated sour cream is reported in Tables 5 and 6. In general, *A. aerogenes* appeared slightly more sensitive to the destructive effects of sour cream than did *E. coli*. The number of *A. aerogenes* B199 was nearly always reduced by at least 50% during the first 24 hr of storage, whereas only 2 samples containing *E. coli* exhibited a similar inactivation of the organism. *Aerobacter aerogenes* was not detectable after 7 days in 7 of the 10 sour creams under test. *Escherichia coli*, on the other hand, survived for 10 days in 6 of the 10 samples examined.

#### Cottage cheese

Tables 7 and 8 record data obtained when cottage

cheese was inoculated with coliforms and then stored. It is evident that survival of *E. coli* and *A. aerogenes* was greater (both in terms of time and numbers) in this product than in yogurt, buttermilk, or sour cream. In fact, growth of coliforms was evident in some of the samples tested. The pH values of most cottage cheese samples declined during storage, but this change often did not appear related to either survival or demise of coliforms in the product.

The maximum storage time before testing cultured milks and cream for coliforms is designated in *Standard Methods for the Examination of Dairy Products* (1) as 48 hr. Because of the rapid inactivation of the 4 test organisms under study when in yogurt and sour cream, meaningful tests could not be carried out after 24-hr storage. Although the reduction in numbers of test organisms in buttermilk after 24 hr was not as great as in yogurt or sour cream, the reduction was significant. After 48 hr of storage, the test organism concentration averaged one-half of the initial population.

For cottage cheese, *Standard Methods for the Examination of Dairy Products* (1) recommends that the coliform count be carried out within 24 hr after manufacture. Although most counts on cottage cheese

were relatively stable for 48 hr, 2 of the 18 samples tested supported a dramatic increase in test culture numbers within this period. If we could assume the growth of the test organisms studied to be typical of that for coliforms found in cottage cheese, the substantial increase in coliform count within 48 hr after manufacture in about 10% of the lots of cottage cheese could result in an adverse regulatory action being applied unfairly if testing were delayed to 48 hr after manufacture. For this reason, it would seem desir-

able to continue to test cottage cheese within 24 hr after manufacture.

#### ACKNOWLEDGMENT

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#### REFERENCE

L. Walter, W. G. (ed.). 1967. Standard methods for the examination of dairy products. American Public Health Association, New York.

## ASSOCIATION AFFAIRS

### REPORT OF THE EDITOR

### JOURNAL OF MILK AND FOOD TECHNOLOGY 1969-1970

#### REVIEW OF VOLUME THIRTY-TWO

Volume 32 of the *Journal of Milk and Food Technology* was completed with publication of the December, 1969 issue. This was a record-breaking volume in that it contained more pages (624) and more papers (87) than any preceding volume of the *Journal*. Forty-seven research papers and 14 technical papers of general interest were published. This material utilized approximately 45% of the total pages, whereas non-technical papers of general interest, association affairs, and news and events occupied 30% of the pages. Advertising, covers, index, standards, etc. made up the remaining 25% of volume 32. This distribution, when compared to that of volume 31, reflects an increase (5%) in space devoted to technical material and decreases in space utilized for non-technical material (2%) and for covers, advertising, etc. (3%). The composition of volume 32 is detailed more completely in Table 1 together with similar information for volumes 30 and 31.

Research papers in volume 32 dealt with a variety of topics including studies on the plate count method, oxidase method, resazurin test, changes during cooking of crab, tests for abnormal milk, composition of apples, egg pasteurization, fat tests for skim milk, cleaned-in-place systems, coliform bacteria, food-plant wastes, microbiology of meats, microbiology of feedstuffs, methods to enumerate molds, microbial quality of imitation dairy products, Cheddar cheese, low-fat spreads, salmonellae in cheese, methods to measure aflatoxins, salmonellae in butter, and other subjects. General interest papers dealt with such topics as interstate milk shipments, fluorescent antibody technique, imitation foods, methods for detection of viruses and of *Clostridium perfringens*, *C. perfringens* food poisoning, recirculation cleaning, vector control, milk powder, testing of milk, solid wastes disposal, and other topics.

#### PRESENT STATUS OF VOLUME THIRTY-THREE

The first six issues of volume 33 contained 25 research

papers, 7 general interest technical papers, and 8 non-technical general interest papers. This compares with 24, 7, and 10 papers in the same categories in the first six issues of volume 32. In addition, 48 pages of E-3A and 3A standards have appeared in volume 33. The first six issues of volume 33 contained 16 more pages than did similar issues of volume 32.

On July 15, 1970 there was a backlog of 48 papers ready for publication. This included 28 research papers and 20 general interest (technical and nontechnical) papers among which are 7 papers from the 1969 annual meeting and 1 from the 1970 annual meeting. In addition, on July 15 there were 16 research papers being reviewed or revised. The backlog would not be as great if 48 pages had not been devoted to standards. As a consequence, it will be necessary to add extra pages to most, if not all, remaining issues in volume 33 so that research papers can be published within six (or less) months after they are received.

#### REVIEW PAPERS

In the Editor's opinion, review papers are extremely helpful to busy people so they can keep up-to-date on developments in fields which are not their immediate concern. Consequently a number of persons (many suggested by members of the Editorial Board) have been asked to prepare reviews on timely topics. Three such review papers, dealing with activation of bacterial spores, nitrites and nitrates in the environment, and carcinogens in the environment, are awaiting publication. Additional review papers should be received in the near future.

#### EDITORIALS

Last year the Journal Management Committee suggested that consideration be given to the regular use of editorials. Consequently, the Editor contacted all members of the Editorial Board to determine their interest in and willingness to prepare editorials. There was no ground-swell of enthusiasm for editorials although some members of the Board favored their use. Many board members felt that editorials in technical journals often are a waste of space, especially if one has to appear each month and no one really has anything worthwhile to say. Some Board members felt that the "Letter to the Editor" could serve the same purpose since