

Heat Resistant Coliform Organisms, with Particular Reference to Butter*

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OVER a period of years various investigators have studied the heat resistance of coliform organisms, particularly with reference to their ability to survive the pasteurization of milk. In general, the results show that the great majority of coliform cultures do not survive the exposures used in milk pasteurization but certain of them indicate that some strains may be unusually heat resistant (1, 3, 4, 5, 6, 8, 9).

The renewed interest in the use of the coliform test as an indication of pasteurization efficiency and sanitation in milk plants, and possibly other dairy plants, has again attracted attention to the heat resistance of the organisms.

In the studies reported herein *Escherichia* organisms of unusual heat resistance were repeatedly encountered. Most of them came from butter, but various other materials also were involved.

PROCEDURE

In the examination of butter for coliform organisms a sample was melted, centrifuged and the fat removed with precautions against contamination. Tubes of gentian violet lactose peptone bile broth were inoculated with 1 ml. and 0.1 ml. portions of the serum. Milk, buttermilk, and cream were added directly to gentian violet lactose peptone bile broth, and water was inoculated into lactose broth; with these materials 10 ml. portions were placed in 10 ml. double strength concentrations of the enrichment media while 1 ml. and 0.1 ml. portions were

added to 10 ml. single strength concentrations. Incubation was at 37° C. Material from a tube showing gas was smeared on eosin methylene blue agar for purification and preliminary identification.

In testing a culture for heat resistance, 0.2 ml. of a 2-day milk culture grown at 37° C. was suspended in 10 ml. sterile skim milk; 2 ml. portions of this suspension were sealed in small glass tubes and exposed for various periods in a water bath at 61.7° C. After exposure, a tube was cooled in ice water, broken, and the contents transferred to a tube of litmus milk for incubation at 37° C.

RESULTS

In connection with cooperative work between the Iowa Agricultural Experiment Station and a butter manufacturing plant, a number of heat resistant coliform strains were isolated. A transfer of one of the more resistant strains (identified as *Escherichia coli*) was studied in considerable detail at the Station. When tested repeatedly over a period of several months, it regularly survived 61.7° C. for 40 minutes although a part of the time it was held at approximately 3° C. The high heat resistance of the organism was verified in two other laboratories also interested in heat resistant coliform species; one of them that investigated its identity reported it was *E. coli*.

Resistant coliform organisms in cheese. Considerable difficulty was encountered with gas formation in edam cheese made in plant A from

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pasteurized milk. Investigation of the defective cheese indicated coliform organisms were the cause and from certain of the cheese *Escherichia* organisms were isolated. Heat tests on the cultures indicated they almost invariably survived 61.7° C. for 30 minutes. Tests on pasteurized milk from the plant showed that at the time it was free of coliform species. Infection of the cheese eventually was traced to defective vats.

Resistant coliform organisms in pasteurized milk. There was reason to suspect the presence of coliform organisms in milk pasteurized in plant B. Pasteurization was conducted in a spray vat with an exposure of 30 minutes at 62.2° C. Immediately after pasteurization the samples were removed aseptically and cooled in ice water. During a period in mid-summer, coliform types occasionally were found in the pasteurized milk. Heat tests were not conducted on the cultures at this time, but a number of them were held at approximately 3° C. without transferring for about 13 months when they were removed and sub-cultured in litmus milk. Four of the cultures grew and were transferred several times. They were tested for heat resistance and one of them survived 30 minutes at 61.7° C.; the others failed to survive 20 minutes.

In a more detailed study of the presence of coliform organisms in milk from plant B, two samples of raw milk and two corresponding samples of pas-

teurized milk were examined weekly from Dec. 10, 1942, to Oct. 7, 1943. With each sample, five 10 ml. portions were enriched at 37° C. in 10 ml. double strength gentian violet lactose peptone bile broth and five 1 ml. portions in single strength broth; during summer months the 10 ml. inoculations of raw milk were replaced with 0.1 ml. inoculations. Tubes containing the smallest inoculation of a sample which gave gas were cultured on eosin methylene blue agar to confirm the presence of coliform organisms; the 10 ml. enrichments of pasteurized milk also were smeared on the agar regardless of gas formation.

In the period outlined, 83 raw milk samples and 83 corresponding pasteurized milk samples were examined. Although coliform organisms regularly were found in the raw milk, they never were found in the pasteurized milk.

Resistant coliform organisms in various materials from a butter plant. In connection with studies on the water supplies of butter producing plants, various materials were examined for coliform organisms and frequently the heat resistance of isolated cultures was determined. At one of the plants (plant C) coliform organisms were repeatedly obtained. Results of heat tests on selected cultures are given in Table 1.

Of the 18 *Escherichia* cultures studied, 16 survived 20 minutes at 61.7° C., 12 survived 30 minutes and 7 survived 40 minutes. The cultures

TABLE 1
HEAT RESISTANCE OF SELECTED COLIFORM CULTURES ISOLATED FROM PLANT C

Material examined	Genus isolated	No. cultures tested	Cultures surviving 61.7° C. for						
			20 min.		30 min.		40 min.		
			no.	%	no.	%	no.	%	
Butter wash water	<i>Esch.</i>	1	0	.0	0	0	0	0	0
Cream	<i>Esch.</i>	4	3	75.0	2	50.0	0	0	0
	<i>Aerob.</i>	1	0	0	0	0	0	0	0
Buttermilk	<i>Esch.</i>	1	1	100.0	0	0	0	0	0
Butter	<i>Esch.</i>	12	12	100.0	10	83.3	7	58.3	

showing the greatest resistance came from butter, although two obtained from cream survived 30 minutes at 61.7° C. Since the butter wash water regularly was chlorinated, it would be expected to yield coliform cultures only rarely; the one culture isolated from it failed to survive 20 minutes at 61.7° C. The one *Aerobacter* culture tested was very low in heat resistance.

Resistant coliform organisms in butter. The high heat resistance of coliform organisms isolated from butter at plant C suggested an investigation of the heat resistance of coliform organisms in butter from other sources. Accordingly, samples of butter were collected from various plants, examined for coliform organisms, and heat resistance was determined on the cultures isolated. Certain of the plants yielded cultures which were highly resistant; Table 2 presents results on two of these plants.

TABLE 2

HEAT RESISTANCE OF COLIFORM CULTURES ISOLATED FROM BUTTER FROM PLANTS D AND E

Plant	Butter sample no.	Genus isolated	Min. cultures survived 61.7° C.
D	1	<i>Aerob.</i>	<20
	2	<i>Aerob.</i>	<20
	3	<i>Esch.</i>	40
	4	<i>Esch.</i>	40
	5	<i>Esch.</i>	40
	6	<i>Esch.</i>	40
E	1	<i>Esch.</i>	40
	2		
	3	<i>Esch.</i>	<20
	4	<i>Esch.</i>	<20
	5	<i>Esch.</i>	30
	6	<i>Esch.</i>	40

All of the six butter samples from plant D yielded coliform organisms; four of them yielded *Escherichia* cultures and two *Aerobacter* cultures. All of the *Escherichia* cultures survived 40 minutes at 61.7° C. while neither of the *Aerobacter* cultures survived 20 minutes.

Five of the six samples from plant E

yielded coliform organisms of the *Escherichia* type. At 61.7° C. two of the cultures survived 40 minutes, one survived 30 minutes but not 40 minutes and two did not survive 20 minutes.

A summary of all the examinations of butter samples, including heat tests on selected cultures, is given in Table 3.

The results indicate that coliform organisms are widely distributed in butter. Of a total of 220 samples from 77 plants, 143 from 65 plants contained these types. The great majority of samples yielded *Escherichia* cultures only; in a few instances both *Escherichia* and *Aerobacter* species were obtained while rarely only *Aerobacter* cultures were isolated.

The studies on heat resistance of selected cultures from the various samples show that a high percentage of the *Escherichia* cultures isolated from butter are considerably more heat resistant than would be expected on the basis of work done by previous investigators on cultures isolated from milk and certain other materials. Of the 92 *Escherichia* cultures from Iowa butter, 58 (63.0 percent) survived 20 minutes at 61.7° C., 35 (38.0 percent) survived 30 minutes and 16 (17.4 percent) survived 40 minutes. While the numbers of *Escherichia* cultures obtained from butter from other states are small (presumably because of the small number of samples examined), it is significant that again considerable numbers of them were heat resistant. Of the 116 cultures selected from all the samples examined, 74 (63.8 percent) survived 20 minutes at 61.7° C., 48 (41.4 percent) survived 30 minutes and 28 (24.1 percent) survived 40 minutes. The 17 *Aerobacter* cultures examined did not show significant heat resistance. All except two of them were killed in less than 20 minutes at 61.7° C. and the two that survived 20 minutes did not survive 30 minutes.

Detailed studies on heat resistant cultures. Although many of the cultures survived 40 minutes at 61.7° C.,

TABLE 3
RESULTS OF TESTS FOR COLIFORM ORGANISMS IN BUTTER FROM ALL PLANTS,
INCLUDING HEAT TESTS ON SELECTED CULTURES

Source of butter samples	No. plants represented	No. samples tested	No. samples positive	Cultures tested for heat resistance		Cultures surviving 61.7° C. for					
				genus	no.	20 min.		30 min.		40 min.	
						no.	%	no.	%	no.	%
Iowa	71	182	117	{ <i>Esch.</i>	92	58	63.0	35	38.0	16	17.4
				{ <i>Aerob.</i>	12	1	8.3	0	0	0	0
Missouri	3	18	17	{ <i>Esch.</i>	15*	8	53.3	8	53.3	7	46.7
				{ <i>Aerob.</i>	4*	0	0	0	0	0	0
Kentucky	1	8	3	{ <i>Esch.</i>	3*	3	100.0	2	66.7	2	66.7
				{ <i>Aerob.</i>	1*	1	100.0	0	0	0	0
Illinois	1	6	4	<i>Esch.</i>	4	3	75.0	1	25.0	1	25.0
Tennessee	1	6	2	<i>Esch.</i>	2	2	100.0	2	100.0	2	100.0
Totals	77	220	143	{ <i>Esch.</i>	116	74	63.8	48	41.4	28	24.1
				{ <i>Aerob.</i>	17	2	11.8	0	0	0	0

* Some samples yielded both *Escherichia* and *Aerobacter* cultures.

the method of determining the heat resistance gave no indication of the number of cells present at any time. Even though a culture is heat resistant, not all the cells are equally so, and the numbers of organisms should decrease as the period of heating increases. To determine the extent of survival in resistant cultures, counts were made on suspensions of organisms before and after exposure at 61.7° C. The suspensions were prepared and heated in the manner already described and counts were made with tryptone glucose extract milk agar, using an incubation of 48 hours at 37° C. The results are given in Table 4.

organisms in the original suspensions were purposely kept low to compare the rates of destruction with those in suspensions having high numbers. The unheated suspensions had 58,000 and 130,000 organisms per ml.; after 20 minutes heating the counts had dropped to 30 and 2,600 per ml., respectively, while after 30 minutes both counts were less than one organism per ml. The percentages of organisms surviving 20 minutes were 0.05 in trial 11 and 2.00 in trial 12. With heavier suspensions of the same cultures in earlier trials (trials 3 and 4), corresponding percentages were 3.25 and 6.67. Thus with fewer organisms in

TABLE 4
NUMBERS AND PERCENTAGES OF *Escherichia* ORGANISMS SURVIVING 61.7° C.
FOR VARIOUS PERIODS

Trial	Bacteria per ml. in original suspension	Bacteria surviving 61.7° C. for							
		20 min.		30 min.		40 min.		50 min.	
		no. ¹	%	no. ¹	%	no. ¹	%	no. ¹	%
1	15,300,000	3,000,000	19.60	245,000	1.60	48,000	0.31	11	0.00007
2	10,500,000	5,500,000	52.40	1,800,000	17.14	29,000	.18	860	.008
3	12,600,000	410,000	3.25	52,000	.41	1,060	.008	7	.00006
4	18,600,000	1,240,000	6.67	248,000	1.33	700	.004	9	.00005
5	9,800,000	50,000	.51	21	.0002	<1	>1
6	9,700,000	14,000	.14	15	.0002	2	.00002	>1
7	10,000,000	364,000	3.64	22,000	.22	340	.003	2	.00002
8	12,000,000	7,100,000	59.16	191,000	1.59	550	.005	>1
9	12,200,000	770,000	6.31	1,360	.011	1	.000008	>1
10	5,100,000	520,000	10.20	18,000	.35	340	.007	>1
11	58,000	30	.05	<1	<1	>1
12	130,000	2,600	2.00	<1	<1	>1

¹ = number per ml.

In trials 1 to 10, inclusive, the unheated suspensions had relatively high counts, from 5,100,000 to 18,600,000 per ml. Although the counts had decreased in each case after 20 minutes heating, they still were high. As the heating continued the numbers dropped off rather rapidly but they were significant in all trials after 30 minutes and in most trials after 40 minutes. In five of the trials organisms survived 50 minutes heating; in one, the count then was still relatively high while in the other four the counts were very low.

In trials 11 and 12 the numbers of

the original suspension there was a greater percentage destruction during the heating. However, the comparisons were not made on the same subculture of each strain which may have been a factor in the results.

DISCUSSION

The results of the studies substantiate the work of earlier investigations in showing that some strains of coliform organisms are considerably more heat resistant than others. The resistant strains belonged to the genus *Escherichia* rather than to the genus

Aerobacter, a fact that may be of significance in considering certain defects of pasteurized milk and cream which may be due to coliform organisms, such as ropiness.

The frequent occurrence of coliform organisms in butter and the high percentage of heat resistant *Escherichia* cultures encountered indicate the need for further study of these organisms in butter. Although it is possible that some of the resistant strains survive pasteurization of the cream and thus gain entrance to the butter, it is not probable that an extensive contamination of butter occurs in this way due to the rather high pasteurization temperatures used with cream for butter; also the cream often is acid which would increase the destruction of organisms with a given exposure. The churn may be an important source of coliform organisms, particularly heat resistant *Escherichia* types. The heat treatment given churns in attempts at cleaning and sterilization may tend to select the more resistant types of coliform organisms which then multiply and are added in significant numbers to the butter during the manufacturing process.

The considerable number of heat resistant coliform organisms isolated and the particularly high resistance of certain of the strains indicate the need for care in interpreting results of coliform tests on pasteurized dairy products. This is especially true with milk if it is to be subjected to a pre-test incubation period of 24 hours at 22.2° C. (2,7). In this type of examination the survival of one or two cells in a comparatively large volume of milk could account for relatively large numbers of the organisms at the end of the incubation and thus would be highly significant.

SUMMARY

Escherichia cultures of comparatively high heat resistance were encountered rather frequently in dairy

products, especially in butter. Of 116 cultures selected from isolations from butter, 74 (63.8 percent) survived 20 minutes at 61.7° C., 48 (41.4 percent) survived 30 minutes and 28 (24.1 percent) survived 40 minutes. Bacterial counts on certain of the resistant cultures indicated that in some of them appreciable numbers of cells still were present after 50 minutes exposure at 61.7° C. Heat resistant *Aerobacter* cultures were not encountered.

The general results suggest that *Escherichia* cultures isolated from pasteurized dairy products should be tested for heat resistance before assuming that pasteurization was inadequate or that contamination had occurred.

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