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Fecal Streptococci in Frozen Foods

I. A Bacteriological Survey of Some Commercially Frozen Foods[1]

EDWARD P. LARKIN, WARREN LITSKY AND JAMES E. FULLER

Department of Bacteriology and Public Health, University of Massachusetts, Amherst, Massachusetts

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Frozen foods, as possible health hazards, have been remarkably free from suspicion. It is possible, according to Fitzgerald (1947), that frozen fruits and vegetables served in raw salads could very well be a source of infection.

Yurchenco, Piepoli and Yurchenco (1954) studied the maintenance of stable infectious bacterial collections. They found that the microorganisms remain stable and give reproducible "mortality rates" after as long as two years in freezing storage. No losses in viability as a result of freezing and thawing were detected. The virulence of the organisms was unchanged during the storage period.

Berry (1946) stressed the need for standardized methods for the examination of frozen foods. The use of *Escherichia coli* as a test organism was not considered feasible because the organism died during storage.

Stock cultures of *E. coli*, *Salmonella typhosa*, and *Shigella paradysenteriae*, inoculated into pasteurized orange concentrate and frozen, were so reduced in numbers at the end of 24 hours that a plate count could not be obtained (Hahn and Appleman, 1952a, b). *Streptococcus faecalis* added from stock cultures or from fresh fecal material outlived all other enteric organisms.

Forty-two cans of commercially packed frozen orange concentrate from three plants were examined by Kaplan and Appleman (1952). All cans contained enterococci, but only four showed the presence of coliform bacteria. The enterococci were apparently more resistant to the storage conditions of the frozen orange concentrate.

Hucker, Brooks and Emery (1952) during their investigation of the bacterial populations of frozen beans, peas, and corn found that a cold-resistant basic flora appeared in the frozen vegetables. Apparently, the bacterial counts at the beginning of storage had no relationship to the quality of the foodstuffs after 6 and 12 months. Sometimes streptococci were the predominant organisms in the frozen foods examined.

By presumptive coliform and enterococcus tests Burton (1949) examined 376 samples of commercially frozen Canadian vegetables and cantaloupe for fecal contamination. The coliform bacteria were apparently more dependable than enterococci for indicating contamination in foods prior to freezing, whereas the enterococci were apparently superior indicators in the frozen food. No coliform bacteria or enterococci were found on vegetables directly after blanching.

An investigation of the incidence of coliform bacteria in frozen vegetables could very easily give erroneous results (Elrod, 1942). A genus of plant pathogens, the *Erwinia*, is closely related to the *Escherichia-Aerobacter* group, and the IMVIC reactions for this genus and their ability to ferment lactose classific them many times as *E. coli*. Thus, fecal contamination was not truly indicated.

Hajna and Perry (1943) made comparative studies of presumptive and confirmative media for bacteria of the coliform group and for fecal streptococci. A new medium, SF medium, highly specific at 45.5 C for fecal streptococci, was developed. A later study by Mallmann and Seligmann (1950) showed that azide dex-

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trose broth was more effective than lactose broth, azide broth, or SF medium for enterococci isolation.

Litsky, Mallmann and Fifield (1953) developed a medium, containing ethyl violet and sodium azide, which was specific for the growth of enterococci. A comparative study of Hajna and Perry's SF medium, the method of Winter and Sandholzer (1946), and the ethyl violet-sodium azide medium showed that the last of these media detected and confirmed 100 to 1000 times as many enterococci as the other types. Azide dextrose broth as the presumptive medium and ethyl violet azide broth as the confirmatory medium were advocated as a more exact indicator of the numbers of enterococci present.

Materials and Methods

A bacteriological survey of some commercially frozen foods was made as the initial step in this study. As many different types of frozen vegetables and fruit juice concentrates as could be found in the Amherst markets were purchased and examined. Wherever possible, tentative procedures for the Examination of Frozen Foods (American Public Health Association, 1946) were followed. The results of this survey are recorded in table 1.

The pH values of the citrus concentrates were obtained by using a Model H Beckman pH meter. The low pH value of the citrus concentrates presented a problem at the initial stage of the investigation. When aliquot amounts of concentrate were transferred to enrichment medium, the bacteria were inhibited and did not produce recognizable changes in the medium. This difficulty was overcome by neutralizing the concentrate before examination.

Fifty-gram samples of the fruit or vegetables were weighed, transferred to 450 ml of sterile tap water, and blended for exactly two minutes in a Waring Blendor. The container was put aside for 3 to 5 minutes to allow foam to subside. The citrus fruit concentrates were pipetted directly into the respective sampling media.

The most probable number (MPN) of coliform bacteria was obtained by adding 10, 1, 0.1, 0.01, and 0.001 ml portions of the blended material to sets of five replicate lactose broth tubes. Transfers were made into brilliant green lactose bile broth from all tubes in which gas had formed within 48 hours. Streaks were made on eosin methylene blue agar plates from the gas-positive lactose tubes.

The MPN of fecal streptococci was obtained by the same procedure as that for the coliform bacteria, except that azide dextrose broth was the presumptive medium and ethyl violet azide broth was the confirmative medium.

In table 1, brilliant green (BGB) lactose bile broth is compared with eosin methylene blue agar (EMB). Results were far from consistent when these two media were used for confirmation from the same presumptive tube. This age-long problem continues to confuse the investigator when he correlates and interprets his experimental data.

Results

The results of the survey are presented in table 1. The total bacteria count per gram of frozen vegetables ranged from 3,000 to 900,000. In the citrus concentrates, the count ranged from 1,000 to 10,000 per ml.

The results of the presumptive and confirmed coliform procedures on the same food products showed great variations. Beans, for example, showed a MPN of 2,400 when the presumptive medium was used, and a MPN of only 92 when transferred to confirmatory media. In corn, the MPN ranged from 54,000 to 92,000 when lactose broth was used, but values of only 35,000 to 54,000 were obtained when BGB broth was used, and 180 when EMB agar was employed. The same trend was noted when spinach, lima beans, mashed potatoes, and orange juice concentrates were tested. The MPN of enterococci was generally higher than the MPN for the coliform bacteria.

It is of interest that lemon juice, lemonade, grapefruit juice, and orangeade concentrates showed no indication of coliform bacteria or enterococci even after the acidity of the sample was neutralized. Two samples of grape juice concentrate showed a MPN of 6.8 and 29, respectively, when EMB was used, whereas BGB broth gave no results.

Discussion

Fecal streptococci, which originate only in the fecal contents of man or warm-blooded animals, were found in all the vegetable samples examined (Ostrolenk and Hunter, 1946; Winter and Sandholzer, 1946). The number of enterococci varied considerably, but enough were present in all samples to be of Public Health significance.

The citrus concentrates had fewer enterococci than the vegetable samples examined. However, the presence of even a few of these organisms is probably of more significance in this type of food than in vegetables, because the frozen vegetables are cooked before they are eaten. But this is not true of the citrus juice concentrates and the frozen fruits. If fecal streptococci are in uncooked frozen foods, then it is possible that other fecal organisms may be present as well.

Fecal bacteria in any foods constitute a possible health menace. The increasing consumption of frozen foods in the United States also increases the possibility that serious, widespread epidemics could result from improperly processed frozen foods.

TABLE 1. *Bacteriological examination of various commercial frozen foods*

Name and Type of Food	pH	Total Bacteria Count per ml or g at 32 C* (4 Days)	Coliform Bacteria			Enterococci, MPN 100 ml or g (19, 20) Ethyl Violet Azide Broth
			MPN per 100 ml or g lactose broth	MPN per 100 ml or g BGB	MPN per 100 ml or g EMB	
Green beans						
Brand 1		3,000	950	0	1.8	2,800
		3,000	470	260	61.0	9,200
		3,000	790	45	18.0	3,500
		3,000	220	—	—	2,200
Brand 2		1,000,000	—	—	—	180,000
		900,000	7.8	—	20	180,000
Brand 3		93,000	2,400	92	92	260
		260,000	2,200	460	460	6,400
Brand 4		200,000	20	—	—	1,300
		135,000	1,100	40	20	54,000
Brand 3		100,000	1,800	100	100	54,000
Brand 4		3,000	170	20	—	1,300
Brand 5		28,000	—	—	—	180,000
Spinach						
Brand 1		3,000	20	—	20	520
		3,000	45	—	—	220
Corn						
Brand 1		700,000	92,000	35,000	—	3,500
		800,000	92,000	35,000	—	2,200
		160,000	54,000	54,000	180	18,000
Lima beans						
Brand 1		23,000	490	170	—	2,200
		35,000	220	170	—	1,700
		24,000	790	790	37	1,100
Mashed potatoes						
Brand 1		11,000	230	230	—	16,000
		50,000	18	—	—	3,500
		45,000	230	45	—	790
Orange juice						
Brand 1	4.4	6,500	240	—	130	18,000
	4.4	8,000	540	—	48	18,000
Brand 6	4.25	3,000	240	—	41	640
	4.25	3,000	>1,800	—	19	170
Brand 4	4.5	3,000	5,400	—	12	72
	4.4	3,000	>1,800	—	45	170
Brand 5	3.2	7,500	2	—	—	1,700
	3.5	4,000	79	4	4	2,800
Brand 7	3.4	3,000	14	—	—	540
	3.3	3,000	15	—	—	540
Brand 8	3.3	3,000	—	—	—	5,400
	3.2	3,000	2	—	—	16,000
Brand 1	3.5	8,500	410	55	54	1,100
	3.5	10,000	920	—	170	2,200
Brand 7	3.4	1,000	240	—	14	1,200
	3.5	1,500	41	—	14	72
Brand 6	3.5	6,000	16,000	5.4	2	18,000
	3.8	6,000	22,000	40	—	18,000
Lemon juice						
Brand 8	1.8	3,000	—	—	—	—
	2.00	3,000	—	—	—	—
Limeade						
Brand 9	2.7	3,000	—	—	—	—
	2.75	3,000	—	—	—	—
Grape juice						
Brand 1	3.2	3,000	1,800	—	29	—
	3.2	3,000	1,800	—	6.8	—
Brand 10	3.2	3,000	1,800	—	—	—
	2.9	3,000	—	—	—	—

* Medium used for total count was tryptone glucose extract agar.

TABLE 1—*Continued*

Name and Type of Food	pH	Total Bacteria Count per ml or g at 32 C* (4 Days)	Coliform Bacteria			Enterococci, MPN 100 ml or g (19, 20) Ethyl Violet Azide Broth
			MPN per 100 ml or g lactose broth	MPN per 100 ml or g BGB	MPN per 100 ml or g EMB	
Grapefruit juice						
Brand 7	3.7	3,000	—	—	—	—
	3.4	3,000	—	—	—	—
	3.7	3,000	—	—	—	—
	3.5	3,000	—	—	—	—
Orange and grapefruit						
Brand 7	3.5	3,000	240	—	11	5.5
	3.4	3,000	240	—	22	5.5
	4.0	3,000	>1,800	—	7.8	3.6
	4.1	3,000	>1,800	—	17	1.8
Orangeade						
Brand 7	3.7	3,000	—	—	—	—
	3.65	3,000	—	—	—	—
Melon balls						
Brand 11		14,000	180,000	1,000	160,000	92,000
		18,000	8,000	1,300	—	54,000
Sliced peaches						
Brand 4		3,000	40	18	—	—
		3,000	460	20	—	—

SUMMARY

Sixty-four samples of commercially frozen fruits, fruit juice concentrates, and vegetables were tested. Both coliform bacteria and fecal streptococci were found in many of the samples. Fecal streptococci were found more consistently, and usually in greater numbers, than coliform bacteria.

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