passage of this bill cannot set aside the high cost of shipment of a bulky refrigerated food that must reach the market promptly, hence distant markets for milk may be an economic illusion.

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MICROBIAL ANALYSIS OF COMMERCIAL[1] FROZEN FISH STICKS

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A total of 78 samples of frozen fish sticks were analyzed for total plate count, coliform count, coagulase-positive staphylococci and members of the Salmonella-Shigella group. Fifteen samples (19%) contained 50,000 organisms or more per g. and 4 had 100,000 or more per g. Coliform counts were generally low, ranging from zero to 35 per g., with 6 samples showing counts of 10 or more per g. Two samples contained coagulase-positive staphylococci and an isolate from one of these samples was positive to salmonella polyvalent sera.

The consumption of prepared pre-cooked or partially cooked frozen foods has increased greatly in recent years. Though regulatory groups and public health officials have expressed concern over the sanitary quality of pre-cooked or prepared frozen foods, there has so far been very little promulgated as official standards for such control *(7, 8)*. The U. S. Army Quartermaster Corps stipulates standards of a total plate count not to exceed 100,000 organisms per g, not more than 10 coliform organisms per g and the absence of pathogens *(4)*.

The standards of the Commonwealth of Massachusetts for these products are somewhat more stringent *(9)*. The total plate count is limited to 50,000 organisms per g, and not more than 10 coliform organisms per g and no coagulase-positive staphylococci or members of the *Salmonella-Shigella* groups should be present. The National Association of Frozen Food Packers has tentatively suggested a standard consisting of 100,000 organisms per g, omitting any maximum allowance for coliforms or staphylococci *(2)*.

Although pre-cooked frozen fish sticks have been marketed commercially since 1953, published bacterial analyses of this product have not been numerous. Larkin ,Litsky and Fuller in 1956 *(5)* examined pre-cooked frozen fish sticks and reported that for most samples coliform counts were less than 20 per g, enterococci were less than 500 per g and total plate counts never exceeded 3,000 per g. They suggested that the breading on the fish sticks might be a major source of contamination. Benarde *(1)* in a later study stated that although breading was found to possess appreciable numbers of organisms, most contaminants were destroyed during processing.

Since the introduction of pre-cooked, frozen fish sticks, the market has expanded greatly and the number of producers has increased. It was thought that re-examination of this commercial product would be of interest.

PROCEDURE

Fish stick samples were purchased in retail outlets in 24 cities across the country, packed and shipped with dry ice, and maintained at temperatures of 0°F or below until examined. Three samples from each of 26 different processors were included.

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Each sample was segmented while still frozen, and portions totaling 50 g blended for 2 min in 450 ml of water *(6)*. Total plate counts were made in plate count agar incubated at 35°C for 24 hr. The tetrazolium flooding technique of Solberg and Proctor *(11)* was employed to distinguish colonies in low dilutions.

Coliform organisms were enumerated by plating in desoxycholate lactose agar. After incubation at 37°C for 24 hr typical colonies were selected and inoculated into brilliant green bile lactose broth. The presence of gas and gram-negative organisms was considered confirmatory. In certain instances a larger aliquot, 10 ml, of the 1-10 slurry was plated in large 150-mm Petri dishes.

Coagulase-positive staphylococci were isolated by surface streaking on mannitol-salt agar. Characteristic yellow colonies were transferred to brain heart infusion broth for the determination of soluble coagulase by the tube method *(6)*.

For the detection of members of the *Salmonella-Shigella* group, 10 ml of the homogenate were mixed with 10 ml of double strength selenite-cystine broth. After 24 hr incubation at 37°C, a loopful from the broth was streaked on both bismuth sulfite and SS agars, and the plates incubated for 48 and 24 hr, respectively, at 37°C. Representative colonies were inoculated on tryptone agar slants which were incubated for 18 hr at 37°C. The isolates were characterized by their reactions in triple sugar iron agar and SIM medium. The presence of salmonella was then tested by agglutination with polyvalent sera (Lederle Labs).

The designation "*Salmonella-Shigella* group" is a term employed by Massachusetts *(9)* and does not imply that these organisms are identical. The isolation procedure will not distinguish *Salmonella* from certain other *Enterobacteriaceae*, notably the Arizona group. Although distinct from *Salmonella*, members of the Arizona group are, due to their infectious capabilities, of public health significance. Since identification of the isolates suspected of being either *Salmonella* or *Shigella* were not verified by official public health laboratories, their identity in this study is considered tentative.

RESULTS AND DISCUSSION

Total plate counts varied from a low of 300 to a high of 1,400,000 organisms per g. (Table 1). Of 78 samples, representing 26 processors, 15 samples (19%) contained 50,000 or more organisms per g. Four of the 15 samples had 100,000 or more organisms per g. These 15 samples were distributed among 8 processors, 6 of which accounted for 13 of the 15 samples. It appears that since the study of frozen fish sticks by Larkin, Litsky and Fuller *(5)* sanitary quality has deteriorated and that this problem is confined to individual manufacturers since a small number of the producers are distributing a bacteriologically inferior product. This is also supported by the fact that 2 of the 6 producers having high total count samples also accounted for 3 out of 5 samples that contained more than 10 coliform organisms per g. (Table 1). The majority of the samples examined did not contain any coliform organisms. The highest coliform count noted was 35 per g.

TABLE 1—MICROBIAL ANALYSIS OF FROZEN FISH STICKS FOR TOTAL PLATE COUNT AND COLIFORM ORGANISMS

Producer	Total Plate Count, No./g		Coliforms/g (range)
	Ave. Count	Range	
A	6,800	5,600-8,000	0
B	8,100	1,100-17,000	0-25
C	4,900	1,800-7,500	0
D	38,000	320-62,000	0
E	26,000	6,800-49,000	0
F[b]	800,000	11,000-1,400,000	0-14
G	23,000	7,300-44,000	0
H	20,000	1,800-38,000	0
I[a]	18,000	4,200-36,000	0-2
J	75,000	51,000-100,000	0-5
K	17,000	1,900-39,000	0
L	3,100	1,800-4,000	0
M	28,000	1,300-75,000	0
N	38,000	13,000-50,000	0-10
O	78,000	1,500-180,000	0-7
P	7,200	1,600-15,000	0
Q[b]	27,000	16,000-44,000	0
R	11,000	2,700-26,000	0
S	11,000	7,000-13,000	0-5
T	3,800	900-8,000	0
U[a]	56,000	16,000-76,000	0-39
V[abc]	17,000	4,200-40,000	0-24
W	29,000	12,000-59,000	0-5
X	12,000	3,800-17,000	0-5
Y	7,200	2,700-11,000	0
Z	13,000	2,200-24,000	0-5

[a]Coagulase-positive staphylococci were present.
[b]Isolates possessed reactions typical of *Salmonella-Shigella* group.
[c]Positive reaction with *Salmonella* polyvalent sera.

Quantitating the number of coliform organisms present in a sample by using one ml of a 1-10 homogenate presents certain difficulties. At this dilution, an average of slightly more than one organism per plate, which is equivalent to more than 10 per g in the original sample, will cause the sample to be considered illegal by certain authorities. Hartman *(3)* had previously noted this difficulty.

The use of a large 150-mm Petri dish should resolve this difficulty by allowing the use of a sample size of 10 ml, thus increasing the reliability of the test. Table 2 presents a comparison of results obtained from 1-ml and 10-ml aliquots. Two samples found to contain more than 10 coliforms per g by the standard technique possessed even higher coliform counts when examined with the large Petri

dish. One sample was found to possess 17 coliforms per g with the large Petri dish although averaging 10 per g when the 100-mm Petri dish was employed.

TABLE 2—COMPARISON OF COLIFORM COUNTS OBTAINED WITH 100 MM. AND 150 MM. PETRI DISHES

No. of Samples	1 ml of Original Homogenate (coliforms/g)	10 ml of Original Homogenate (coliforms/g)
4	0	0
1	0	2
1	5	1
1	10	4
1	10	17
1	15	32
1	25	53

Coagulase-positive staphylococci were isolated from one sample, of producers I and V. In a subsequent study by the authors of frozen raw and precooked shrimp *(10)*, an enrichment technique involving cooked meat media containing 10-% NaCl followed by selection on egg yolk agar was found to be greatly superior to mannitol-salt agar for isolation of coagulase-positive staphylococci. Mannitol-salt agar is not efficient for detection when these organisms are present as a minor portion of the bacterial population. The question of whether or not the possession of coagulase activity is a sufficient criterion for characterizing an enterotoxin-producing strain of staphylococci is discussed by the authors elsewhere *(10)*.

Isolates from 5 samples gave reactions typical of members of *Salmonella-Shigella* groups and, of these, one isolate from producer V reacted with *Salmonella* polyvalent sera. This sample, although having a moderate total plate count, had, in addition to a positive *Salmonella* isolate, a high coliform count, and contained coagulase-positive staphylococci.

The majority of the samples of frozen fish sticks analyzed in this study were of acceptable bacteriological quality, but a number of exceptions did occur. A total of 20 samples distributed among 12 processors did not meet the sanitary standards required by Massachusetts. Twelve samples obtained from 8 producers would have been in violation if the allowable total plate count requirement were to increase to 100,000 organisms per g.

The procedures used in this study have been recommended by various investigators and are intended to be indicative of sanitary conditions during processing and storage. Attempts to find a correlation between various tests normally employed, such as total plate count, coliform and pathogens have not been overly successful. This is not surprising when one reflects on all the numerous vectors for contamination indigenous to a food plant.

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