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FAECAL INDICATOR ORGANISMS IN FROZEN PRAWN PRODUCTS:

I. Incidence And General Distribution.

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[A general survey carried out on several brands of frozen prawn products has shown that along with the standard plate count (SPC), the numbers of pathogenic organisms like *Escherichia coli*, enterococci and coagulase positive staphylococci have also to be taken into consideration for the evaluation of the quality of these products. No correlation could be established between the total plate count and the numbers of *E. Coli*, enterococci or staphylococci. Enumeration of enterococci, however, is advocated as a better index of faecal contamination of the products than *E. coli*.]

Enumeration of the viable bacteria in fish muscle has frequently been used as a means of ascertaining to what extent bacterial spoilage has proceeded therein and for getting an idea about its keeping quality. Along with the determination of total plate count, tests for the presence of pathogenic organisms like *E. coli* type I, enterococci and coagulase positive staphylococci have recently been introduced for the inspection of various types of food products. Investigations are now being carried out in several conntries for determining the limits of tolerance of these organisms in various types of frozen products, particularly precooked or partially cooked foods (Fitzgerald 1947, Frechette and Michael 1961, Rayman *et. al.* 1955) and also for standardising the methods of detection and estimation of these organisms in frozen foods (Borgstrom 1956, Barnes 1956 a, b & 1959, Fanelli and Ayres 1959, Raj and Liston 1961 a, b). A high incidence of *E. coli* or if any other organisms of faecal origin in a processed fishery products is viewed far more seriously than a high standard plate count, as the latter may sometimes be due to surface contamination by harmless bacteria.. In the establishment of quantitative bacterial standards for food products the effects of variations due to environmental conditions and that due to differences in processing and storage temperatures should also be taken into consideration. Frozen prawn presents an additional problem since it is sold cooked or raw, peeled or unpeeled and with or without breading. Cooked frozen prawn may even be consumed without further cooking after thawing.

There are a few reported studies of the incidence and survival of bacteria of public health significance in frozen sea foods. Gunderson *et. al.* (1954) analysed 27 samples of frozen raw breaded shrimp and found 950,000—14,000,000 organisms per gram, 150 — 14,000 coliform organisms per gram, presence of coagulase positive staphylococci and absence of salmonella in all samples. In an analysis of 91 samples of commercial frozen shrimp consisting of raw or cooked shrimp, that had been breaded, shelled or left unshelled, Silverman *et. al.* (I961) found that the total bacterial counts ranged from 50 - 9,000,000 organisms per gram, no direct correlation between total plate counts and coliform counts except for the precooked samples in which both were low, presence of coagulase positive staphylococci in 75% of the frozen samples and presence of Salmonella and Shigella organisms in a limited number of the samples. Kachikian *et. al.* (1959) surveyed 144 samples of frozen and breaded shrimp representing 24 brands and reported that total counts varied from 22,500 - 54,000,000 organisms per gram, 68% of samples contained less than 100 coliform bacteria per gram and presence of faecal streptococci in all the samples, their numbers varying from a few bacteria per gram to 13,500 per gram. These authors also suggested the use of the standard plate count as the primary test procedure and the enterococci as an adjunct in evaluating the sanitary quality of frozen breaded shrimp.

The work described here was undertaken as part of a general study of public health bacteriology of frozen prawn products and was designed to provide information on the nature of the raw material received in factories, how the preprocess treatment affects the bacterial quality of the raw material, changes induced in the microflora by storage in ice and by different methods of handling and on the effects of freezing and subsequent prolonged low temperature storage.

Materials and Methods

An initial survey of 92 samples of frozen prawn (headless, P & D, and cooked) was carried out to determine the extent of contamination of these products by faecal indicator organisms. Samples were collected at random from seven sources which include a few suspected sources also, and tested for standard plate count and for the presence of coliforms, enterococci and staphylococci. Ten gram samples from the frozen blocks were homogenised in waring blenders with 100 ml. of sterile sea water and serial dulutions were made from this blended sample. For the determination of total viable count, sea water agar was used after a comparative study of sea water agar and nutrient agar. Aliquots were plated in duplicate and incubated at 30°C for 48 hr.

Desoxycholate lactose agar (APHA 1958) was used for the enumeration of coliform organisms by a plate count method. Typical coliform colonies were counted after incubation of these plates for 24 hr. at 37°C. Representative colonies from desoxycholate agar plates were streaked on eosine-methylene blue (EMB) agar plates. Colonies showing the characteristic metallic sheen on EMB agar plates were then subcultured into lactose broth tubes. The tubes were incubated for 24-48 hr. at 37°C and cultures from those tubes showing gas production within 48 hr. were carried on to nutrient agar slants. Each culture was gram stained and subjected to the IMViC procedure.

The presence of enterococci was ascertained by the use of sodium azide agar. The plates were incubated for 48 hr. at 37°C. Ten colonies were selected

at random from each plate showing a count of between 30 - 300 colonies, inoculated into Todd-Hewitt broth and incubated for 48 hr. at 37°C. The presence of enterococci was confirmed by (1) ability to survive at 60°C for 30 min, (2) ability to grow in the presence of 6.5% sodium chloride and (3) ability to grow at pH 9.6.

Mannitol salt agar was used for the isolation and identification of staphylococci. Plates were incubated for 48 hr. at 37°C. Ten colonies were picked at random both from the surface and subsurface and streaked on Chapman's agar plates. The presence of yellow to lemon pigment and fermentation of mannitol were considered to tentatively indicate the presence of coagulase positive staphylococci.

Results and Discussion

The results obtained with 92 samples of frozen prawn collected from different sources are shown in Table I. The data are presented as the average values for the microbial determinations. The distribution of bacteria varied from a minimum of 40,000 organisms per gram to a maximum of 7,200,000 organisms per gram for P & D, from 14,000 to 3,100,000 organisms per gram for headless and from 4,100 to 2,000,00 organisms per gram for cooked frozen prawn.

TABLE I

The bacteriological content of frozen prawn

Index No.	Source	Nature of sample	No of samples	Average No. of organisms per gram				
				S.P.C. (X10⁴)	Entero-cocci	Staphy-lococci	Coli-forms	E. coli
1-11	A	P & D	11	118.6	3,880	20,470	1,250	0,8
12-15	A	Headless	4	89.45	1,100	13,030	..	..
16-19	A	Cooked	4	40.25	38	12,150	..	..
20-22	B	P & D	3	30.3	150	30,000	7	Nil
23-28	B	Headless	6	11.92	2,030	16,170	10	Nil
29-36	C	P & D	8	40.33	1,290	4,190	48	8
37	C	Headless	1	1.4	Nil	990	..	..
38-46	C	Cooked	9	70.38	120	27,940	28	Nil
47-57	D	P & D	11	16.88	190	2,230	20	2.0
58	D	Headless	1	3.38	295	..	380	5.0
59-63	E	P & D	5	144.56	43	120,190	..	..
64	E	Headless	1	42.0	18,080	1,650	..	..
65-82	E	Cooked	18	35.62	220	6,900	9	Nil
83-89	F	P & D	7	338.0	16,690	40,610	207	14
90	F	Headless	1	11.0	325	Nil	12	Nil
91	G	P & D	1	22.0	100	4,600	180	Nil
92	G	Headless	1	28.0	100	760	108	Nil

In Table II the percentage of samples falling within the indicated ranges of bacterial counts are listed. Out of the total samples 21 per cent had a bacterial

count of less than 100,000 and 78 per cent contained less than one million. This is comparable to the figures reported by Kachikian *et. al.* (1959) who reported that in 144 samples of breaded shrimp 7 per cent contained less than 100,000 organisms per gram and 61 per cent less than one million.

TABLE II

Numbers of viable bacteria in frozen prawn

Range of Bacterial Count (No. of organisms / g.)	% of Samples		
	P & D	Headless	Cooked
Less than 100,000	18	33	19
$1.0 \times 10^5 - 5.0 \times 10^5$	39	60	52
$5.0 \times 10^5 - 1.0 \times 10^6$	9	Nil	19
Over 1.0×10^6	34	7	10

The minimum, median, "three quarter", and maximum values for each of the numerically determined bacterial categories are represented in Table III (The "three quarter" value is that below which 75% of all specimens would conform). Values in the first and last quartile show the extreme contrast between the quality of the heavily contaminated specimens and the quality of very good specimens that can be attained under the prevailing commercial conditions. This applies particularly to the enterococci and staphylococci which at the "75%" point occur in the respective numbers of 1660 and 19560 organisms per gram respectively for P & D, 1350 and 10830 organisms per gram for headless and 100 and 19500 organisms per gram for the cooked frozen prawn. Most of the samples showing high counts were obtained from sources where external contamination of the material was known to occur in some form or other. In these cases the prawns were either washed repeatedly with heavily contaminated water, left unprotected in the open for long periods with flies covering the surface or prepared in heavily contaminated premises. Very low values are encountered for coliforms and enterococci in more than 50% of the samples, although staphylococci counts are comparatively high, and these samples were from sources where the preprocess preservation and subsequent processing are carried out under standard conditions.

TABLE III

Some distribution values for bacteria of specified "indicator" categories in frozen prawn.

	Bacterial values - Number per gram				
	Standard plate count.	Coliforms	E. coli	Enterococci	Staphylococci
P & D					
Minimum	4.0×10^4	Nil	Nil	Nil	Nil
Median	3.5×10^5	33	Nil	223	5,325
"$\frac{3}{4}$ value"	1.1×10^6	150	Nil	1,660	19,560
Maximum	7.2×10^6	2,300	77	39,000	317,000

	Bacterial valuas - Number per gram				
	Standard plate count.	Coliforms	E. coli	Enterococci	Staphylococci
Headless					
Minimum	1.4 x 10^4	10	Nil	Nil	Nil
Median	1.2 x 10^5	60	Nil	325	1,550
"$\frac{3}{4}$ value"	1.86 x 10^5	108	Nil	1,350	10,830
Maximum	3.1 x 10^6	377	4	18,080	60,900
Cooked					
Minimum	4.1 x 10^3	Nil	Nil	Nil	Nil
Median	3.25 x 10^5	18	Nil	Nil	2,450
"$\frac{3}{4}$ value"	5.9 x 10^5	27	Nil	100	19,500
Maximum	2.0 x 10^6	50	Nil	1,220	64,000

The incidence of the various indicator organisms in the sample examined is represented in Table IV. In all the three types of frozen prawn under study more than 50% of samples contained 100 or less than 100 coliforms per gram and except in a few samples *E. coli* was absent. In contrast to the coliform bacteria, enterococci and staphylococci were more predominant, the latter being present in very large numbers in the majority of samples, even in the cooked frozen specimens. In general, enterococci and staphylococci far exceeded the number of coliform organisms in the same sample. Compared to the fresh frozen samples, cooked frozen prawn contained fewer enterococci (77% of cooked frozen prawn contained 100 or less than 100 organisms per gram while the percentage of samples containing the same number of organisms in the P & D and headless varieties were respectively 41 and 40) although no appriciable difference was noticed in the incidence of staphylococci. This is highly significant in the light of recent suggestions (Larkin *et. al.* 1955, 1956; Ostrolenk *et. al.* 1947, Kereluk and Gunderson 1959, Zaborowski *et. al.* 1958) that enterococci counts give a better index of faecal contamination in frozen food products.

TABLE IV

Incidence of Coliforms, E. coli, enterococci and Staphylococci in frozen prawns

Range of Bacterial count; Number of organisms per gram	Percentage of samples containing the indicated number of, in											
	P & D				Headless				Cooked			
	Coliforms	E. Coli	Enterococci	Staphylococci	Coliforms	E. Coli	Enterococci	Staphylococci	Coliforms	E. Coli	Enterococci	Staphylococci
Nil	17	79	30	6.5	Nil	75	27	14	33	Nil	58	6
1 - 5	Nil	4	Nil	Nil	Nil	25	Nil	Nil	Nil	Nil	Nil	Nil
5.1 - 25	25	8.5	Nil	Nil	50	Nil	Nil	Nil	25	Nil	3	Nil
25.1 - 100	25	8.5	11	Nil	Nil	Nil	13	Nil	42	Nil	16	Nil
100 - 1,000	29	Nil	26	13	50	Nil	27	29	Nil	Nil	16	32
1,000 - 10,000	4	Nil	20	39	Nil	Nil	27	21	Nil	Nil	7	26
10,000 - 100,000	Nil	Nil	13	35	Nil	Nil	6	36	Nil	Nil	Nil	36
Over 100,000	Nil	Nil	Nil	6.5	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

The general relationship between coliforms, enterococci and staphylococci count with the total microbial population of the frozen prawn is represented in Table V. No marked correlation was noted between the total plate count and the other three groups.

TABLE V

Relationship of Coliforms, enterococci and staphylococci to standard plate count

A. Percentage of samples with various coliform: total count ratios

Coliforms per gram	Total count per gram			
	10^3-10^4	10^4-10^5	10^5-10^6	Over 10^6
Nil		7.5	12.5	Nil
1 - 10		2.5	2.5	5.0
10 - 10		5.0	22.5	17.5
Over 100		2.5	10.0	12.5

B. Percentage of samples with various enterococcus: total count ratios

Enterococci per gram	Total Count per gram			
	10^3-10^4	10^4-10^5	10^5-10^6	Over 10^6
Nil	2.0	11.0	22.0	4.3
0 - 100		2.0	10.0	2.0
100 - 1,000		4.3	15.0	3.0
1,000 - 10,000		2.0	9.0	5.4
Over 10,000		Nil	1.0	7.0

C. Percentage of samples with various Staphylococcus: total count ratios

Staphylococci per gram	Total count per gram			
	10^3-10^4	10^4-10^5	10^5-10^6	Over 10^6
Nil		2.0	4.5	1.0
0 - 100		Nil	Nil	Nil
100 - 1,000	2.0	6.0	12.0	1.0
1,000 - 10,000		8.0	21.0	3.0
Over 10,000		3.0	20.0	16.5

The results presented show that some of the samples examined contained numbers of bacteria sufficiently high to indicate quality deterioration and established an objectionably high degree of contamination due to unsanitary conditions. Special attention has to be paid to this problem, since limits of tolerance for pathogenic organisms in frozen fishery products have been tentatively set up by many foreign laboratories.

The raw material used for preparation of the experimental products were also examined at random. To study the effect of using polluted water in the pre-process preparation of the raw material, a few samples were treated with water taken from the backwaters near Cochin, known to be highly contaminated. Data collected from these samples showed that such raw materials took up the faecal indicator organisms and that even after freezing these samples showed a very high total count and a high incidence of coliforms and enterococci. Results of a few series of experiments carried out are represented in Table VI and of specially treated samples in Table VII.

TABLE VI

Numbers of bacteria, Coliforms, Enterococci and Staphylococci in fresh prawns

(Bacterial count expressed as number of organisms per gram.)

Description of sample	Standard plate count x 104	Entero-cocci	Staphylo-cocci	Coliforms M. P. N.	E. coli
1. *Metapenaeus affinis* P D	1,000	57	52,250	460	58
2. *Metapenaeus affinis* fantail	64.2	3,160	26,550	4,090	70
3. *Metapenaeus affinis* butterfly	21.75	90	43,800	105	6
4. *Metapenaeus affinis* fantail 41-50	30.25	50	590	4,080	Nil
5. *Penaeus indicus* P & D 71-90	35.82	280	4,390	73	Nil
6. *Metapenaeus affinis* P & D 50-60	31.4	62	340	1,230	234
7. *Metapenaeus affinis* P & D	297.7	425	24,970	155	20
8. *Penaeus indicus* headless	77.0	1,075	8,460	9,350	Nil
9. *Metapenaeus affinis* P & D 81-100	262.5	900	5,860	1,440	12
10. *Metapenaeus affinis* fantail 31-40	15.82	438	420	3,060	206
11. *Penaeus indicus* P & D 40-50	750.0	Nil	382,500	265	23
12. *Metapenaeus affinis* butterfly 31-40	393.5	140	15,500	4,060	550

TABLE VII

Numbers of bacteria, coliforms, enterococci and staphylococci in fresh prawns handled under unsanitary conditions

(Bacterial count expressed as number of organisms per gram.)

Description of sample	Standard plate count x 104	Entero-cocci	Staphylo-cocci	Coli-forms	E. coli
1. *Metapenaeus affinis* P & D	83.95	6,640	45,870	1,090	94
2. do	58.9	12,470	57,700	19,000	Nil
3. *Penaeus indicus* P & D	275.0	16,000	..	1,490	306
4. *Penaeus indtcus* P & D 40-50	137.0	3,490	Nil	11,280	1,270
5. *Penaeus indicus* P & D	84.7	162	6,400	36,000	85
6. *Penaeus tndicus* P & D	367.0	3,320	Nil	1,720	46

It is seen that here again very wide fluctuations occur in the total counts as well as in the counts of the different types of pathogenic organisms, which reflect upon the process of catching and subsquent handling of the raw material. In the case of coliform organisms, freezing and subsequent frozen storage may cause a substantial reduction or even their complete elimination. However, this is not the case with the other two types of pathogens.

Summary & Conclusions

The survey carried out on frozen prawn products as well as raw materials show that faecal indicator organisms like *E. coli & Enterococci* as also *Staphylococci* are present in varying intensities in these products. It is further seen that incidence of these organisms is quite independent of the total organisms in the products and that it depends mostly on the conditions of preprocess preparation of the material. These data also indirectly show that total bacterial plate count of a product alone cannot be depended upon to predict either the quality of the frozen product or the hygienic conditions of the factory and that the levels of the above organisms are also to be considered.

Among the three indicator organisms considered in this study enterococci seem to be more reliable as an index of faecal contamination as these undergo the least fluctuations during preparation and processing. The exact nature of the changes of these organisms during various stages of preparation and processing will be dealt with in detail in a subsquent paper.

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References

American Public Health Association—*Recommended methods for the Microbiological Examination of Foods,* New York. (1958)

Barnes, E. M. J. Appl. *Bact. 19.* 193, (1956 a)

Barnes, E. M.—*J. Gen. Microbiol., 14,* 57, (1956 b)

Barnes E. M.

J. Sci. Food. Agri. 10, 656, (1959)

Borgstrom, G. *Advances in Food Research 6.* 163, (1955)

Fanelli, M. J. and Ayres J. C. *Food Technol. 13,* 294, (1959)

Fitzgerald, G. A. *Food Industry. 19,* 623, (1947)

Frechette, A. L. and Michael, G. A.—Rules and Regulations relative to the storage and distribution of frozen foods. Dept. Public Health, Bureau of Consumer Products Protection, Division of Food and Drugs, Boston Mass. (1961)

Gunderson, M. F., McFadden, H. W. and Kyle, T. S. *The Bacteriology of Commercial Poultry Processing,* Burgess Publishing Co., Minneapolis, Minn. (1954)

Kachikian, R., Fellers, C. R. and Litsky, W. *J. Milk and Food Technol. 22,* 310, (1959)

Kereluk, K. and Gunderson, M. F. *Appl. Microbiol. 7,* 320, (1959)

Larkin, E. P., Litsky, W. and Fuller, J. E. *Appl. Microbiol. 3,* 98, (1955) Larkin, E. P., Litsky, W. and Fuller, J. E. 1956 *Am. J. Public Health, 4,* 464, (1956)

Ostrolenk, H., Kramer, N. and Cleverdon, R. *J. Bact. 53,* 197, (1947)

Raj, H. and Liston, J. *Appl. Microbiol. 9,* 171, (1961 a)

Raj, H. and Liston, J. *Appl. Mtcrobiol. 9,* 295, (1961 b)

Rayman, M.M., Huber, D.A. and Zaborowski, H. *Natl. Acad. Sci.* National Research Council, Washington, D. C., 55–67, (1955)

Silverman, C. J., Nicherson, J. T. R., Duncan, D. W., Davis, N. S., Schachter, J. S. and Joselow M. M. *Food Technol. 15,* 455, (1961)

Zaborowski, H., Huber, D. A. and Rayman, M. M. *Appl. Microbiol. 6,* 97, (1958)