

Faecal Indicator Organisms in Frozen Prawn Products

II. SURVIVAL OF THE ORGANISMS DURING FREEZING AND FROZEN STORAGE

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[The rate of survival of different types of faecal indicator organisms like *Escherichia coli*, enterococci etc. during freezing and frozen storage has been studied. Peeled and deveined prawns inoculated with a mixed culture of the above organisms were subjected to freezing and storage at -10°F and examined for over four months.]

Introduction

The microbiological condition of a frozen fish product is dependent upon various factors such as the nature of raw material, its pre and post process treatments, the sanitary condition of the processing factories, the rate and nature of freezing (Luyet and Gehenio, 1940), the temperature and time of storage (Kereluk and Gunderson, 1959; Larkin *et al.* 1955), the original numbers, types and stages of growth of microorganisms present (Hess, 1934; Record and Taylor, 1953), thawing processes and physical protection offered by the food (Squires and Hartsell, 1955; Woodburn and Strong, 1960). Recent studies (Brown and Gibbons, 1950; Burton, 1949; Larkin *et al.* 1955, 1956; Raj and Liston 1961c) show that some of the faecal indicator organisms have greater ability to survive freezing and frozen storage and can therefore predict the extent of contamination of the material than any other factor. Because of their ubiquity in nature and their possible destruction during freezing and frozen storage *E. coli* is being considered as a poor index, although their presence in large numbers even after storage for long periods is a sure sign of extreme contamination.

Recent investigations (Lekshmy and Pillai, 1964) on frozen prawns have shown clearly that enterococci and *E. coli* will be found in large numbers in prawns processed in unhygienic surroundings, and that their incidence can directly be correlated to the degree of sanitation. This led to the present studies on the rate of survival of these organisms during freezing and frozen storage for different periods with a view to find out whether the same trend of correlation is maintained throughout.

Experimental Procedure

Cultures of *E. coli* type I, *Aerobacter aerogenes* types I & II, *E. freundii* types I & II, *S. faecalis* and *S. faecalis* var *liquefaciens* were inoculated into 1% peptone water and incubated at 37°C for 24 hr. Six different dip solutions were prepared containing varying amounts of the bacterial suspensions. Fresh peeled and deveined prawn (*Penaeus*

indicus) were soaked in the suspension for 15 min. and drained. The treated samples were packed in 500 gram lots and frozen (at -14°F in 8 hrs.). The samples after freezing were stored at -10°F . Samples were removed periodically from the frozen storage and tested for the number of coliforms and enterococci present. The procedures for the preparation of samples and enumeration of the viable bacteria, coliforms, *E. coli* and enterococci were the same as those described previously (Lekshmy and Pillai, 1964).

Results and Discussion

The percentage survival of coliforms, *E. coli*, enterococci and total viable bacteria after freezing and during frozen storage is represented in Table I.

TABLE I. — Effect of freezing and frozen storage (-10°F) on the microorganisms present in peeled and deveined prawns.

Serial number	Organism tested	Count before freezing*	% survival after freezing	% survival † after storage for 128 days
1.	Total viable bacteria	1.87×10^6	12.1	79.0
	Enterococci	7.60×10^4	67.1	60.0
	Coliforms	1.50×10^6	6.1	0.5
	<i>E. coli</i>	4.80×10^4	5.6	1.7
2.	Total viable bacteria	5.13×10^7	4.2	59.2
	Enterococci	1.71×10^6	70.2	100.0
	Coliforms	6.80×10^6	0.9	0.62
	<i>E. coli</i>	1.06×10^6	2.4	1.2
3.	Total viable bacteria	2.16×10^7	11.1	50.0
	Enterococci	1.30×10^6	84.6	95.4
	Coliforms	3.02×10^6	25.0	0.06
	<i>E. coli</i>	8.41×10^5	7.5	0.7
4.	Total viable bacteria	3.80×10^7	5.7	30.0
	Enterococci	7.36×10^5	94.8	66.0
	Coliforms	5.10×10^6	15.7	0.08
	<i>E. coli</i>	8.29×10^5	24.1	0.26
5.	Total viable bacteria	1.86×10^7	10.2	50.0
	Enterococci	1.42×10^6	55.0	87.6
	Coliforms	4.20×10^6	1.2	1.4
	<i>E. coli</i>	4.72×10^5	2.8	4.5
6.	Total viable bacteria	9.90×10^6	14.1	48.4
	Enterococci	1.20×10^6	41.0	81.3
	Coliforms	1.70×10^6	2.1	0.7
	<i>E. coli</i>	8.50×10^5	3.1	0.9

* Bacterial count expressed as number of organisms per gram muscle.

† Percentage of the microorganisms that survive after freezing.

The above results indicate that there is appreciable reduction during freezing of both coliforms and *E. coli* (Over 90%). Enterococci however exhibit greater degree of survival, the maximum reduction observed being 59%. The rate of destruction of the organisms during frozen storage is represented in Figure I. The results obtained on duplicate samples from one of the experimental blocks for standard plate count and enterococci count are shown in Table II, while the fluctuations in the average counts in all the blocks are given in Table III.

TABLE II. — Effect of frozen storage on inoculated frozen prawns

No. of days of storage	SPC*			Enterococci*		
	Sample I	Sample II	Average	Sample I	Sample II	Average
2	5.3617	5.3502	5.3559	4.7168	4.7152	4.7160
7	5.2878	5.2553	5.2715	4.6937	4.6866	4.6901
21	5.2041	5.3483	5.2762	4.7160	4.6848	4.7004
56	5.2304	5.1761	5.2037	4.6415	4.6243	4.6329
94	5.1461	5.1461	5.1461	4.5855	4.5051	4.5453
128	4.9912	5.4150	5.2031	4.4232	4.5378	4.4805

* Expressed as log. bacterial count.

TABLE III. — Fluctuations in the average bacterial load in different blocks during storage.

	SPC*			Enterococci*		
	2-21 days	56-128 days	2-128 days	2-21 days	56-128 days	2-128 days
1	5.3010	5.1844	5.2427	4.7021	4.5529	4.6275
2	6.2698	6.1058	6.1878	6.0469	6.0315	6.0391
3	6.2845	6.0769	6.1807	5.9690	6.0259	5.9972
4	6.1528	5.9801	6.0215	5.7393	5.6701	5.7047
5	6.1976	6.0352	6.1164	5.8008	5.8057	5.8068
6	6.0168	5.8439	5.9313	5.6429	5.5706	5.6067

* Expressed as log. bacterial count.

Figure I reveals that throughout the storage period enterococci showed no significant increase or decrease. There was an initial fall in the total plate count followed by more or less constant levels during subsequent storage. This may be mainly due to the fact that the predominant flora in the frozen prawn after the first few days were enterococci. Slight differences noticed in the bacterial counts from one sampling period to another may be attributed to the possible errors in measuring out the serial dilutions from the homogenised samples, the settling of the bacterial cells in the dilution tubes during sampling and

the possible differences in the amounts of the microorganisms adsorbed onto the individual prawns. In the case of all the six blocks under examination both coliforms and *E. coli* showed a decided decrease in numbers throughout the storage period. Compared to the reduction in the number of coliforms, reduction in the number of *E. coli* is less so that as the storage period increases the percentage of *E. coli* in total coliforms gradually increases. The one type that stands out unaffected by storage is enterococci and therefore these have to be considered as superior than any other organisms tried in the series for use as an index of sanitation. This is in agreement with the results obtained by Raj & Liston (1961 a & b).

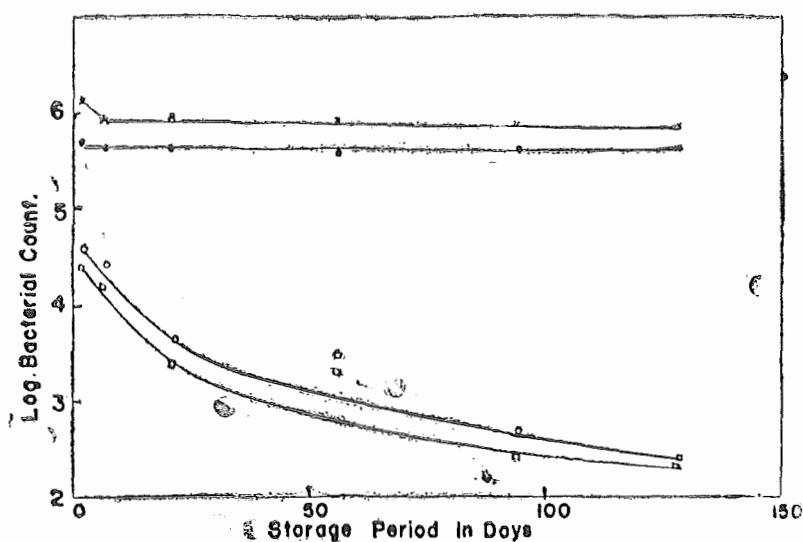


Fig. 1. Effects of storage at -10°F on the flora of peeled and deveined fresh frozen prawn.

× — × Standard Plate Count
 ● — ● Enterococci count
 ○ — ○ Coliforms
 □ — □ *E. coli* Count.

Results of examination of duplicate samples from the same block as presented in Table II show that the distribution of the organisms has been more or less uniform in the block. The averages of bacterial counts (both for SPC and for enterococci) during different intervals (Table III) further show that the fluctuations in the enterococci during the latter half of storage (between 56 and 128 days) are not as high as in the case of total counts.

Summary

The effect of freezing and frozen storage on the viability of coliform organisms and enterococci when present together in frozen, peeled and deveined prawn was determined. Enterococci showed the maximum survival both on freezing and during frozen storage at -10°F for 128 days. Coliform organisms underwent a significant reduction

and what remained in the frozen block after 128 days storage was in most cases less than 1% of the number present originally in the frozen block.

Acknowledgement

The author wishes to express her grateful thanks to Dr. A. N. Bose and Dr. V. K. Pillai for their very valuable advice and suggestions during the course of this investigation.

References :—

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| Brown, H. J. & Gibbons, N. E., | <i>Can. J. Res.</i> , F. 28, 107 (1950) |
| Burton, M. O., | <i>Food Res.</i> 14, 434 (1949) |
| Hess, E., | <i>J. Biol. Board, Canada.</i> 1, 95 (1934) |
| Kereluk, K. & Gunderson, M. F., | <i>Appl. Microbiol.</i> 7, 327 (1959) |
| Larkin, E. P., Litsky, W. & Fuller, J. E., | <i>Appl. Microbiol.</i> 3, 102 (1955) |
| | <i>Amer. J. Public Health.</i> 4, 464 (1956) |
| Lekshmy, A. & Pillai, V. K., | <i>Fishery Technology.</i> 1, 48 (1964) |
| Luyet, B. J. & Gehenio, P. M., | <i>Biodynamica.</i> 3, 33 (1940) |
| Raj, H. & Liston, J., | <i>Appl. Microbiol.</i> 9, 171 (1961a) |
| | <i>Ibid</i> 9, 295 (1961b) |
| | <i>Food Technol.</i> 15, 429 (1961c) |
| Record, B. A. & Taylor, R., | <i>J. Gen. Microbiol.</i> 9, 475 (1953) |
| Squires, R. W. & Hartsell, S. E., | <i>Appl. Microbiol.</i> 3, 40 (1955) |
| Woodburn, J. M. & Strong, D. H., | <i>Appl. Microbiol.</i> 8, 109 (1960) |