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Evaluation of Certain Factors Affecting the Total Plate Count of Frozen Seafoods

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The total plate count (TPC) is considered to be an important index to determine the hygienic quality of the product. Different parameters that influence the recovery of micro-organisms during bacterial enumeration of frozen seafoods were evaluated. The parameters studied were plating technique, incubation temperature, incubation period and the diluent. The results were satistically analysed and it was found that spread plate technique yielded higher bacterial count than pour plate technique. An incubation temperature of 30°C was found to facilitate greater bacterial recovery than conventional 37°C. Comparing incubation periods of 24, 48, 72 and 96 h it was found that an incubation period of 48 h was sufficient for frozen marine products.

The most important part of any proposed microbiological standard is the total plate count (TPC) of bacteria present in the food. Several authors have shown the value of TPC in reflecting the hygienic condition occured during processing (Abrahamson, 1960; Dack, *et al.*, 1960; Thatcher, 1960). A low bacterial count indicates enhanced shelf-life. It also points to the sanitational level of processing and pre-processing conditions. (Eliott & Michener, 1961). Some authors suggested bacterial plate count as an index of determining the degree of adulteration by decomposing material (Lepper *et al.*, 1944). Eliott & Michener (1961) in their review on microbiological standards emphasized the significance of total plate count in quality control programmes.

Sampling and analytical procedures are the major problems in the enforcement of setting up bacteriological standards (Anon 1960; Appleman, 1957). The physiological state of bacteria present in the food and nature of food material are thought to exert some influence on TPC. Only a limited information is available on the evaluation of methodology for bacterial enumeration of frozen foods (Hartmann & Huntzberger, 1961; Nottingham *et al.*, 1975). The present study attempts to evaluate various factors in plating procedure that may affect the TPC of frozen fishery products.

Materials and Methods

A variety of samples comprising frozen fish, prawns, fish fillets and minced fish were studied. The samples consisted of common varieties of fish and prawns available in the Cochin region, and were procured from local factories.

The effects of four parameters namely, sampling method, incubation period, incubation temperature and diluent on enumeration of bacteria were investigated. Each of the parameter in the methodology is mentioned and discussed under appropriate section. Unless otherwise specified the scheme of analysis adopted was according to ISI procedure (IS: 2237, 1971). The data on different types of products were pooled and analysed using analysis of variance technique. Since sample variation was significant in certain instances the coefficient of variation was also determined wherever necessary.

In course of analysis about 300 cultures were also isolated. The temperature of isolation was 30°C and 37°C. A set of plates were also incubated at 8°C to study the quantitative and qualitative nature of the psychrotrophic flora of frozen fish. The cultures were maintained on sea water agar slants. They were identified to the generic level.

The scheme put forward by Okuzumi *et al.* (1980) and that of Surendran & Gopakumar (1981) were made use of for classification purposes. Each isolate was also tested for its ability to grow at a series of temperatures ranging from 0 to 56°C. The method adopted was the same as that used for bacteria from raw fish (Thampuran & Iyer, 1979).

Results and Discussion

Zobell & Conn (1940) pointed out the harmful action of agar at 45°C on marine bacteria. Nottingham *et al.* (1975) have also suggested that the lower count obtainable with pour plates could be attributed to the inability of some psychrotrophs to survive in the hot agar media used for pour plating. Being associated with cold environments, there is considerable chance for the accumulation of psychrotrophs in frozen fishery products. Our study shows that the psychrotrophic count at 8°C lies in the range

of 1.1×10^4 to 3.0×10^4 while the count at room temperature (30 ± 1°C) and 37°C are 5.2×10^4 to 4.5×10^5 and 3.8×10^4 to 2.8×10^5 respectively. By observing the growth temperature ranges of bacteria isolated from frozen fishery products, they could be placed into two major groups, the psychrotrophs and the mesophiles. The percentage of these groups occuring in frozen fishery products is as given in Table 1. In frozen foods stress due to intense cold can also cause the microbial flora to be more sensitive to warm agar temperatures leading to decreased recoveries from pour plates.

Table 1. *Percentage of psychrotrophs and mesophiles in fishery products*

Type	Growth temperature range of isolate	Percentage at RT
Psychrotrophs	0 to 30 or 0 to 37	58
Mesophiles	20 to 45	42
Thermophiles	30 to 56	Nil

Based on these results the spread plate method and pour plate method were compared for their efficiency in bacterial recovery, the principlal difference between the two being the temperature of the agar media used. Spread plate method employs preset plates of agar cooled to room temperature while pour plate method uses agar at 43–45°C. Analysis of variance of the results are given in Table 2.

Table 2. *Effect of plating method and incubation temperature on bacterial count of frozen fish: Analysis of variance*

Source	S.S.	df	m.S.	F
Total	14.7414	47		
Temperature	0.6399	1	0.6399	68.04**
Methods	0.4904	1	0.4904	52.17**
Samples	12.5108	11	1.1370	120.96**
Samples × temperatures	0.7437	11	0.0676	7.19
Samples × methods	0.2484	11	0.0225	2.39
Temperature × methods	0.0038	1	0.0038	1
Error	0.1044	11	0.0094	

** Significant at 1% level

Significant difference existed between methods, spread plate giving a higher count than pour plate method. There is also significant difference in bacterial count between samples. This is to be expected as the samples belonged to varied products and were drawn from different sources. The interaction, sample x temperature is significant at 1% level. But the other two interactions sample x method and temperature x method are not significant at 5% level.

Incubation temperature

During bacterial sampling of frozen fishery products, incubation of petridishes at room temperature (30 ± 1°C) gave a higher count than incubation at 37°C. Analysis of variance of the results are given in Table 2. The experiment was carried out by keeping sets of plates at room temperature and 37°C simultaneously. In order to find out whether qualitative nature of the flora exerted any influence on bacterial count, about 200 cultures were isolated from plates incubated at room temperature and 37°C and identified to the generic level. The percentage of the bacteria isolated at room temperature and 37°C are given in Table 3.

Table 3. *Major bacterial groups present at different incubation temperatures* (as %)

	8°C	RT	37°C
Pseudomonas	16	13	9
Moraxella	9	8	2
Acinetobacter	11	6	8
Flavobacterium & Cytophaga	2	5	1
Micrococcus	52	57	65
Enterobacteriacea	2	1	2
Alcaligenes	1	2	2
Bacillus	7	7	5
Unidentified	—	1	4

It is quite clear from the Table that greater numbers of Gram negatives are recovered at the lower temperature of incubation. A similar finding was also reported by Kawabata *et al.* (1975) who showed that recovery of *Moraxella* spp. and *Pseudomonas* spp. is higher at the lower temperature of incubation (25°C) while the number of *Micrococcus* spp. lesser at 25°C compared to that at 37°C. They also found that the count at 25°C was 2 to 1000 times greater than the mesophilic count at 37°C for frozen shrimp imported from tropical areas.

Incubation period

The effect of incubation period on bacterial count was determined by incubating the petridishes for 4 days and noting the bacterial count after 24, 48, 72 and 96 h. Analysis of variance of the data are given in Table 4.

Frozen fish showed significant difference ($P < 0.01$) in the increase in bacterial count after the four periods

of incubation. The least significant difference was 0.2124 and the mean logarithmic count after 24, 28, 48, 72 and 96 h were 1.5489, 1.9231, 1.3651 and 0.9377 respectively. The increase in bacterial count after 48 h was significantly high compared to the other three. The coefficient of variation in bacterial count after the four periods were 28, 19.08, 27.86 and 50.11%. There is also greater consistency in count after 48 h The sample variation is significant at 1% level.

Table 4. *Effect of incubation period on bacterial counts: Analysis of variance*

Source	S.S.	df.	m.S.	F
Total	13.5903	47		
Incubation period	6.0294	3	2.5809	56.61**
Samples	6.3904	11	0.5809	16.36**
Error	1.2705	33	0.355	

** Significant at 1% level

Effect of diluent

Effect of five diluents namely, distilled water, peptone water containing 0.1% peptone, phosphate buffer (IS:2237, 1971) and Ringer's solution (Robert Cruickshank, 1962) on their efficiency in bacterial recovery was studied for frozen fishery products using isotonic saline (0.85% NaCl) as control. The results of the analysis of variance are presented in Table 5. The study points out that the effect of diluent was significant at 1% level and that saline, phosphate buffer and peptone water gave significantly higher counts compared to the other two.

Table 5. *Effect of diluents on bacterial count: Analysis of variance*

Source	S.S.	df.	m.S.	F
Total	3.5001	59		
Diluents	1.2989	4	0.3247	8.12**
Error	2.2012	55	0.0400	

** Significant at 1% level

Table 6. *Mean logarithmic count of different diluents*

Distilled water	2.0161
Peptone water	2.2485
Phosphate buffer	2.3519
Ringer's solution	1.9979
Isotonic saline	2.2983

Among the three diluents that gave higher counts, phosphate buffer was giving the maximum recovery of bacteria though the mean logarithmic count was not significant statistically (Table 6). Still, the study shows that for practical purpose phosphate buffer can be considered to be the diluent of choice.

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