

## Effect of Incubation Period on Plate Count of Raw Iced and Frozen Fish

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The total viable counts were estimated in one hundred and sixty five samples of raw, iced and frozen fish using incubation periods of 24, 48, 72 and 96 h. For raw fish, 24 h and for iced and frozen fish 48 h incubation of the plates were found to be adequate. Variation between samples was significant at 1% level for raw iced and frozen samples.

A careful analysis of the factors involved in the enumeration of bacteria for various types of food is always an essential requisite for attaining greater reliability in the assessment of product quality and for the standardisation of procedures. Considerable amount of work has been done on this aspect with respect to meat and milk products. (Huhtanen *et al.* (1972); Huhtanen *et al.* (1976); Nottingham *et al.* (1975); Pedraja & Mengelis (1964); Roughley *et al.* (1974). But enough attention has not been given to the evaluation of methods to determine the total viable count or total plate count of sea foods (Hartmann & Huntzberger, 1961). This paper deals with the effect of incubation period on bacterial recovery on raw, iced and frozen samples.

### Materials and Methods

165 fishery products, 50 raw, 58 frozen and 57 iced fish were analysed. Fish procured from landing cites were analysed within 2 h after collection. Part of fish were kept in ice boxes ( $0 \pm 1^\circ\text{C}$ ). Frozen samples were either purchased from factory or prepared in the laboratory.

10 g of fish cut aseptically and homogenated in a sterile mortar with 90 ml of normal saline. Serial dilutions were prepared in n-saline and one ml of the appropriate dilution was pipetted into duplicate plates and mixed with agar media. For raw and iced fish, sea water agar and for frozen fish tryptone agar were used. The composition of sea water agar was peptone 1%, ferric phosphate 0.05 mg, agar powder 2% in sea

water (pH 7.2 sterilization at  $1.05 \text{ kg/cm}^2$  for 30 min) and that of tryptone glucose agar, beef extract 0.03%, dextrose 0.1%, tryptone 1.0%, sodium chloride 0.5% in distilled water (sterilization at  $1.05 \text{ kg/cm}^2$  for 30 min, pH 7.2). The plates were incubated at  $30 \pm 1^\circ\text{C}$  for four days and the bacterial counts made after 24, 48, 72 and 96 h.

### Results and Discussion

Some representative values of the total plate count after 24, 48, 72 and 96 h for raw, iced and frozen fish are shown in Table 1. The data of different varieties of fish were pooled and analysed by analysis of variance (Tables 2, 3 and 4) after converting the count at 24 h and subsequent increase in the bacterial count at 48, 72 and 96 h to their logarithmic values.

The data indicates that for raw fish, there is significant difference ( $p < 0.01$ ) in the bacterial count at the four periods of incubation. The least significant difference at 1% level for the logarithm of bacterial count is 0.2558 and the mean logarithmic increase in count after 24, 48, 72 and 96 h of incubation are respectively 2.4906, 2.3310, 1.7668 and 1.5365 which shows that there is no significant difference in count between 24 and 48 h for raw fish. The coefficient of variation of count at the four incubation periods were 14.24%, 20.33%, 27.98% and 31.88%, respectively. Also, the consistency in count is more pronounced at 24 h of incubation than at the other three periods. The variation between samples was significant at 1% level.

**Table 1.** Total bacterial count after 24, 48, 72, 96 h incubation (Colony count/g  $\times 10^3$ )\*

|                                                   | Bacterial count after (h) |      |      |      |      |    |    |    |        |     |     |     |
|---------------------------------------------------|---------------------------|------|------|------|------|----|----|----|--------|-----|-----|-----|
|                                                   | Raw                       |      |      |      | Iced |    |    |    | Frozen |     |     |     |
|                                                   | 24                        | 48   | 72   | 96   | 24   | 48 | 72 | 96 | 24     | 48  | 72  | 96  |
| Sardine ( <i>Sardinella logiceps</i> )            | 249                       | 448  | 506  | 322  | 12   | 38 | 52 | 53 | 28     | 99  | 121 | 128 |
| Horse mackerel ( <i>Caranx crumenophthalmus</i> ) | 420                       | 737  | 903  | 952  | 18   | 58 | 72 | 80 | 18     | 63  | 95  | 100 |
| Jew fish ( <i>Pseudosciaena</i> sp.)              | 306                       | 520  | 562  | 577  | 5    | 16 | 22 | 26 | 55     | 185 | 216 | 221 |
| Cat fish ( <i>Tachysurus</i> sp.)                 | 415                       | 690  | 777  | 800  | 9    | 21 | 35 | 39 | 31     | 101 | 128 | 132 |
| Prawn ( <i>Penaeus indicus</i> )                  | 958                       | 1680 | 1706 | 1733 | 18   | 57 | 75 | 78 | 44     | 166 | 195 | 199 |

\* Only representative values given

**Table 2.** Analysis of variance in bacterial count at 4 incubation periods in raw fish

| Source             | S.S.    | d.f. | m.s.   | F        |
|--------------------|---------|------|--------|----------|
| Total              | 16.5691 | 47   |        |          |
| Incubation periods | 7.3871  | 3    | 2.4624 | 46.90 ** |
| Samples            | 7.4511  | 11   | 0.6774 | 12.90 ** |
| Error              | 1.7309  | 33   | 0.0525 |          |

\*\* Significant at 1% level

**Table 3.** Analysis of variance of increase in bacterial count in iced fish

| Source             | S.S.    | d.f. | m.s.   | F        |
|--------------------|---------|------|--------|----------|
| Total              | 12.7790 | 47   | —      | —        |
| Incubation periods | 5.2394  | 3    | 1.7465 | 57.64 ** |
| Samples            | 6.5403  | 11   | 0.5946 | 19.62 ** |
| Error              | 0.9993  | 33   | 0.0303 | —        |

\*\* Significant at 1% level

**Table 4.** Analysis of variance of increase in bacterial count in frozen fish

| Source             | S.S.    | d.f. | m.s.   | F        |
|--------------------|---------|------|--------|----------|
| Total              | 13.5903 | 47   | —      | —        |
| Incubation periods | 6.0294  | 3    | 2.0098 | 56.61 ** |
| Samples            | 6.3904  | 11   | 0.5809 | 16.36 ** |
| Error              | 1.1705  | 33   | 0.355  | —        |

\*\* Significant at 1% level

In iced fish, there is significant difference ( $p < 0.01$ ) in the bacterial count at the four periods of incubation. The least significant difference at 1% level for logarithm of bacterial count is 0.1944 and the mean logarithmic increase in bacterial count after 24, 48, 72 and 96 h of incubation were 1.3434, 1.6937, 1.3922 and 0.7792 respectively. The increase in bacterial count after 48 h of incubation was significantly high compared to the increase in the other three periods. The coefficient of variation of the increase in bacterial count for the four periods of incubation were 30.50%, 24.42%, 27.55% and 57.29% respectively and the consistency of the count was more pronounced after 48 h of incubation. The variation between samples was significant at 1% level.

Like the other two types, frozen fish also showed significant difference ( $p < 0.01$ ) in the increase in bacterial count after the 4 periods of incubation namely 24, 48, 72 and 96 h. The least significant difference in this case was 0.2104 and the mean logarithmic count after 24, 48, 72 and 96 h of incubation 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 of incubation are 28%, 19.08%, 27.86% and 50.11% respectively. There is greater consistency in count after 48 h of incubation than the other three periods. The variation between samples is also significant at 1% level.

These results point out that for raw fish 24 h of incubation can be taken as the appropriate period for estimating the bacterial load, while for iced and frozen fish 48 h of incubation seems adequate. The criteria for selection were the increase in bacterial count at the respective incubation periods along with their consistency at that period.

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