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A RAPID PROCEDURE FOR THE APPROXIMATION OF BACTERIAL LOAD IN FISHERY PRODUCTS

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[The production of colour by homogenised fish material in a simplified sugar medium containing an acid indicator has been made use of for the rapid approximation of bacterial load in such products. The medium thus developed contains poptone, tryptone, yeast extract, sodium chloride and beef extract besides dextrose. The time of colour production is influenced to some extent by the level of sodium chloride in the medium; and is almost always inversely proportional to the bacterial load in the homogenate.]

The search for a quick and reliable method for accurate determination of quality of fresh and processed fishery products has been going on in many countries for the past several years. This has led to a closer study of the mechanism of biochemical changes taking place in fish during spoilage and of the usefulness of some of the spoilage products for quality assessment. Glycogen, lactic acid, trimethylamine, total volatile bases, volatile reducing substances, volatile acids etc., are some of the factors exhaustively tried in this connection. While many of these show excellent correlation with spoilage under specified conditions none can yet be considered complete and dependable for general application.

Several authors (Shewan, 1949; Hobbs, 1953) still agree that bacterial count is by far the best of all the known indices for assessing spoilage in fish. These authors have shown that by the time decomposition has reached the point where it is detectable as such to the human senses, the bacterial counts are one to ten millions per gram. Hobbs (*loc. cit*) further states that foods suspected of causing food poisoning gave counts greater than ten million per gram. Continued studies have also clearly established (Anon., 1960 Mossel, 1953) that a low bacterial count invariably parallels freedom from food poisoning pathogens. However, quality assessment by bacterial count also is not free from limitations. A correctl interpretation and correlation with spoilage is often impossible due to severa reasons (Elliot and Michener 1960). It also has the disadvantage that it takes too much time and requires elaborate preparations for plate count determinations which limits its value for routine work. In a fish processing factory the raw materials will have to be graded for quality as and when they arrive without much loss-

of time, and in the absence of reliable quick methods they are forced to depend still on organoleptic and visual characteristics.

In order to overcome this disadvantage a few attempts aimed at methods for rapid approximation of bacterial counts have been made in recent years. Novak et. al. (1956) made use of bromothymol blue at 35°C and a milk medium containing methylene blue for this purpose. These authors noticed definite differences in the time taken for colour changes in the medium when an extract of shrimp was added, depending upon the total bacterial count. It was found that a sample of shrimp with a total plate count of ten million and above produced a colour change within two hours in the former and within one hour in the latter. Uno and Tokunga (1954) made use of resazurin for similar work.

The present communication deals with the investigations carried out using a sugar medium for the purpose of rapid assessment of bacterial loads in fresh, ice-stored and frozen prawn products. The ability of most of the predominent types of micro-organisms present in fresh and spoiling fish products to produce acid from sugars has been taken advantage of in this method. Particular emphasis is given to standardize the procedure in such a way that it could be adopted for day to day work in fish processing factories.

Materials and Methods

In all the experiments prawns collected from the landing centres. from the local market or from the processing factories were used. Twenty gram muscle was homogenised with aged sterile sea water to make up 100 cc. and the homogenate was used. Total bacterial plate count was determined by plating out serial dilutions of the homogenate in triplicates on sea water agar and incubating for fortyeight hours at room temperature. In all the experiments 10cc. each of the sterile medium at a final pH of 7.0 was used.

Experimental

The initial trials were carried out in a medium prepared in distilled water with the following percentage composition: Peptone 0.5, Tryptone 0.25, maltose 0.5, lactose 0.5, dextrose 0.15, yeast extract 0.05, Beef extract 0.1, NaCl 0.58, and Andred's indicator 0.8 cc. Definite quantities of muscle homogenised with sterile sea water were added to triplicate tubes and incubated at 37°C for production of pink colour.

In Subsequent work a simplified medium was used containing all the above constituents in the same percentage composition except maltose and lactose. The tubes were incubated in triplicates with definite quantities of inoculum at 37°C and at room temperature.

Subsequently efforts were made to bring out the effect of sugars by trying media without any sugar, dextrose alone and in combination with maltose and lactose. The effect of sodium chloride in the medium was also studied by keeping its concentration at different levels in the experiments. The following five media were tried :

(1) Medium without any sugar

(2) Sugar mixture

(3) Dextrose alone without salt

(4) Dextrose with 0.58% salt

(5) Dextrose with 3.5% salt

To study the relative merits of the above media in colour production, homogenates from several series of raw materials containing varying bacterial loads were inoculated to all the media simultaneously and incubated at 37°C and room temperature.

To determine the optimum salt concentration in the medium same quantities of muscle homogenate in sea water were incubated simultaneously at room temperature with media containing dextrose and 0.5, 1.5, 2.5, 3.5 and 5% sodium chloride.

In a few series of experiments the prawn meat was added direct to the medium without homogenisation in order to examine whether the procedure could thus be simplified. The results obtained in each series were compared with the corresponding values obtained from the same quantity of inoculum added as homogenate.

To account for the highly significant difference in results between the two prodedures mentioned above, some experiments were carried out with media containing dextrose and 0.58% salt and dextrose and 3.5% salt, in which the same concentration of inoculum was added as a homogenate in sea water, as a lump and as a lump with enough sea water added to make the resultant salt concentration same as when the homogenate is added, and incubated at room temperature.

Results

In all the experiments carried out the time taken for the production of pink colour in the media was found to be inversely proportional to the bacterial load of the material under test.

The results obtained with the initial medium at 37°C in a series of experiments is shown in Figure 1. The following general trend (Table I) was found in almost all the series tried.

TABLE I

Time for colour production at 37°C in sugar mixture in relation to bacterial count

Bacterial Count	Time taken for the production of pink colour in minutes
100,00,000 and above	Below 100
10,00,000	100 - 150
1,00,000	150 - 210
10,000	210 - 250
1,000 and below	Above 250

Statistical analysis of the data shows that the scatter diagram (Fig. 1) gives a straight line with the following equation,

$$Y = (\log y) = 8.523 - 0.0174\ X$$

$$X^2_{30} \text{ (Calculated)} = 3.0483$$

$$X^2_{30} \text{ (5\% table value)} = 43.77$$

It can be said with probability 0.95 that a straight line is the best fit for the given data.

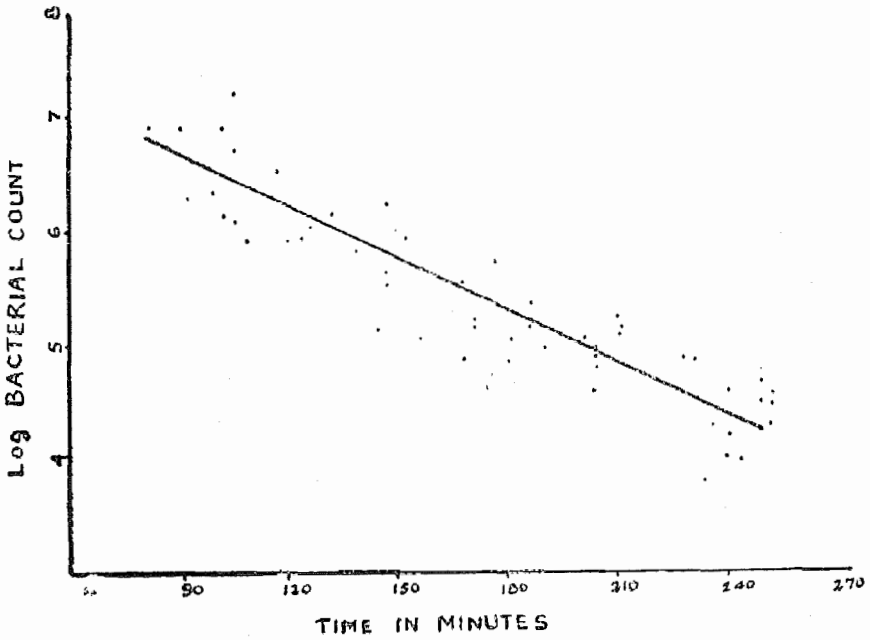

Fig. 1. Time of colour production and bacterial count in sugar mixture.
Y = (Log. Y) = 8.523 — 0.0174 X

Figures 2 and 3 represent respectively the results obtained with dextrose containing 0.58% salt incubated with inoculum at 37°C and room temperature.

The general trend obtained in these series is as follows: (Table II)

TABLE II

Time for colour production in medium with Dextrose and 0.58% salt at 37°C and room temperature in relation to bacterial count

Bacterial Count	Time taken for the production of pink colour, in minutes	
	at 37°C	at room temperature
100,00,000 and above	90	120
10,00,000	90-145	120-160
1,00,000	145-195	160-240
10,000	195-245	240-280
1,000 and below	above 245	above 285

These results reveal that there is a difference between the timings at room temperature and at 37°C, the former being always higher.

Analysis of the data shows that as in the case of the sugar mixture the time taken for colour production bears a close relationship with the bacterial count obtained in the above two series (30 observations each from the two series using the same concentration of inoculum being considered for analysis purpose). The

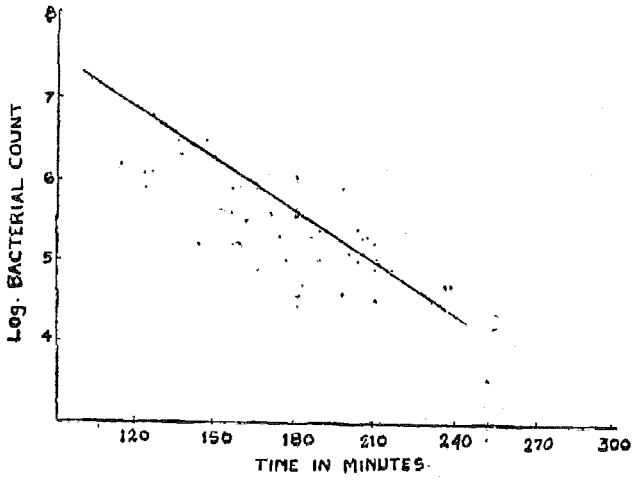

Fig. 2. Time of colour production and bacterial count in medium with dextrose and 0.58% NaCl at 37°C.
Y = (Log. Y) = 7.403 — 0.01019 X.

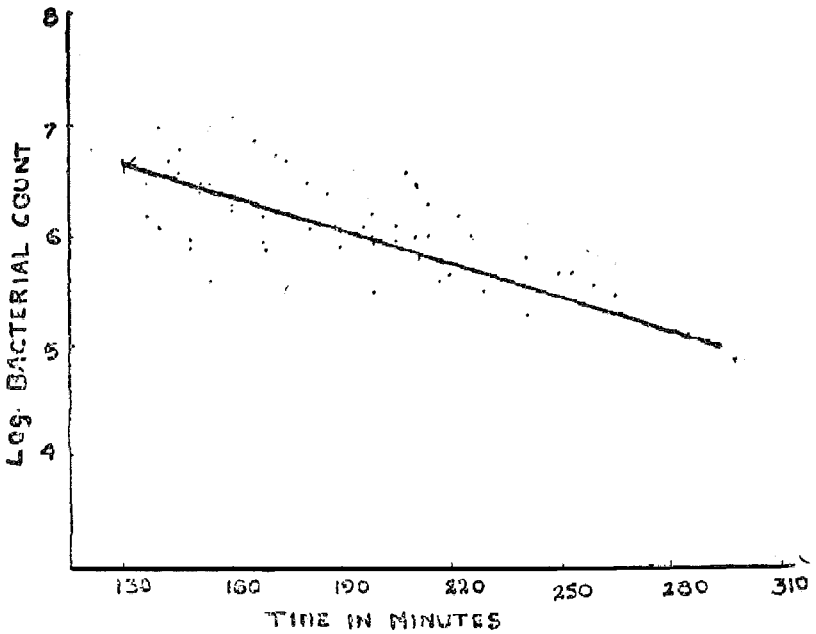

Fig. 3. Time of colour production and bacterial count in medium wit dextrose and 0,58% NaCl at room temperature.
Y = (Log. Y) = 8.735 — 0.01616 X.

scatter diagrams (Figs. 2 and 3) of both the series give straight lines with the following equations :

$$Y = (\log y) = 7.403 - 0.01019\ X \text{ (for 37°C) and}$$

$$Y = (\log y) = 8.735 - 0.01616\ X \text{ (for 28°C)}$$

Calculated value for x^2_{27} being 2.00 and 1.06 respectively against table value of 40.11 thus indicating the fitness of the lines for the data. The apparent difference in timings noticed in Table above, however, is found to be not significant at 5% level when the F test is applied, the calculated value of 1.104 for F (29, 29) being lower than the table value of F at 5% level of significance.

Figures 4 and 5 represent the results obtained with the different media at room temperature and at 37°C respectively for the same concentration of inoculum. The general trend obtained in these experiments is given in Table III.

TABLE III

Time for colour production in different media at 37°C and 28°C in relation to bacterial count

Bacterial count	Medium without sugar	Sugar mixture		Dextrose without salt		Dextrosa with 3.5% salt		Dextrose with 0.58% salt	
		28°C	37°C	28°C	37°C	28°C	37°C	28°C	37°C
7.26 x 10⁶	No colour production till 8 hrs. both at 28°C and 37°C.	152	127	147	132	147	117	167	147
3.67 x 10⁶		165	145	175	150	147	125	135	120
1.21 x 10⁶		177	155	192	177	179	145	198	178
6.0 x 10⁶		155	135	165	140	137	115	125	110

Analysis of the data obtained shows that the scatter diagrams (Figures 4 and 5) give straight lines with the following equations:

$$\left.\begin{aligned} Y &= 6.496 - 0.001\ X\ 28°C \\ Y &= 7.357 - 0.006\ X\ 37°C \end{aligned}\right\} \text{ for sugar mixture.}$$

$$\left.\begin{aligned} Y &= 8.48 - 0.002\ X\ 28°C \\ Y &= 0.252 - 0.038\ X\ 37°C \end{aligned}\right\} \text{ for glucose without salt.}$$

$$\left.\begin{aligned} Y &= 8.344 - 0.011\ X\ 28°C \\ Y &= 8.235 - 0.013\ X\ 37°C \end{aligned}\right\} \text{ for glucose with 3.5\% salt.}$$

$$\left.\begin{aligned} Y &= 8.145 - 0.010\ X\ 28°C \\ Y &= 7.835 - 0.009\ X\ 37°C \end{aligned}\right\} \text{ for glucose with 0.58\% salt.}$$

Analysis of data showed that glucose alone with 3.5% salt appears to be a better medium for colour production than the other three.

The results obtained for the same concentration of inoculum at room temperature in media containing dextrose and varying concentrations of salt is shown in Table IV.

TABLE IV

Time for colour production in media containing dextrose and varying concentration of salt

Bacterial Count	0.5% salt	1.5% salt	2.5% salt	3.5% salt	5.0% salt
$3.5x10^5$	210	215	225	205	265
$1.4x10^6$	152	160	173	144	200

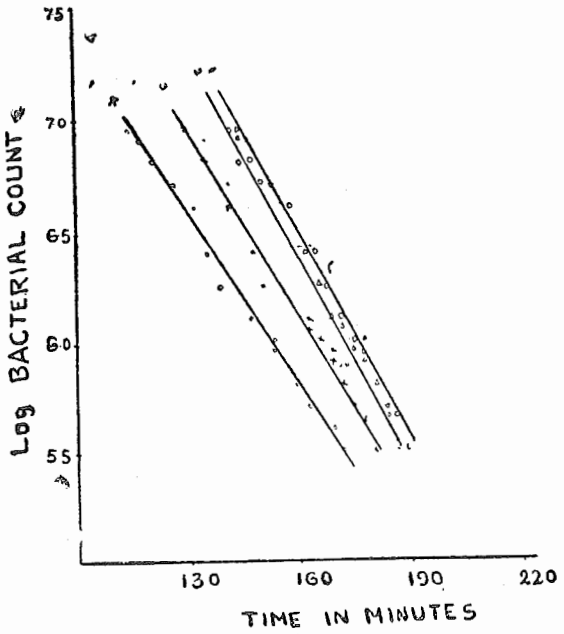

Fig. 4. Comparison of efficiency of four different media in colour production at room temperature.

1. Dextrose alone without salt.
   Y = (Log. Y) = 8.48 — 0.002 X.
2. Sugar mixture Y = (Log. Y) = 6.496 — 0.001 X.
3. Dextrose with 0.58% NaCl.
   Y = (Log. Y) = 8.145 — 0.010 X.
4. Dextrose with 3.5% salt.
   Y = (Log. Y) = 8.344 — 0.011 X.

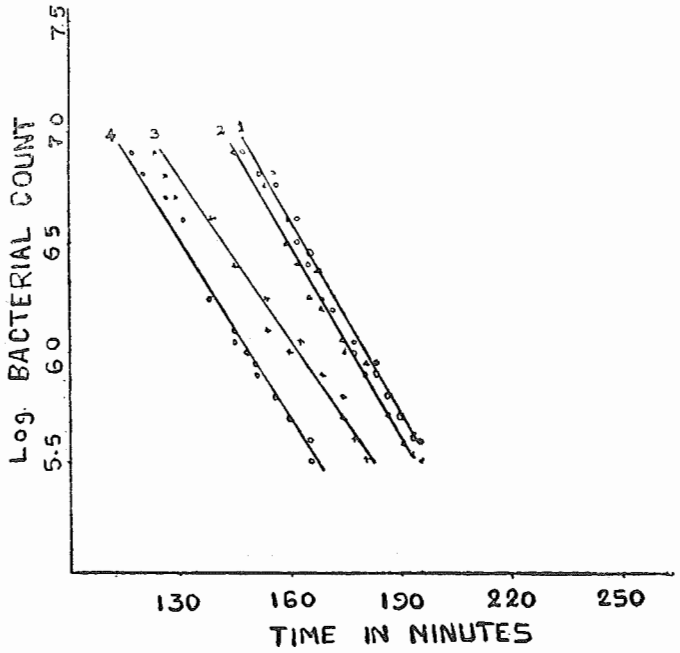

Fig. 5. Comparison of efficiency of four different media in colour production at 37°C.

1. Dextrose alone without salt.
   Y = (Log. Y) = 2.52 - 0.038 X.
2. Sugar mixture.
   Y = (Log. Y) = 7.357—0.006 X.
3. Dextrose with 0.58% NaCl.
   Y= (Log. Y) =7.835—0.009 X
4. Dextrose with 3.5% NaCl.
   Y= (Log. Y)= 8.235—0.013 X.

In all the experiments where the inoculum was added as a lump, the results were always higher than when the same concentration of inoculum was added as a homogenate. The results obtained are shown in Figure 6. Typical results showing the general trend are given in Table V.

TABLE V

Time for colour production at 28°C in medium containg dcxtrose and 0.58% salt for the same concentration of inoculum added as a homogenate and as a lump

Bacterial Count	Time taken for colour production in minutes	
	As a homogenate at 28°C	As a lump at 28°C
1.71×01^{7}	127	240
2.85×10^{6}	167	280
$2{\cdot}41 \times 10^{7}$	115	275
4.0×10^{6}	150	302
1.66×10^{5}	287	410
4.3×10^{6}	134	278

Statistical analysis of the data showed a high significance at 5% level between the two series. The mean and the variance for a few results in both the series are given in Table VI.

TABLE VI

Statistical analysis of a few results obtained in media containing dextrose and 0.58% salt at 28°C for the same amount of inoculum added as a lump and as a homogenate.

As a homogenate		As a lump	
Mean	Variance	Mean	Variance
$\bar{X}_1 = 264.25$	1.584	$\bar{X}_1 = 374.5$	389.67
$\bar{X}_2 = 295.00$	4.000	$\bar{X}_2' = 393.25$	62.247
$\bar{X}_3 = 308.5$	1.000	$\bar{X}_3' = 402.25$	284.25
$\bar{X}_4 = 324.25$	1.584	$\bar{X}_4' = 414.50$	188.334
$\bar{X}_5 = 341.5$	1.670	$\bar{X}_5' = 424.00$	236.667

Since the mean as well as the variance are widely different in each case the 't' test and F test showed a high significance at 5% level, showing that there is a definite variation in the time for colour production in the medium when the inoculum is added as a lump and as a homogenate.

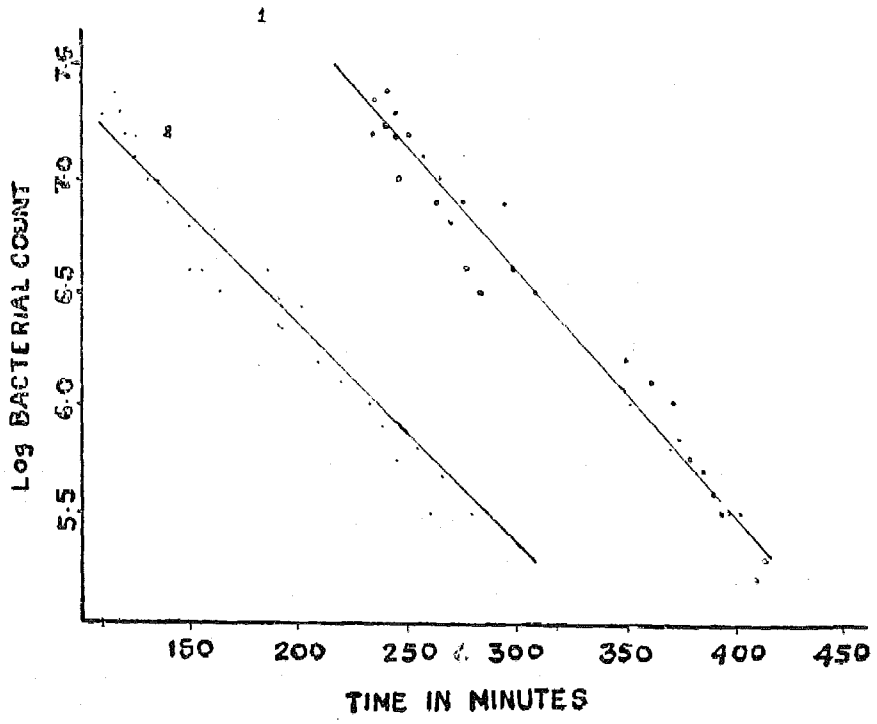

Fig. 6. Colour production for the same bacterial count when material is added as lump and as homogenate, at room temperature.
1. As lump. 2. As homogenate.

The results of the experiment where the same concentration of inoculum was incubated as a homogenate, as a lump and as a lump with enough sterile sea water added to make the resultant salt concentration the same as in the one where the homogenate was added, in media containing dextrose with 0.58% salt and 3.5% salt are shown in Table VII.

TABLE VII

Time for colour production for the same concentration of inoculum added as a homogenate, as a lump, and as a lump with sea watet in media containing dextrose and 0.58 and 3.5% salt at 28°C.

Bacterial Count	Dextrose with 0.58% salt			Dextrose with 3.5% salt		
	Homogenate	Lump	Lump+S.W.	Homogenate	Lump	Lump+S.W.
5.0 x 107	175	267	187	163	169	177
2.5 x 107	185	282	202	179	186	190

It can be seen from the table that there is significant difference between the results where the inoculum was added as a homogenate and as a lump in medium with dextrose and 0.58% salt. There is also significant difference between the series even when the salt concentration is equalised in both cases.

Discussion

The production of a pink colour in a medium containing dextrose can be used as a method for the approximation of bacterial levels in fishery products. Though the time for colour production, viz., 1.5 hr. to 4 hr. for bacterial levels ranging from 10 million to 1,000 organisms is still a little too high for practical use under commercial conditions, it is a definite improvement on the conventional plate count techniques which take a minimum of 48 hours. The results so far obtained clearly indicate that where absolute values are not required the method can be used to advantage for a rapid approximation of bacterial range in a fishery product.

The absence of colour production in the medium without any sugar shows that it is the breakdown products of the sugar that brings about the colour change. The decrease in timings in the four media for the same concentration of inoculum was in the following order: Glucose without salt—Sugar mixture—glucose with 0.58% salt—glucose with 3.5% salt. Slightly higher timings obtained with sugar mixture than with dextrose alone in presence of 0.58% salt indicate that it is immaterial whether dextrose alone is used or in combination with maltose and lactose. Since the lowest results were obtained with a medium containing dextrose with 3.5% salt, it can be assumed that a sodium chloride concentration of 3.5% is the optimum and that the best medium for this test is dextrose with 3.5% salt. This is further proved in the series where different salt concentrations were used with dextrose (Table IV). The highly significant differences in the results obtained for the same concentration of inoculum added as a homogenate and as a piece (Table V) can be explained by the difference in the resultant salt concentration.

When a homogenate in sea water is added to the medium at a salt concentration of 0.58% the resultant salt concentration increases, while in the case where the inoculam is added as a lump the salt concentration remains unchanged. The significant differences in timings obtained between the homogenate and the lump even after equalising the final salt concentration (Table VII) need some explanation. This may be due to the more even distribution of bacteria in the homogenate than the muscle lump, the effect of surface bacteria, the effect of particle size, the effect of oxidation-reduction potential developed or due to some active substances formed during the homogenising process. Investigations are in progress to elucidate this aspect as well as the role of individual strains of bacteria on colour production.

Summary

The usefulness of a sugar medium for the rapid approximation of bacterial counts in fishery products has been investigated. A simplified formula for the reaction medium has been worked out and tried extensively in experiments. The method is found to give satisfactory results for rapid assessment of bacterial ranges in fresh, iced and frozen prawn products.

Acknowledgement

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