FACTORS INFLUENCING COLOUR PRODUCTION FROM THE SUGAR MEDIUM USED FOR THE RAPID APPROXIMATION OF BACTERIAL COUNTS IN FISHERY PRODUCTS

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[Results of the studies carried out to elucidate the factors influencing colour production from the sugar medium used for the rapid approximation of bacterial counts in fishery products are reported. The effect of particle size, trace elements, salt soluble protein and non-protein fractions, rate of mulipication of bacteria, in the medium, surface bacteria and the rate of colour production by individual strains of bacteria were studied. It is observed that the best results are obtained when a sea-water homogenate is used.]

Introduction

The use of an improved sugar medium containing dextrose and an acid indicator has been shown to be useful for the rapid assessment of the bacteriological quality of fish products and the procedure and results were published elsewhere (Cyriac Mathen et al., 1964). In the course of this investigation it was observed that the time for the production of the colour was slghtly less for the seawater homogenate (SWH) of prawn muscle than for the same weight of muscle added as a lump. It, therefore, appeared essential to elucidate the factors, if any, other than the bacterial count responsible for colour production. The present communication deals with the investigations carried out on this aspect. The factors studied were :

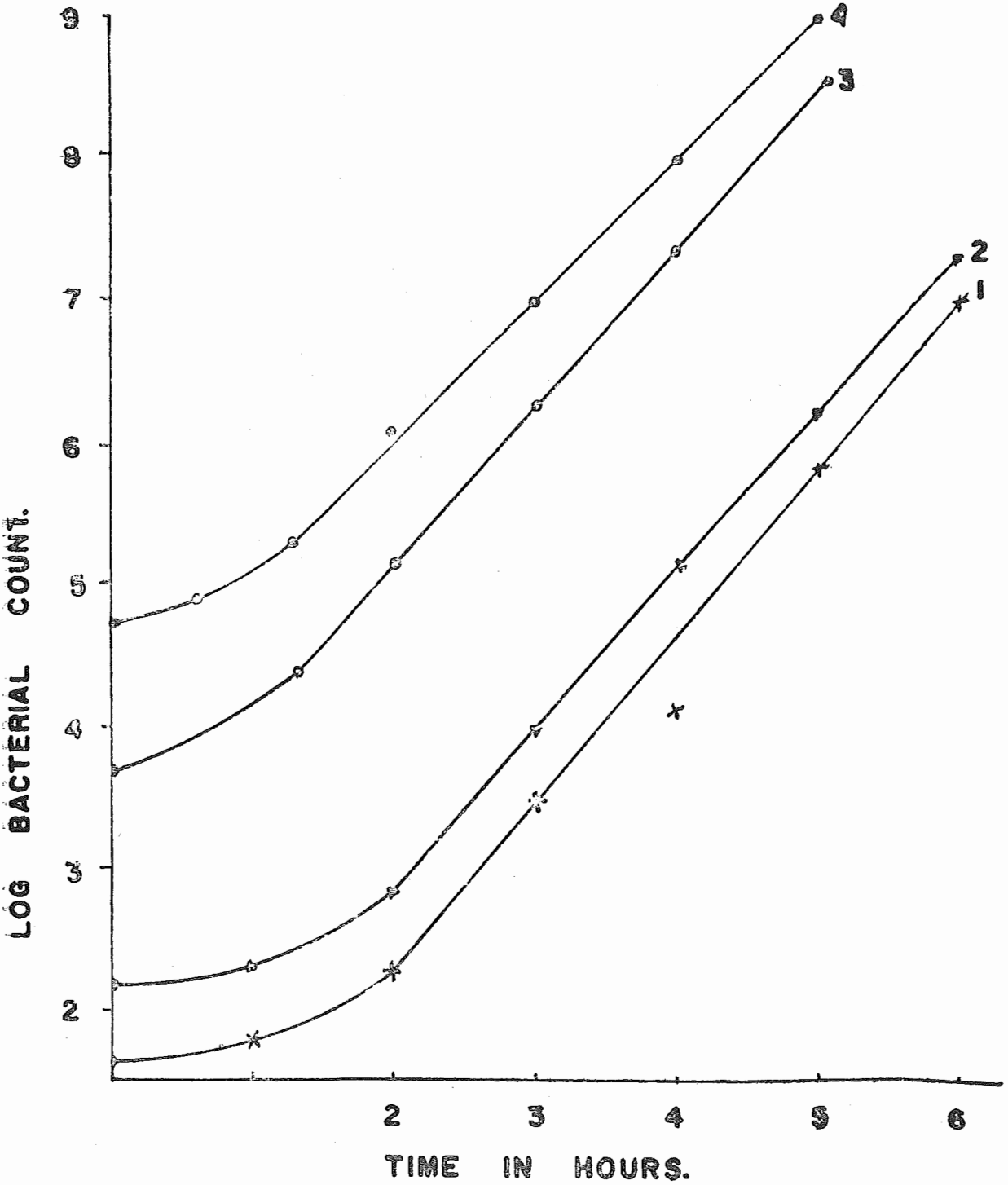
1. The effect of particle size.
2. The rate of multiplication of bacteria in the medium when added as a lump and as a seawater homogenate (SWH)
3. The distribution of bacteria in the muscle
4. The effeffct of trace elements
5. The effffect of protein and non-protein fractions
6. The rate of mlultiplication of bacteria when introduced as a SWH and as a distilled water homogenate (DWH)
7. The distribution of glucose fermentors in SWH and DWH
8. The behaviour of individual strains of bacteria.

Materials and Methods

In all the tests prawn collected locally was used. The standard plate count (SPC) was obtained by plating serial dilutions of the homogenates (SWH or DWH) on to seawater agar in duplicates and incubating for 48 hours at room temperature. The sugar medium used was one containing dextrose and 3.5% sodium chloride at a final pH of 7.0.

To examine whether varying blending time and consequently varying particle size has any effect on the colour production, prawn muscle was blended with sterile seawater in a waring blender for different intervals ranging from 2 seconds to 5 minutes. The homogenate was tested for colour production and for SPC.

To check whether any growth stimulating factors get released in the process of homo--genization and consequently accelerate the



Rate of multiplication of bacteria in the sugar medium.

1. Muscle lump; 2 & 4 Muscle homogenate in seawater.

3. Muscle homogenate in distilled water.

Fig. 1.

rate of multiplication of bacteria compared to the muscle lump, solutions from tubes inoculated with SWH and muscle lump were plated for SPC at definite intervals.

When the inoculum was added as a lump only the surface comes in contact with the medium and hence only the bacteria on the surface can react. Therefore it was felt essential to determine the proportion of bacteria on the muscle surface compared to the total bacterial count. With this purpose prawn muscle was washed twice repeatedly by shaking for five minutes each with sterile sea water (1 : 5) and the SPC of the washings and washed muscle were determined. Also unwashed muscle as a lump (UML) and as SWH were tested for colour production. The entire procedure was repeated using distilled water in place of sea water as the washings were found highly turbid.

When DWH was tested for colour production the time was very much higher in relation to SPC. This observation opened up poosibilities of interference by trace elements in the sea water and of the protein and non-protein fractions extractable in sea water, by the destruction of glucose fermentors in distilled water or by the longer lagphase when distilled water is used. To test these phenomena the following trails were made :

To determine the effect of trace elements DWH were tested for colour production with and without the addition of trace elements.

In order to study the effect of protein fraction on colour production, sterilized sea water extracts containing protein fraction from prawn muscle were tried. In conjunction with these the following were also studied.

1. Comparing colour productions of distilled water, sea water and isotonic saline homogenates and homogenate prepared in distilled water and again blending with 3.5% sodium chloride
2. Studying the colour production of mixed cultures with and without the addition of SWH.
3. The lag phase in DWH and SWH were studied by plating out both the solutions at definite intervals.

The percentage of glucose fermenting type of bacteria in both DWH and SWH were determined by testing the colonies picked up from both plates for colour production after 48 hours of incubation at 28°C.

Some strains of bacteria isolated from fresh and spoiling shrimp muscle were tested for colour production by inoculating 48 hours' culture at pH 7.0 into the sugar medium and noting the time for colour production. Individual strains as well as a mixture of strains were used. The SPC was also determined.

Results and Discussion

The blending time, SPC and time for colour production obtained in a few series are shown in Table I.

The results clearly indicate that a blending time above four seconds does not materially affect either the total plate count or the time for colour production. This shows that particle size has no effect on colour production and that a blending time above four seconds will be sufficient to get proper distribution of bacteria in the usual plating out procedure.

The relation between time interval and multiplication of bacteria in SWH and muscle lump is shown in Fig. 1. The rate of multiplication is almost identical in both cases, showing that the enhanced colour production with SWH is not due to more rapid multiplication of bacteria.

The total plate counts of sea water washing (SWW) and of muscle after washing are shown in Table II. From Table II it is clear that between 86 to 98% of the total bacteria can be removed by washing. This amount probably constitutes the surface bacteria. This observation is in accordance with the results obtained by Georgala (1957) who found that 80 to 90% reduction in bacterial load occurs by careful washing. Side by side it was also noted that washing more than twice did not remove more bacteria from the muscle.

TABLE — I. RELATION OF BLENDING TIME WITH SPC AND TIME FOR COLOUR PRODUCTION.

Expt. No.	Blending time in seconds	SPC|gm.	Time for colour production at 28°C in minutes
	30	9.05×10^6	120
I	60	7.02×10^6	122
	90	7.60×10^6	122
	120	9.50×10^6	120
	180	8.00×10^6	122
	240	7.60×10^6	122
	300	7.90×10^6	122
II	5	3.15×10^6	113
	10	3.04×10^6	113
	15	2.89×10^6	113
	20	3.10×10^7	113
	25	3.03×10^6	113
	30	3.10×10^6	113
III	2	6.40×10^6	129
	4	6.50×10^6	127
	7	6.00×10^6	127
	13	6.50×10^6	127

TABLE — II. DISTRIBUTION OF BACTERIA IN THE PRAWN MUSCLE

Sea water washings	SPC|gm. of muscle Washed muscle	Total	% of surface bacteria
1.38×10^5	1.50×10^4	1.53×10^5	90.2
4.40×10^5	8.20×10^3	4.48×10^5	98.1
1.80×10^3	3.05×10^2	2.11×10^3	98.5
1.64×10^4	2.20×10^3	1.84×10^4	88.0
2.50×10^6	2.90×10^5	2.79×10^6	89.4
7.10×10^6	5.35×10^5	7.63×10^6	93.1
8.55×10^6	1.32×10^6	9.87×10^6	96.7

The time for colour production for SWH, UML, SWW, washed lump (WML) and washed muscle homogenate in sea water (WMH) is shown in Table III, together with the SPC of unwashed muscle at 28°C. It is clear from Table III that the time for colour production from SWH is the lowest. The unwashed muscle added as lump takes the same time as the SWW and those for WML and WMH are the same. These results indicate that the higher time observed for unwashed muscle lump is due to the fact that surface bacteria alone react in the particular case, though the question of some activating substances being washed out during the washing process is not avoided.

TABLE — III. TIME FOR COLOUR PRODUCTION FOR THE SAME WEIGHT OF MUSCLE ADDED IN DIFFERENT FORMS

SPC/g. of UWM	Time for colour production at 28°C in minutes				
	SWH	UML	SWW	WML	WMH
7.90×10^6	124	139	137	190	190
7.01×10^6	128	145	145	188	190
1.82×10^7	113	131	125	168	168
1.38×10^5	175	213	212	245	247
8.55×10^6	120	140	138	192	192
2.50×10^6	143	159	157	202	200
1.64×10^4	233	252	245	287	285

The results obtained when the washing was done with distilled water are shown in Table IV. These results show that when homogenisation or washing is done with distilled water the timings are very high. The lower time obtained for unwashed muscle lump and even for distilled water washed muscle lump show that some change takes place during the distilled water treatment that affects colour production.

TABLE — IV. TIME FOR COLOUR PRODUCTION FOR THE SAME WEIGHT OF MUSCLE WHEN DISTILLED WATER IS USED FOR HOMOGENISATION OF WASHING.

Unwashed muscle homogenate in distilled water		Unwashed muscle lump	Distilled water washings		Distilled water washed muscle lump	Distilled water homogenate of distilled water washed muscle	
1.22×10^4	500	192	1.45×10^4	525	360	1.05×10^4	530
7.50×10^4	480	210	4.10×10^3	517	500	1.65×10^3	650
4.97×10^3	612	282	1.32×10^3	659	590	4.80×10^3	625

The standard plate counts obtained for the same sea water homogenate when serially diluted with sea water and distilled water and plated on to sea water agar are shown in Table V. These results show that there is 90 to 99% destruction of bacteria when diluted with distilled water.

TABLE — V. SPC FOR THE SAME MATERIAL WHEN SEA WATER OR DISTILLED WATER WAS USED FOR DILUTIONS.

SPC in sea water	SPC in distilled water	% destruction
9.80×10^6	4.80×10^5	94.7
1.16×10^7	9.55×10^5	91.8
1.30×10^{10}	5.30×10^7	99.4
6.75×10^6	4.30×10^4	99.4
7.10×10^6	8.20×10^4	99.0

The addition of Cu, Mn. K, Mg, P, I etc. or a mixture of all these to the DWH did not show any lowering of the time for colour production.

The addition of creatine phosphate to DWH did not lower the time for colour production.

Distilled water homogenate when tested for colour production with and without the addition of heat sterilized sea water homogenate, the time for colour production was the same.

The time for colour production from the same amount of homogenates in distilled water, 3.5% sodium chloride solution, isotonic saline, sea water and distilled water homogenate with 3.5% sodium chloride added, is shown in Table VI. The results for SWH and homogenates in 3.5% sodium chloride and isotonic saline are almost identical while the result for DWH closely follow that for DWH with 3.5% sodium chloride. This proves that the protein fraction soluble in 3.5% sodium chloride solution could not have produced any significant effect on colour production.

TABLE — VI. COMPARISON OF THE TIME FOR COLOUR PRODUCTION OF HOMOGENATES PREPARED IN DISTILLED WATER, SEA WATER, 3.5 % SODIUM CHLORIDE SOLUTION, ISOTONIC SALINE AND DWH WITH 3.5 % SODIUM CHLORIDE.

DWH	SWH	3.5 % NaCl	Isotonic saline	DWH plus 3.5% NaCl
198	115	110	115	200
335	88	85	78	335
406	206	205	202	406
335	150	150	155	330

The rate of multiplication of bacteria in distilled water and sea water are shown against time in Fig. 1. The same trend is found in both cases. It was observed that when the count was 1.0×10^7 or near about SWH produced colour, while in DWH there was no colour production even when the count was 1.0×10^8 or more.

The standard plate counts in seawater and distilled water and the percentage of glucose fermentors in them are shown in Table VII. These results show that only 30 to 40% of the bacteria isolated from DWH ferment glucose. It is also observed that the bacterial strains from SWH gave much higher intensity of colour than those from DWH.

TABLE — VII. DISTRIBUTION OF GLUCOSE FERMENTORS IN SWH AND DWH

SPC in SWH	% of glucose fermentors	SPC in DWH	% of glucose fermentors
1.30×10^{10}	100	5.30×10^7	61
6.75×10^6	100	4.30×10^4	32
5.30×10^6	100	3.90×10^4	34
6.15×10^6	100	4.00×10^4	41
7.10×10^6	100	8.20×10^4	30

The time for colour production in relation to bacterial count for some strains isolated from SWH is shown in Table VIII. It is clear from Table VIII that individual strains vary very much from one another in the rate of colour production and a mixture of them give time almost similar to that given by a sea water homogenate of prawn muscle.

TABLE — VIII. RELATION BETWEEN BACTERIAL COUNT AND TIME FOR COLOUR PRODUCTION OF INDIVIDUAL STRAINS OF BACTERIA AND PURE MIXED CULTURES A 28°C

Strain No.	Bacterial count in the inoculum	Time for colour production in minutes at 28°C
1	9.10×10^3 to 1.34×10^7	330 to 165
2	5.50×10^5 to 7.62×10^7	240 to 120
3	9.80×10^6 to 1.64×10^9	190 to 78
4	3.42×10^4 to 2.01×10^7	279 to 140
5	2.16×10^5 to 5.28×10^8	178 to 63
6	4.50×10^5 to 1.41×10^8	217 to 63
7	1.63×10^7 to 4.86×10^8	75 to 33
Pure mixed cultures of strain No. 1 to 7	9.10×10^6 to 8.80×10^8	78 to 37

The pure mixed culture contained the strains in the following percentages: Strain No. 1, 3.0%; Strain No. 2, 4.1%; Strain No. 3, 2.9 %; Strain No. 4, 2.7 %; Strain No. 5, 3.2 %; Strain No. 6, 4.7 %; and Strain No. 7, 79.3 %.

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References

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Discussion

Dr. M. V. Rajagopal enquired that as the standard plate count is not an index of quality how the particular test based on the standard plate count will be useful in judging the quality.

Shri Mathen replied that if the test had given a positive result within 10 or 15 minutes, then it would indicate the sanitary aspect of the material.

Dr. A. N. Bose also agreed that the standard plate count alone is not the correct index of the quality of the raw material, except under standard conditions. The count will certainly serve as an index of sanitation. He further pointed out that the test is based on one of the biochemical reactions common to the majority of the organisms occurring in fish products — namely, the production of acids from sugars.

Shri Gopinatha Pillai suggested that this test needs much more study and correlation with other factors. Dr. A. N. Bose intimated that the Institute is trying to find out some correlation of this particular test with specific group of bacteria like E. coli and enterococci and the author informed that no conclusions can be drawn yet on this aspect, as the studies are only in progress.

Dr. Bose further pointed out that it has been shown that no single index can give a correct picture of the quality of a product. It would be advisable to look for a combination of two or three indices for this purpose.

Shri Mitra said that in different countries work has been done and is in progress to find out a rough and ready method for determination of quality but so far none has been totally successful.