EFFECT OF DIFFERENT MEDIA ON THE DETERMINATION OF THE NUMBER OF MICRO ORGANISMS IN FROZEN PRAWNS (SHRIMP)

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[The Indian standards Institution have recently laid down specifications for the grading of frozen shrimp of which the bacterial examination of the product forms a significant part. Work carried out in this regard in our laboratories have shown that various improvements could be made in the composition of the medium to obtain a more true picture of the total bacterial count. Changes in the medium for total bacterial count have, therefore, been suggested and the reasons for the same discussed.]

Introduction

The ISI specifications for the microbiological examination of frozen prawns has specified the medium with the following composition: Agar 15.0 gm., Peptone 5.0 gm., Ferric phosphate 0.5 gm., Aged Sea Water 1000 ml. Investigations carried out at the laboratory has, however, shown that the bacterial flora usually found in the frozen prawn grow very slowly and in much smaller numbers as compared with other standard media. A systematic study was, therefore, undertaken to determine the growth of bacteria present in the frozen prawns in two other media as compared to the medium prescribed by the I.S.I.

Experimental procedure

Samples of frozen prawns of the following category :

a) Butterflied (BF)

b) Peeled and Deveined (SD)

c) Cooked and peeled (PS)

d) Peeled Deveined and Cooked (SCPS)

were selected at random from the cold storage rooms and used as raw material. All these samples were plated in duplicate in the three media.

The three media used for plating the samples had the following composition.

1) I.S.I. Medium — Peptone 5.0 gm., ferric phosphate 0.5 gm., agar 15.0 gm., and aged sea water 1000 ml.*

2) Tryptone Beef Extract Medium — Tryptone 5.0 gm., beef extract 3.0 gm., glucose 1.0 gm., agar 15.0 gm., and distilled water to 1000 ml.

3) Tryptone Beef Extract Medium — Same composition as above except that aged sea water* was used for making up the medium to volume.

The aged sea water* used in media 1 & 3 had the following Composition.

NaCl	..	27.50 gm.
$MgCl_2$	..	5.00 gm.
$MgSO_4$	..	3.00 gm.
$CaCl_2$	..	0.50 gm.
KCl	..	1.00 gm.
$FeSO_4$	..	Trace
Distilled water	..	1000 ml.

* *Private communication from Dr. V. K. Pillay, Central Institute of Fisheries Technology Ernakulam, India.*

Results and Discussion

The following table gives the comparative growth in the Three different media used for plating of frozen prawns

TABLE I

COMPARATIVE GROWTH ON THREE DIFFERENT MEDIA USED FOR PLATING OF FROZEN PRAWN

Expt. No.	Item	ISI Medium	Tryptone Beef Ext. Med.	Tryptone Beet Ext. & Aged Sea Water Media
1.	BF	5.4×10^4	6.9×10^5	2.3×10^5
2.	PS	7.0×10^3	1.7×10^5	1.2×10^3
3.	BF	2.2×10^4	2.4×10^5	2.2×10^5
4.	BF	1.5×10^4	4.1×10^5	3.0×10^5
5.	BF	3.2×10^4	6.6×10^3	4.2×10^5
6.	BF	6.2×10^4	5.7×10^4	2.4×10^5
7.	BF	1.4×10^4	2.4×10^5	2.4×10^5
8.	BF	1.8×10^4	7.8×10^4	7.0×10^4
9.	PS	1.0×10^4	4.6×10^4	3.6×10^4
10.	SCPS	1.2×10^4	5.9×10^3	1.6×10^5
11.	CPS	4.0×10^3	2.0×10^4	1.6×10^4
12.	FS	1.0×10^5	3.8×10^5	3.8×10^5
13.	CPS	4.2×10^5	2.3×10^6	5.3×10^5
14.	SCPS	6.0×10^4	2.9×10^5	2.4×10^5
15.	PS	7.9×10^4	1.1×10^5	1.0×10^5
16.	SCPS	1.0×10^4	2.0×10^5	2.0×10^5
17.	CPS	8.0×10^3	6.7×10^4	5.5×10^4
18.	PS	3.0×10^4	4.4×10^5	4.2×10^5

It is seen that in all the samples the tryptone beef extract medium and the tryptone beef extract medium containing sea water gave significantly higher counts than the one prescribed by the ISI. More number of organisms/gm. grew in the tryptone beef extract made with aged sea water.

It was also seen that the samples plates with the ISI medium (medium 1) always took more than 72 hours (sometimes as much as 96 hours) before the plates could be counted. In contrast, media 2 and 3 did not have to be kept more than 48 hrs. for full development.

It is evident from the results that the medium prescribed by the ISI for determining the total plate count does not give a true picture of the actual bacterial population. The majority of bacterial population found in the shrimp processed in the factory are contaminants from various sources inside the plant. While the ISI medium may be a good one for isolating bacteria of marine origin from the prawns it does not seem to be proper one for determining the total bacterial population present in the prawns.

As the ISI specifications are meant to be a sanitary safeguard in order to determine that the number of bacteria do not exceed tolerable level and that the pathogenic bacteria are not present, it would be in the best interests of the industry to use a medium easily available and also one which will facilitate

the growth of maximum number of bacteria in a short time. The presence of salts in aged sea water will definitely be detrimental to the growth of bcteria entering as contaminants. It is, therefore, suggested that the tryptone glucose beef extract medium without aged sea water be substituted as the Media in the ISI specifications for enumerating the total number of organisms per gram in place of the one prescribed.

Reference

1. ..... *Indian Standards specifications for frozen prawns* (shrimp), I.S. 2337-1962, Indian Standards Institution, New Delhi, (1962).

Discussion

Presenting his paper Dr. Rajgopal reported that compared to the sea water agar medium recommended in I.S.I. standards for the determination of standard plate count, he got consistently higher counts with Tryptone — Beef extract agar, with or without sodium chloride. He therefore urged the need for review of the ISI methods.

Dr. Pillai pointed out the ISI standards and the methods recommended therein have been prepared after careful experimentation and comparative study on the efficiency of the various media commonly used for the purpose. He mentioned that the seawater medium advocated in the standards will give the best results in the case of marine products as most of the organisms are of marine origin needing salt for optimum growth. Contamination of the products by terrestrial organisms is not ruled out but he pointed out that even in this case such organisms will grow in the presence of sodium chloride. On the other hand growth of marine organisms may be limited in nutrient media and the like without sodium chloride.

Miss A. Lekshmy presented data on over a hundred series in which the counts on seawater agar have been compared with that on other media including the one recommended by the author and showed that in all cases the counts on sea water agar were higher. She agreed, however, that the colonies developing on Tryptone — Beef extract agar were bigger. The differences in the few series reported by the author, she explained, are probably due to the errors in counting, the incubation temperature etc.

Shri Velankar pointed out that when fish is plated out on to nutrient agar or to other media having no sodium chloride, the colonies are likely to spread.

The Fisheries Development Adviser, Shri Mitra made a suggestion that in the light of the observations made by the author, the C.I.F.T. should carry out some more work on this aspect.

Recommendations

1. More intensive studies be carried out to evolve effective measures against the formation of black spot in fresh and ice stored prawns and for the prevention of drip and dehydration in forzen prawns.
2. Studies be directed to find out the causes and methods of prevention of flavour losses in ice-stored and frozen prawns.
3. Studies on development of quick methods for accurate determination of the quality of prawns be further pursued.
4. As far as possible all processing work be centralized in factories especially in the case of cooked peeled prawns which are prone to stricter bacteriological standards.
5. Stricter cleaning schedules as proposed by the C.I.F.T. should be adopted by all Processing and Peeling centres.
6. The method given in ISI standards for the determination of total plate counts in frozen prawn be re-examined in the light of more data to be gathered.

7. A pilot plant, followed by a commercial unit be designed and constructed to demonstrate the economic feasibility of the tunnel drying techniques of dry prawn pulp production.

8. The actual quantity of ice needed and the probable distributions of ice factories required for the future development of fish / prawn processing industries be clearly assessed.

9. The research work carried out at the Research Institutes be coordinated with the practical needs of the industry. A small committee of the representatives of the industry and the research institutes will enable greater coordination between both.