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85. STUDY ON THE BACTERIAL QUALITY OF EDIBLE OYSTER, *CRASSOSTREA MADRASENSIS* AND ITS PURIFICATION

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ABSTRACT

Edible oysters (*Crassostrea madrasensis*) collected periodically during the years 1984-1986 from Central Marine Fisheries Research Institute farm at Tuticorin and from the natural beds were studied for their bacterial quality. Seawater samples from the surrounding environs were also simultaneously collected and analysed for physical, chemical and bacteriological parameters. The oyster samples were subjected to purification by employing different methods. The total bacterial count of cultured oysters and natural bed edible oysters ranged between 10^3 to 10^4 organisms per ml of oyster fluid. The T.B.C. of the sea water around cultured oysters and natural bed oysters ranged between 10^2 to 10^3 organisms per ml of seawater. Faecal coliforms were found to be very low and within permissible limits. The pathogenic bacteria *Salmonella*, *Streptococci* and *Staphylococci* were absent. The variations in pH, temperature, salinity and dissolved oxygen of the seawater samples were insignificant. The edible oysters were subjected for purification by employing different purification methods among which chlorination was found to be better.

INTRODUCTION

It is known that protected bays and estuaries offer suitable grounds for culture of oysters. The increase in pollution in these waters pose a threat to oyster farming. The discharge of industrial effluents and sewage in these water bodies necessitates purification of oysters before marketing. Because the oysters are filter feeders they can concentrate pathogenic bacteria that may be present in the surrounding waters. The authors in this paper have dealt the bacterial flora and purification of oysters collected at Tuticorin during the years 1984 to 1986. Also the bacterial flora of natural bed oysters collected from Tuticorin, Ennore and Pulicat were studied and reported in this paper.

MATERIAL AND METHODS

The oyster samples were collected from Central Marine Fisheries Research Institute Farm at Tuticorin at regular intervals alongwith the farm water samples. On few occassions oysters were also collected from natural beds from Tuticorin, Pulicat and Ennore backwater and examined for bacterial flora. A sample of 3 to 5 oysters were used for collecting the oyster liquid which was blended with equal amount by the weight of the Normal Saline solution so that 2 ml of oyster liquid contained 1 g of oyster meat. Plate count of the oyster liquid and farm water were determined by preparing duplicate plates of Tryptone glucose yeast extract agar. The plates were incubated at 37°C for 48 h (Presnell and Kelly 1961 & APAA 1976). E. C. broth with incubation at 44.5°C and Eosine Methylene Blue Agar were used for the enumeration of faecal califorms (APHA 1976) and for *E. coli*, Tergitol -7 Agar were used. For the enumeration of *Salmonella*, Brilliant Green Agar, Bismuth Sulphite Agar and Triple Sugar Iron Agar were used. For enumeration of coagulase +ve staphylococci, Baird parket Agar and for faecal streptococci, K F Agar were employed. Various authors have described different purification methods for oysters. Eyles et al(1984) described purification methods for commercial depuration of oysters. The purification plant was of the recirculating type employing UV light for sterilisation. Mahadevan (1980) has described the depuration of oyster for 24 h in running seawater and also use of Chlorination at 3 ppm for 1 h. Subsequently they were dechlorinated before disposal for marketing. The purification of oysters by the authors were carried out by the methods described as under. (i) By keeping the oysters in filtered sea water for 24 h with the change of water once in 12 h. (2) The oyster samples were purified by keeping them in aerated seawater

for 24 h and for 48 h with the change of sea water once in 12 h. (3) The depurated oysters in filtered sea water were subjected for chlorination at 2, 2. 5, 3, 4 and 5 ppm for 3 h and after that these samples were washed thoroughly in filtered sea water. The purified oysters were then examined for bacterial quality.

RESULTS AND DISCUSSION

Bacterial flora of farm oysters

Oysters (*C. madrasensis*) and oyster farm water collected from Central Marine Fisheries Research Institute Farm. Tuticorin were examined for total bacterial count and pathogens *F. coliforms, E. coli, F. streptococci* and *Salmonella*. The data are given in Table 1. The total viable count of the oysters ranged from 3.43×10^2 to 1.9×10^4/g and the TBC of the farm water ranged from 1.6×10^2 to 2.9×10^3 ml. The TBC of the surrounding seawater was conststently lower than the corresponding counts of oysters. This is in agreement with the findings of Durairaj et al (1983). The pathogenic bacteria were found to be absent except faecal coliforms in all the oysters and seawater samples. The faecal coliforms were noticed on few occasions and the count was very less and with in the permissible limits. This also is in agreemnt with the findings of Durairaj et at (1983). The values of faecal coliforms were found to be between 3 to 38/100/gm in oyster and in farm seawater it ranged from 3 to 23/ml. According to A PHA (1976) the permissible limits of coliforms is 230/100 g, in depurated oysters. Coliform counts of water were reported to be maximum under low salinity conditions (Pressnell & Kelly 1981). Though low salinity conditions were not observed in Tuticorin waters, higher counts of *E. coli* were observed during the periods of lower salinity. During the period July to September 1986 the minimum salinity recorded was 32 ppt and during this period a maximum of 39 counts of *E. coli* were recorded in the oyster and 23 in the seawater sample.

David Hussong et al (1981) have indicated that coliform MIN counts of oysters were found to increase significantly during October and early November of each year when the temperature was maximum. Similar findings were recorded at Tuticorin when maximum coliform count of 39 in oysters and 23 in seawater were

TABLE 1. *Data on bacterial flora of edible oysters and farm water collected from Central Marine Fisheries Research Institue farm, Tuticorin*

Period	Oyster				Farm Seawater			
	TBC/g		F. coliforms/100g		TBC/ml		F. coliforms/100g	
	Min	Max	Min	Max	Min	Max	Min	Max
1. 1984 Apr - Jun	26.95×10^2	9×10^3	Nil	Nil	7.8×10^2	16.5×10^2	Nil	Nil
2. 1984 Jul - Sep	4.9×10^2	8.5×10^2	Nil	Nil	3.6×10^2	6.2×10^2	Nil	Nil
3. 1984 Oct - Dec	3.43×10^2	7.5×10^2	Nil	Nil	3.8×10^2	4.2×10^2	Nil	Nil
4. 1985 Jan - Mar	23.2×10^2	1.9×10^4	Nil	Nil	5.4×10^2	20.5×10^2	Nil	<3
5. 1985 Apr - Jun	5.8×10^2	50.8×10^2	Nil	Nil	3.2×10^2	19×10^2	Nil	Nil
6. 1985 Jul - Sep	16×10^2	5.6×10^3	Nil	15	2.9×10^2	2.9×10^3	Nil	8
7. 1985 Oct - Dec	5.2×10^2	18.4×10^2	3	14	6.2×10^2	8×10^2	Nil	4
8. 1986 Jan - Mar	6.8×10^2	34.8×10^2	11	36	1.6×10^2	20.8×10^2	7	12
9. 1986 Apr - Jun	12.2×10^2	24.2×10^2	9	39	2.8×10^2	12.5×10^2	3	21
10. 1986 Jul - Sep	18.2×10^2	32.3×10^2	11	39	1.8×10^2	16.5×10^2	3	23
11. 1986 Oct - Dec	11.510^2	43.5×10^2	9	11	4.0×10^2	20×10^2	3	11

regarded when the temperature was maximum at 34.5°C during July 1986. The low TBC load of the oysters (10^2 to 10^4/g) and surrounding seawater (10^2 to 10^3/ml) and the complete absence of pathogens except faecal coliforms on few occasions, showed that the oysters were free from pollution in Tuticorin waters.

Bacterial flora of natural bed oysters

The samples of oysters from natural beds and also the surrounding seawater samples were collected on few occasions from Tuticorin, Pulicat and Ennore back waters and examined for bacterial flora and the data are given in Table 2. The TBC of the natural bed oysters near Tuticorin is ranged from 3.43×10^2 to 5.5×10^3 g and the TBC of the surrounding seawater ranged from 1.6×10^2 to 2.5×10^3 ml. The faecal coliforms of the natural bed oysters from Tuticorin ranged from nil to 39/100g and that of the seawater nil to 28/100 ml The total bacterial load of Pulicat oysters and backwaters were 6.2×10^3/g and 2.8×10^3/ml and in case of Ennore oysters and backwaters the total bacterial load were 11.6×10^3/g and 9.8×10^3/1 ml.

From the data given in the table 2 it is noted that the TBC content of Ennore oyster and backwaters was on higher side than the other two places viz Tuticorin and Pulicat. This is because of the discharge of seawage and industrial effluents in the Ennore backwater from the factories situated near Ennore. Coliform 9/100 ml were recorded in oysters collected from Ennore. The other pathogenic bacteria were however nil in Tuticorin, Ennore and Pulicat natural bed collections.

Hydrological parameters of the seawater samples

During the period under report, farm water samples and natural bed seawater samples were collected along with the oyster samples. Dissolved oxygen, salinity, pH of the water samples were determined and the temperature were noted. The Hydrological parameters are given in Tables 3&4. There was however no marked fluctuation in pH and other hydrological parametersr The analysis of water was carried out as per the methods of AOAC (1970)

Purification of oysters

From the data given in Table 5 it was noted that there was reduction in bacterial counts as a result of purification by the methods already described. Depending on the feasibility any one of these methods can be successfully

TABLE 2. *Data on bacterial flora of natural bed oysters and seawater samples collected near Tuticorin, Pulicat and Ennore seawater*

Date	Place	Oyster		Sea water	
		TBC/gm	F. coliforms/100gm	TBC/gm	F. Coliforms/100ml
1. 12-5-84	Tuticorin	$6x10^2$	Nil	$1.6x10^2$	Nil
2. 29-6-64	,,	$5.5x10^3$	Nil	$20.25x10^2$	Nil
3. 17-9-84	,,	$5.41x10^2$	Nil	$6.2x10^2$	Nil
4. 11-10-84	,,	$5.0x10^2$	Nil	$4.6x10^2$	Nil
5. 4-12-84	,,	$3.43x10^2$	Nil	$4.4x10^2$	Nil
6. 7-1-85	,,	$1.9x10^3$	Nil	$5.4x10^2$	Nil
7. 17-6-85	,,	$3.9x10^3$	Nil	$2.5x10^3$	Nil
8. 12-5-86	,,	$24.2x10^2$	39	$4.5x10^2$	28
9. 25-9-86	Ennore	$11-6x10^3$	9	$9.8x10^3$	Nil
10. 25-8-86	Pulicat	$6.2x10^3$	Nil	$2.8x10^3$	Nil

TABLE 3. *Hydrological parameters of the oyster farm sea water at Tuticorin*

Period	Temperature °C		Dissolved Oxygen ppt		pH		Salinity ppt	
	Min	Max	Min	Max	Min	Max	Min	Max
1984 Jan-Mar	27.0	27.6	4.5	5.4	7.2	8.5	35.0	46.0
1984 Jul-Sep	29.4	30.8	4.0	5.6	8.1	8.5	33.5	33.5
1984 Oct-Dec	26 0	27.0	4.6	5.0	7.9	8.3	34.0	36.5
1985 Jan-Mar	26.0	28.1	5.8	6.0	8.4	6.4	35:7	36.0
1985 Apr-Jun	28.0	31.0	Nil	5.2	7.5	8.4	36.4	40.0
1985 Jul-Sep	29.5	33.0	2.6	5.2	8.1	8.3	36.0	36.1
1986 Jan-Mar	29.0	32.0	0.8	3.2	8.0	8.0	36.0	36.2
1986 Apr-Jdn	32.0	34.5	2.2	3.0	8.0	8.8	38.0	38.5
1986 Jul-Sep	31.0	34.5	2.0	4.6	8.0	9.5	32.0	33.0
1986 Oct-Dec	28.6	30.2	3.2	4.4	8.4	8.5	32.3	33.0

TABLE 4. *Hydrological parameters of the natural bed seawater samples collected near Tuticorin, Pulicat and Ennore backwater*

Date	Place	Temperature °C	Dissolved Oxygen	pH	Salinity ppt
1. 12-1-84	Tuticorin	24.2	4.0	7.2	35.2
2. 29-6-84	,,	30.0	6.6	8.1	33.5
3. 17-9-84	,,	26.6	4.8	8.7	33.4
4. 11-10-84	,,	29.4	5.2	8.3	35.2
5. 4-12-84	,,	27.6	4.7	8.3	32.0
6. 7-1-85	,,	23.8	6.3	8.4	35.5
7. 17-6-85	,,	26.4	2.5	8.5	39.0
8. 12-5-86	,,	34.2	2.2	8.0	38.5
9. 25-8-86	Ennore	32.2	4.4	8.4	33.2
10. 25-8-86	Pulicat	30.0	1.6	8.6	35.5

utilised for purification of oysters before marketing. Mahadevan (1980) has suggested depuration of oysters for 24 hours in seawater followed by chlorination with 3 ppm for one hour and it was subsequently washed in seawater before marketing. Balachandran et al (1981) have suggested the method of chlorination upto 5 ppm at the end of depuration in seawater for a period of 16-18 h. Ray (1984) has suggested a depuration period of 36-48 h for purification of oysters. He is of the view that chlorination is effective at 2 to 3 ppm levels and the expensive method of chlorine depuration makes it less attractive when compared with other forms of purification. The observations recorded by the present authors (Table 5) indicate that the bacterial count of the oysters could be brought down effectively either by washing

TABLE 5. *Data on purification studies of oysters*

	Date	Raw Oyster	Seawater sample	Filtered seawarer sample	Oyster after washing in filtered sea water for 24 h	OYster after 24 h aeration in filtered sea water	Cyster after 48 h aeration in filtered sea water	Particulars: Depurated oysaer after chlorinated for 3 h and then throughly washed 2 ppm (a)	2.5 ppm (b)	3.0 ppm (c)	4 ppm (d)	5 ppm
1.	9-4-85	$9.2x10^2$	$5.2x10^2$	90/ml	$4.2x10^2$	$3.5x10_2$	$2.4x10^2$	$3.4x10^2$	$2.8x10^2$	$1.2x10^2$	ND	ND
2.	18-4-86	$25.8x10^2$	$19.8x10^2$	80/ml	$9.8x10^2$	$8.7x10^2$	$4.6x10^2$	$9.6x10^2$	$8.8x10^2$	$1.6x10^2$	ND	ND
3.	23-5-85	$4.4x10^2$	$3x10^2$	75/ml	$3.8x10^2$	$3.5x10^2$	$3.1x10^2$	$3.6x10^2$	$2.8x10^2$	$1.4x10^2$	ND	ND
4.	15-6-85	$126x10^2$	$33x10^2$	85/ml	$112x10^2$	$86x10^2$	$62x10^2$	$42x10^2$	$26x10^2$	$16x10^2$	ND	ND
5.	16-7-85	$13.8x10^2$	$8.2x10^2$	90/ml	$8.8x10^2$	$6.8x10^2$	$5x10^2$	$6.8x10^2$	$4.2x10^2$	$4x10^2$	ND	ND
6.	17-6-85	$78x10^2$	$25x10^2$	90/ml	$62x10^2$	$24x10^2$	$22x10^2$	ND	ND	ND	$18x10^2$	$11xv0_2$
7.	26-6-85	$88x10^2$	$32x10^2$	90/ml	$68x10^2$	$48x10^2$	$36x20^2$	ND	ND	ND	$28x10^2$	$24x10^2$
8.	16-7-85	$9.6x10^2$	$4.2x10^2$	85/ml	$6.4x10^2$	$9.4x10^2$	$8.2x10^2$	$6.8x10^2$	$5.8x10^2$	$4.2x10^2$	ND	ND
9.	8-1-86	$12.5x10^2$	$3.4x10^2$	75/ml	$9.75x10^2$	$8.75x10^2$	ND	$6.8x10^2$	$4.4x10^2$	$2.2x10^2$	ND	ND
10.	18-2 86	$8.75x10^2$	$4.1x10^2$	90/ml	—	$6.2x10^2$	ND	$5.4x10^2$	$4.5x10^2$	$3.2x10^2$	ND	ND

them in filtered seawater for 24 h or keeping them in aerated seawater for 48 h. The bacterial quality could be further improved by chlorination at the end of depuration, The average reduction of the total bacterial count was 46.14% in the oysters, kept in filtered seawater for 24 h. 61.51% by aerating the oysters in filtered sea water for 48 h and the average reduction was 76.90% by chlorinating at 3 ppm level. As the initial TBC load in the oysters is well below the permissibe levels, the purification of oyster could be done either by keeping the oysters in filtered aerated seawater upto 48 h with change of water once in 12 h or by chlorination at 3 ppm level at the ene of depuration in filtered seawater. The chlorinated oysters were thoroughly washed before marketing.

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