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80. REMOVAL OF PATHOGENIC BACTERIA AND GRITTINESS FROM CLAM *(VILLORITA CYPRINOIDES)* AND MUSSEL *(PERNA VIRIDIS)* MEANT FOR PROCESSING BY A BIOLOGICAL MEHOD

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ABSTRACT

Live clams (*Villorita cyprinoides*) from Vembanad Lake (Kerala) and live mussels *(Perna viridis)* from Calicut (Kerala) were examined for their bacteria and sand content. It was observed that the flesh of these bivalves harboured large bacterial populations including faecal coliforms, *Escherichia coli* and faecal streptococci. Also their intestines contained mud and sand (acid insoluble ash) sufficient to impart muddy flavour and gritiness to the meat. A cleansing operation to achieve the elimination of pathogenic/indicator bacteria, mud and sand from the clam and mussel meat has been worked out. process involves depuration of the bivalves in their clean natural water for 18-24 h, followed by chlorination of the system to 5 ppm level and maintaining in that condition for an additional 2 h. By this process, there was complete removel of pathogenic/indicator bacteria from their muscle. Also, the acid insolucle ash decreasep to negligible levels.

INTRODUCTION

Molluscan shell fish such as clams, mussels, and oysters are filter feeders. They filter large volume of water during their feeding activities and while doing so, they may concentrate within their bodies bacteria such as coliforms, faecal streptococci, and salmonella or enteroviruses present in the water. Human infections caused by bacterial, viral and protozoan pathogens have been traced to the ingestion of shellfish, harbouring such pathogens.

Kaysner (1981) reported the incidence of faecal coliforms, mainly comprised of *Escherichia coli* in the clams from Bering sea. *Klebsiella pneumoniae* were recovered in increased numbers from summer harvested Louisiana oyster (Boutin et al 1986). Thison and Fleet (1980) found that edible oysters (*Crassostrea commercialis)* harvested from the major cultivation areas in New South Wales, Australia were contaminated with food poisoning organisms like *Bacillus cerius, Clostridium perfringens, Vibrio parahaemolyticus* and *Salmonella*. Metcalf et al (1979) found that clams bioaccumulated faeces-associated natural viruses mainly in their hepatopancreas and siphon tissues. Pillai (1980) reported that the suspended cultured mussels (*Perna indica*) from Vizhinjam harboured large populations of coliforms, *E. coli*, faecal streptococci and coagulase positive staphylococci. Most of these microorganisms are of faecal origin and cause great health hazards.

In addition to bacteria, the intestines of clams and mussels are often loaded with mud and sand, which impart a muddy flavour and grittiness to the meat if retained within. Therefore a cleansing operation to achieve depuration and elimination of bacteria is a very important step that should precede processing of these molluscan meat.

Nowak (1970) described the mechnism of purification of bivalves in water. Stroud (Torry Advisory Note No. 84) advised cleansing of oysters in recirculating sea water treated with U. V. light or in static water. Prabhu and Balachandran (1980) reported about the practice of holding clams in cages in clean areas in the sea in Canada. Balachandran and Surendran (1984) studied the depuration of live clams (*Vilotita* sp). In this paper, a simple method of purifying clams and mussels, so as to remove bioaccumulated bacteria and grittiness from their muscle, by the process of depuration is described.

MATERIAL AND METHODS

Live clams (*Villorita cyprinoides*) from their natural beds in Vembanad lake near Vaikom

(Keraia) and live mussels *(Perna viridis)* from the shallow sea of Calicut (Kerala) were collected and brought immediately to the laboratory, keeping in water from the same area of collection Water and bottom mud samples from the same area of harvesting of clams and mussels. were separately collected in sterile bottles, Depuration of clams and mussels was carried out in the respective water from the habitat of clams and mussels, potable water, sodium chloride solution made up to the strength of water from their respective natural habitat, as also all these chlorinated to 5 ppm level. Effect of all these on mortality, bacterial quality, removal of sand and contents of glycogen in the meat of both clam and mussels were studied.

Moisture, crude protein, fat and acid insoluble ash of the clam and mussel meat were determined according to AOAC (1975) methods and glycogen by the method of Umbriet et al (1959). Total bacterial count (TPC) was determined using trypton-glucose-beaf extract agar (TGA). The plates were incubated at 28 ± 2°C (room temperature, RT) for 48 h. and counts taken.

Total coliforms and faecal coliforms were determined using a three dilution-three tube replication of lactose broth (LB) in a standard most probable number series (MPN) with an incubation temperature of 37°C. Confirmation of total coliforms was done by MPN method using brilliant green lactose bile broth 2% (BGLB) incubated at 37°C. Loopfuls of culture from positive BGLB tubes were transferred to EC broth tubes and incubated for 48 h. at 45 ± 0.5°C. Tubes with positive growth and gas production showed the presence of faecal coliforms. Tests for *E. coli* were done for cultures from positive EC tubes by first transferring to eosine methylene blue agar (EMB), followed by IMVIC tests. Faecal streptococci were determined using KF streptococci agar. The plates were incubated at 37°C for 48 h. and dark red colonies and colonies with red and pink centre were counted as faccal streptococci colonies (APHA 1970). *Staphylococcus aureus* was determined by the direct direct plating method using Baird Parker agar.

RESULTS AND DISCUSSION

Proximate composition and bacterial quality of raw clam meat and mussel meat, as well as the bacterial profile of the water and bottom mud samples from the natural habitat of clams and mussles are presented in Tables 1 and 2 respectively.

Both clams and mussels harboured a large population of bacteria of the order of a million per gram of raw muscle. The clams carried a total coliform load of 10^3-10^4/g muscle, of which *E. coli* formed the major constituent. Also a heavy load of faecal streptococci was found in its flesh. But, total coliforms in the shucked raw meat of mussel were comparatively less, being only in the order of hundreds. Both *E. coli* and faecal streptococci in the mussel meat were less than those in the clam meat. It can be seen from the Tables (1 and 2) that the environmental water and mud of both clams and mussel carried these bacteria, but to a lesser extent. Due to their filter feeding activities, both clams and mussels have concentrated these bacteria in their body. Coliforms including *E. coli* and faecal streptococci are faecal indicator bacteria. Their presence in the muscle show that there are chances of other pathogens like *Salmonella*, from faecal material, to be present in these bivalves.

Both clam and mussel meat contained acid insoluble ash (sand), 0.38% in clam meat and 0.33% in mussel meat. This acid insoluable ash imparts grittiness to the meat, making it unsuitable for human consumption.

Ability of the contaminated shellfish to rid themselves of the baecteria when placed in clean water is well known and has been extensively exploited in artiificial depuration system (Hartland and Timoney 1979). The same principle is made use of in cleansing clams and mussels, in our studies too.

Live clams were depurated in clean water from the natural habitat, filtered through a cloth 100 kg of clams were kept in 60 l of water, keeping a water column of 7.5 cm above clams. After intervals of 18 h 24 h and 48 h samples were drawn for estimation of acid insoluble ash (sand) and bacteriological examination of raw shucked meat. Results are presented in Table 3.

TABLE 1. *Proximate composition and bacterial quality of clam meat and bacterial profile of water and mud from the clam habitat*

	Raw meat	Water	Mud
Moisture %	78.50	—	—
Fat % (DWB)	2.52	—	—
Protein	10.09	—	—
Ash %	0.86	—	—
Acid insoluble ash (sand) %	0.38	—	—
Glycogen %	6.68	—	—
Total bacterial count	1.2×10^6/g	5.5×10^4/ml	9.06×10^3/g
Total coliforms	6.3×10^3/g	100 ml	25/g
E. coli	3×10^3/g	52 ml	16/g
Faecal streptococci	8.5×10^3/g	2.1×10^2/ml	82/g
Coagulase + ve staphylococci	Nil	Nil	Nil

TABLE 2. *Proximate composition and bacterial quality of mussel meat and bacterial profile of water and bottom mud from the mussel habitat*

	Raw meat	Water	Mud
Moisture %	78.24	—	—
Protein %	12.61	—	—
Fat % (DWB)	3.02	—	—
Glycogen %	7.90	—	—
Ash %	0.82	—	—
Acid insoluble ash (sand) %	0.33	—	—
Total bacterial count	5.3×10^6/g	6.2×10^4/ml	3.12×10^3/g
Total coliforms	2.3×10^2/g	93/ml	210/g
E. coli	1.15×10^2/g	75/ml	63/g
Faecal streptococci	180/g	42/ml	93/g
Coagulase + ve staphylococci	Nil	Nil	Nil

TABLE 3 *Effect of depuration of live clams in natural water for various periods on the bacterial quality and and acid insoluble ash (sand) content of raw clam meat*

	Before depuration	After depuration for 18 h	After depuration for 24 h	After depuration for 48 h
Total bacterial count/g	4.22×10^6	6.52×10^5	4.68×10^5	3.12×10^3
Total coliforms/g	8.31×10^3	8.50×10^2	3.50×10^2	1.16×10^2
E. coli/g	2.74×10^3	1.06×10^2/g	98	Nil
Acid insoluble ash (sand) %	0.38	0.06	0.01	0.01

TABLE 4. *Effect of depuration of live mussels in natural water for various periods on the bacterial quality and acid insoluble ash (sand) content of raw mussel meat*

	Before depuration	After depuration for 18 h	After depuration for 24 h	After depuration for 48 h
Total bacterial count/g	5.3×10^6	6.81×10^5	4.72×10^5	3.03×10^3
Total coliforms/g	2.3×10^2	118	93	24
E. coli/g	115	24	9	Nil
Faecal streptococci/g	280	118	115	28
Acid insolube ash %	0.33	0.026	0.012	0.01

TABLE 5 *Effect of depuration of live clams in different systems for 18 h (over night) on the quality clam meat*

	Raw meat before depuration	Natural water (lake water)	Natural water chlorinated at 5 ppm level	Potable water	Potable water chlorinated at 5 ppm level	Sodium chloride solutions (1.03%)	Sodium chloride solution (1.03% chlorinated at 5ppm level
Total bacterial count/g	5.8×10^6	3.82×10^5	8.91×10^5	5.62×10^5	5.81×10^5	4.14×10^5	4.31×10^5
Total coliforms/g	3.8×10^4	2.19×10^3	7.06×10^3	4.74×10^3	5.03×10^3	3.89×10^5	3.48×10^3
E. coli/g	2.2×10^2	Nil	Nil	12	Nil	Nil	Nil
Faecal streptococci/g	5.14×10^4	4.82×10^3	9.10×10^3	9.20×10^3	1.01×10^4	8.84×10^3	6.62×10^3
Acid insaluble ash (sand) %	0.34	0.012	0.024	0.056	0.094	0.015	0.026
Glycogen %	6.68	5.9	5.6	5.6	5.2	5.6	5.0

TABLE 6 *Effect of depuration of live mussels in different system for 18 h (over night) on the quality of mussel meat*

	Raw meat before depuration	Natural water (sea water)	Natural water chlorinated at 5 ppm level	Potable water	Potable water chlorinated at 5 ppm level	Sodium chloride solution (2.3%)	Sodium chloride solution (2.3%) chlorinated at 5ppm level
Total bacterial count/g	8.3×10^6	4.38×10^5	6.1×10^5	7.82×10^5	9.86×10^5	4.9×10^5	5.62×10^5
Total coliforms/g	6.71×10^2	105	238	218	486	118	118
E. coli/g	230	93	108	124	138	92	105
Faecal streptococci/g	486	124	118	230	238	108	114
Acid insoluble ash (sand) %	0.42	0.02	0.032	0.088	0.18	0.02	0.06
Glycogen %	5.36	3.82	4.54	4.60	4.22	4.90	4.63

TABLE 7 *Bacterial quality of clam meat after depuration for 24 h in natural water and subsequent chlorination of the system and keeping for 2 h*

Bacterial counts	Raw meat before depuration	After depuration	After depuration and chlorination
Total bacterial count/g	5.8×10^6	3.82×10^5	2.92×10^5
Total coliforms/g	3.8×10^4	2.90×10^3	1.02×10^3
E. coli/g	2.2×10^2	Nil	Nil
Faecal streptococci/g	5.1×10^4	4.82×10^3	2.96×10^3

TABLE 8. *Bacterial quality of mussel meat after depuration for 24 h in natural sea water and subsequent chlorination of the system and keeping for 2 h.*

Bacterial counts	Raw meat before depuration	After depuration	After depuration and chlorination
Total bacterial count/g	7.46×10^6	3.29×10^5	1.06×10^5
Total coliforms/g	1.13×10^3	2.04×10^2	118
E. coli/g	230	Nil	Nil
Faecal streptococci/g	2.81×10^3	1.18×10^2	93

Similarly mussels were depurated in clean filtered sea water from the natural habitat and after intervals, samples were analysed for sand and bacterial quality. Results are presented in Table 4.

The primary aim of depuration of live clams and mussels was removal of sand and gritty matter. Not only that this is achieved by this starvation method, but there is very good improvemen tin the bacterial quality of their meat as evidenced by the data presented in Tables 3 and 4.

Studies were also carried out on alternate depuration systems, to find out their ralative efficiency. In addition to the water from the natural habitat of clams and mussels, potable water from municipal water supply, sodium chloride solutions made up to the strength of natural water (1% in the case of clams and 2.3% in the case of mussels) and the above systems chlorinated to 5 ppm level were used for depuration of both clams and mussels. The results are summarised in Tables 5 and 6.

Live clams when kept in water remained with shell slightly agape so that they can filter enough water.

However, it was noticed that when the water is chlorinated, the shells remained tightly closed until such time that the available chlorine disappeared from the system. Until this time no activity leading to depuration took place. This should be the reason why the extent of depuration leading to the decrease in sand and bacteria, was comparatively less in the chlorinated systems than the corresponding untreated systems. The data definitely showed that the best results in depuration is obtained when both clams and mussels are allowed to starve in clean water from their respective natural habitats.

Upto a period of 24 h no mortality was observed in the natural water, in the case of both clams and mussels. But in other media, some mortality though negligible was observed. in other systems, the minimum mortality was observed in sodium chloride solutions of the same strength as the respective natural water.

Acid insoluble ash (sand) content in the muscle could be brought down to an insignificant level by depuration in the water. The retention of glycogen which is the source of stored energy also was the highest in this

system. The total bacterial number in the muscle was brought down considerably. There was 90% decrease in the total bacterial count in total faecal indicator bacteria. The *E. coli* was completely eliminated with 24h of depuration in natural water.

In another series of experiments live clams and mussels were depurated in their respective natural water for 24h and then the system was chlorinated to 5 ppm and maintained like that for another 2h. The bacterial qualities of their meats at different stages in this experiment are presented in Tables 7 and 8. Though depuration in chlorinated water was found to provide no significant improvement (Tables 5 and 6) the bacterial qualities of the meats of clams and mussels were considerably improved in the case of treatment with chlorine for 2h after depuration in natural water for 24h.

It follows from these experiments that for achieving best results in removing the grittiness and eliminating pathogenic bacteria from the clam and mussel meat meant for further processing or consumption, they should be depurated for 24h in clean water from their respective habitat followed by chlorination of the system to 5 ppm level and maintaining like that for 2 more h.

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