Combined effect of lime (*Citrus aurantitolia*) and drying on reducing bacteria of public health significance in Edible Oyster (*Crassostrea madrasensis*)

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Abstract Combined effect of lime and drying on bacteria of public health significance in Edible Oyster (*Crassostrea madrasensis*) from Munambam coastal belt (Kerala, India) were studied (without depuration). Samples were examined for Total Plate Count (TPC), *Staphylococcus aureus* (hygiene indicator), Total coliforms, Faecal coliforms, *Escherichia coli*, (faecal indicator) Faecal Streptococci (faecal indicator), *Salmonella*, *Vibrio cholera* and *Listeria monocytogenes*. The fresh oyster meat though did not confirm to the specifications laid by National shellfish sanitation programme (NSSP), after treatment with lime with and without drying found to show significant reduction in counts and meet the required standards. Prevalence of faecal indicators in the fresh sample indicated faecal pollution in the area. The isolation of potentially pathogenic bacteria, *V. parahaemolyticus* in fresh sample indicates high risk of people consuming and handling oysters in raw and semi processed form and also it may lead to cross contamination. The present study indicates that treatment with natural organic product like lime and simple preservation technique, drying can effectively reduce the bacterial load. The study also revealed that TPC of water and soil collected from the site from where oysters were collected was less than from the meat.

Keywords Edible oyster · Lime · Drying · Microbiological quality

Introduction

Sea foods are of immense commercial importance all over the world. Indian marine products are preferred by most sophisticated market of developed countries. Apart from prawns and fishes that are exported in large quantities, bivalve molluscs are also gaining demand in overseas countries. As the commercial potential of the molluscs are increasing they are now being farmed to meet the needs. Increasing need for the oyster meat demands for better preservation methods. Oyster is a good source of proteins, vitamins and trace elements (Tack et al. 1992; Ruwa and Polk 1994). The oysters are having high demand for the preparation of oyster extract powder. Taurine is one of the oyster's key nutrients and is effective for cholesterol reduction, prevention of anemia, liver counter poison, diabetes prevention and sight recovery.

The micro flora present in oysters depends on the environment, feeding habits and mode of harvesting and handling. One of the main concerns for oyster industry is the health risk associated with the consumption of oysters. Oysters are filter feeders that tend to concentrate microbes present in surrounding waters some of which can cause severe illness in human beings (Depaola et al. 1997; Cook et al. 2002 and FDACFSAN 2005). Since oysters are eaten alive, raw, or poorly cooked, they can act as vectors for pathogenic microbes. Currently, there is high consumer demand for oysters that are safe but still retain their original flavor, nutrient content, texture, and appearance. In addition, they are expected to be additive-free as well as presenting a longer shelf life.

Salt curing and drying are traditional methods of utilization of seafoods and play an important role in the socio-economic status of fishers. Utilisation of seafoods in

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cured/dried form ranks next only to fresh seafoods (Wood 1981). The drying of different varieties of fishes is one of the world's oldest known preservation methods, and dried fish has a storage life of several years. Various food preservation techniques have been utilized to improve the microbial safety and extend the shelf life of seafoods in general, including freezing, chemical preservation, salting, and smoking (Jay 1998; Nickelson et al. 2001; Wood 1981). Smoking usually extends shelf life of fish due to the reduced moisture content and the effects of imparted phenolic compounds (Efiuvwevwere and Ajiboye 1996). Another shelf life promoting strategy involves salting with NaCl or with chemical preservatives. The drying the fish or any kind of processing fishes is putting down enzymatic or microbiological activity either in the presence or absence of salt. Seafood, particularly bivalve shellfish, features highly in statistics of food-borne disease (Potasman et al. 2002). The predominance of oysters and clams as vehicles of seafood-borne disease is probably due to the fact that these shellfish are filter feeders and selectively accumulate bacteria and viruses from the surrounding waters (Richards 1988), and they are normally eaten whole in raw condition or following a very mild heat treatment (Gram and Huss 2000; Lees 2000). The consumption of raw or lightly cooked shellfish not only has implications for disease transmission but also limits processing methods suitable for these products. The largest shellfish associated epidemic occurred in Shanghai, China, in 1988 was due to the consumption of shellfish harvested from a harbor receiving untreated domestic sewage (APHA 2001). Traditional preservation methods, such as heat, have detrimental effects on the taste and appearance of shellfish, and are therefore unacceptable to many consumers. Only limited information is available in the literature on the shelf life extension of *Crassostrea madrasensis*. The shelf life of oysters depends on many factors such as storage conditions, intrinsic factors of the animal and qualitative and quantitative microbial flora. Oysters are highly perishable and have short shelf life, which causes substantial practical problems for their distribution. Several investigators have reported significant reduction in *E.coli* levels during depuration (Reilly and Barle 1987). The objective of the present study is to know the combined effect of lime and drying on the natural bacterial fauna of public health significance.

Materials and methods

Collection of edible oysters

Edible Oysters (*Crassostrea madrasensis*) of commercial size, i.e. measuring 10–12 cm in shell length were collected

from the Munambam coastal area, of Ernakulam district in Kerala during the month of March–May, 2010 with the assistance of some fisherwomen labourers who are skilled in this work. Oysters were shucked by using a clean sanitized sickle and the meat was transferred to a sterile container. It was immediately washed in potable water and meat was transferred to a sterile stainless steel container. This container was kept inside a plastic box containing flake ice and taken to the laboratory without any delay (within 75 min).

Collection of water and soil samples

From the site from where harvest was made, water sample was collected in sterile glass bottles for analysis. Soil from the site was also collected aseptically in a sterile container.

Extraction of lime

Lemon was purchased from the market. The juice was extracted by cutting the lemon horizontally by using a sterile knife and squeezing it into a sterile beaker. It is then filtered using a filter paper.

Sample preparation and analysis

Soon after reaching the laboratory, the shucked meat of oysters were divided into three groups of 250 g each (F1, F2 & F3) and three groups of 2 Kg each (D1, D2 & D3). F2 and D2 were treated with 5% lime (v/w) and F3 and D3 were treated with 10% lime (v/w). F1 was taken as such for further analysis. F2 and F3 were taken for further analysis, 2 h after the application of lime. D1, D2 and D3 were dried aseptically under sun by keeping the sample in stainless trays with a net covering, so as to reduce the moisture content <10%. The coding pattern of sample is as follows. Fresh Edible oyster without any treatment was coded as F1, Fresh Edible oyster treated with 5% lime (v/w) as F2, Fresh Edible oyster treated with 10% lime (v/w) as F3, Dried Edible oyster as (D1), Edible oyster treated with 5% lime (v/w) and dried as D2 and Edible oyster treated with 10% lime (v/w) and dried was coded as D3. These samples were also subjected to microbiological analysis. Total Plate Count, Faecal streptococci and *S. aureus* were quantified by the plating method (FDA 1998). The Total coliforms, Faecal coliforms, *Escherichia coli* by the 3 tube MPN method (APHA 2001). Faecal Streptococci (faecal indicator), Qualitative analysis of *Vibrio cholera*, *Vibrio parahaemolyticus* and *Listeria monocytogenea* were done as per Sanjeev (2007), and *Salmonella* as per Joseph (2007).

Statistical analysis

All analysis were carried out in triplicate. The data on Total Plate Count, Total coliform, Faecal coliform, *E. coli*, Faecal streptococci, *Staphylococcus aureus* were subjected to statistical analysis using SAS package (Version 9.2). The ANOVA was done to compare the effect of treatments on Total plate count and Faecal streptococci. Post hoc tests were performed using Turkey's procedure to compare significant difference among treatments at 5% level.

Results and discussion

Table 1 depicts the mean logarithmic value of Total Plate count (TPC) of oyster with and without treatment. The oysters in fresh condition were found to harbor TPC at higher levels than recommended (APHA 2001). TPC for edible oyster at the wholesale level has been set as $<5.0 \times 10^5$ /g. After treatment with lime the TPC level has come down to acceptable level. The initial higher load in the present study was due to the higher load of contamination from the site from where it was harvested. The oysters were not collected from farm but from open coastal area. Drying with or without lime has considerably reduced the bacterial load.

Total plate Count in fresh edible oyster was found to be 5.7×10^5 . As per National shellfish sanitation programme (NSSP) standard for the acceptance of shellfish for the market is fixed as 500,000/g. Lakshmanan et al. (1984) and Nambiar and Iyer (1990) reported that TPC varied from 10^3 cfu/g to 10^7 cfu/g in fishes sold in retail markets of cochin. On application of lime one log cycle reduction was noticed in both fresh and dried oysters. Compared to fresh oyster there was 2 log cycle reductions was noticed in dried sample. Hence it can be assured that drying can reduce the bacterial load, and

hence shelf life can be extended. From the table it is also clear that application of lime will reduce bacterial load to considerable level irrespective of whether it is fresh or in dried condition.

Regarding the keeping quality of edible oysters, combined effect of lime and drying is much more than without any preservation. The lime contains citric acid which is having antioxidant property. So it will enhance the shelf life of the product. It can also prevent or retard the development of off odours and flavours as citric acid can chelate trace metals which will catalyse these reactions.

The ANOVA for TPC data reveals that there is significant difference between the treatments, viz, F1, F2, F3, D1, D2 and D3. When post-hoc tests were performed these treatments were grouped into 4 groups (a, b, c and d). Group a has only one treatment F1, Group b has F2 and F3. Group c has D1 and Group d has D2 and D3. The analysis revealed that the treatments F1 is significantly different from other treatments. It was seen that F2 and F3 has same effect on TPC, but the TPC of these treatments are significantly different from the rest of the treatments.

This higher total plate count in fresh samples will indicate poor hygienic condition of the habitat. In order to have a clear indication of the environment from where it was caught, water quality was also assessed. The result of the analysis is presented in the Table 2. The quality of shellfish harvest waters are mainly the responsibility of each states Shellfish Control Authorities (FDA 1998).

To develop a growing area standard more indicative of faecal pollution, the proposal of FDA was that the median faecal coliform MPN value for a sampling station shall not exceed 14 per 100 ml of sample and not more than 10% of the samples shall exceed 43 for a 5 tube three dilution test, or 49 for a 3 tube, 3 dilution test. As per National shellfish sanitation programme (NSSP) standard fresh or frozen shellfish are considered to be satisfactory if faecal coliform MPN does not exceed 230/100 g

Table 1 Microbial quality of fresh and treated oyster samples

Sample code	TPC $\text{Log}_{10}\text{cfu/g} \pm \text{SE}^*$	MPN/g (range)			Faecal streptococci count/gm ($\pm \text{SE}$)	<i>Staphylococcus aureus</i> /gm ($\pm \text{SE}$)
		Total coliforms	Faecal coliforms	<i>E. coli</i>		
F1	5.74 ± 0.065^a	460–1100	240–460	4	34 ± 1.15^a	$1.31 \times 10^2 \pm 0.092^a$
F2	4.64 ± 0.098^b	150–240	150–240	<3	30 ± 1.05^a	ND
F3	4.46 ± 0.058^b	93–150	75	<3	29 ± 1.73^a	ND
D1	3.31 ± 0.025^c	150	93–75	<3	20 ± 1.73^b	ND
D2	2.28 ± 0.022^d	75–93	39–43	<3	17 ± 0.58^b	ND
D3	2.24 ± 0.017^d	39–43	7–15	<3	16 ± 1.15^b	ND

*SE-Standard error. Different letters indicate different groups

ND-not detected

Table 2 Microbiological parameters of water and soil collected from the harvesting site

Particulars	Water	Soil
TPC ($\pm$ SE)	4×10^5 /ml (± 0.35)	3.7×10^5 /g (± 0.17)
<i>Staphylococcus aureus</i> ,	ND	ND
Total coliforms (range)	460–210 MPN/100 ml	210–150 MPN/g
Faecal coliform (range)	210–150 MPN/100 ml	210–150 MPN/g
<i>Escherichia coli</i> (range)	23–15 MPN/100ML	23–15 MPN/g
Faecal <i>Streptococci</i> ($\pm$ SE)	2/ml(± 0.57)	4/g(± 0.57)
<i>Vibrio cholera</i>	ND	+
<i>Vibrio parahaemolyticus</i>	+	+
<i>Listeria monocytogenes</i>	ND	ND

*SE-Standard error. Different letters indicate different groups

ND-not detected

The range of change in counts of fecal indicator organisms in edible oyster with and without treatments is given in Table 1. From the table it can be seen that total coliform, faecal coliform and *E. coli* counts in fresh edible oyster is above the level recommended. But the application of lime has considerably brought down the coliform level to the acceptable range. Drying was also found to be effective in bringing down the coliform counts.

Total coliform and faecal coliforms were found to be more in fresh oyster samples than the water and soil collected from its habitat. This may be because of the bio accumulative behavior of the oyster. The safety of shellfish is predicted on the densities of indicator organisms, primarily the coliform group, present in the growing waters.

Faecal streptococci was present in all samples. The maximum count was observed in fresh oyster sample F1 and showed some reduction with application of fresh lime and drying (Table 1). Quantity of Faecal streptococci was very less in water and soil. But there was bioaccumulation of Faecal streptococci in oysters. ANOVA for Faecal streptococcus shows that the treatments applied are showing significant reduction in counts at 1% level. The post-hoc tests group these treatments into groups a and b.

Staphylococcus aureus could not detected from the water and soil of the habitat of oyster. In fresh oyster there was the presence of *Staphylococcus aureus* at a concentration of 1.31×10^2 /gm. This indicates that *Staphylococcus aureus* was transferred to the sample during handling. After the application of lime no *Staphylococcus aureus* was detected in either fresh or dried samples. Hence it can be concluded that *S.aureus* is sensitive to citric acid and water activity

The result of the qualitative analysis to detect the presence/absence of *V parahaemolyticus*, *Vibrio cholera*, *Salmonella* and *Listeria monocytogenes* in oyster samples Fresh (F1, F2 & F3) & Dried (D1, D2 & D3) has revealed that *V parahaemolyticus* were present only in F1 sample and were absent in all other samples. The study

also revealed that both drying as well as the application of lime could neither detect *Vibrio spp*, *Salmonella* nor *L. monocytogenes*. According to Hackney and Dicharry (1988), out of 11 species pathogenic to human beings only 6 namely, *Vibrio cholerae*, *V parahaemolyticus*, *V vulnificus*, *V mimicus*, *V hollisae* and perhaps *V furnissi* are associated with food borne illness. Karunasagar et al. (1987) identified and isolated pathogenic *V vulnificus* from molluscan shellfish.

The FDA has a zero tolerance for *L. monocytogenes*, *Salmonella* spp and *V.cholerae* in ready to eat products and raw shellfish. The action level for *V.parahaemolyticus* is 10,000/g

Conclusion

Oysters are highly valued and most abundant harvested shellfish in the world. Many commercially important shellfish are filter feeders and hence concentrate microbes from the surrounding waters. Consumers require safe, minimally processed, additive-free food with an extended shelf-life. The high incidence of faecal indicator organisms and presence of *Vibrio parahaemolyticus* in fresh sample should be regarded as public health concern as there is chance for cross contamination and multiplication of these bacteria during post-harvest handling at ambient temperature. Application of lime with or without drying was found to reduce the bacterial load to considerable extent. After harvest if samples were subjected to depuration it will decrease the initial bacterial load which in turn will enhance food safety and extended shelf life. 5% (v/w) of lime is sufficient for waters with moderate pollution. Good handling practices including depuration should be followed immediately after harvesting to reduce the bacterial load so as to avoid public health risk. The study also points out the risk in consuming oysters without any treatment in raw or partially cooked form as it may harbor bacteria of public health significance.

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