# Investigating the effect of storage temperature and hot-water treatment on the microbial dynamics in edible oyster (*Saccostrea cucullata*)

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**Abstract:** Oysters are an important sea food all over the world apart from shrimp and crabs. They are usually sold as a live product and can be stored for several weeks before consumption. Temperature abuse during oyster post-harvest handling may allow multiplication of natural spoilage microflora as well as pathogens, which is a potential threat to consumers and/or compromising product quality. In this study, the effect of storage temperatures (25°C, 4°C, 0°C and –10°C) and boiling on the microbial quality of shell stock and shucked meats of oysters (*Saccostrea cucullata*) were examined. The load of total bacteria, fungi, coliform, fecal coliform, *Salmonella* sp. and *Streptococcus* sp. were comparatively higher in shucked meat than in the shell-stock at all the storage temperature (except *Vibrio* sp.) and number of the spoilage bacteria is directly proportional to the storage time but decreased with lowering of the temperature. Microbial count also observed at various interval of treatment with boiling water (100°C). During boiling water treatment of the shell-stock and shucked meats, the decimal reduction time, D-value ($D_{100°C}$) for total microbes was found to be 2.7 min and 2.4 min respectively. The results

indicated that oysters must be stored for a limited time as shell-stock and during cooking it should be prepared as shucked meats, and washing with boiled water is a simple method for making it microbes free.

**Keywords:** oysters; *S. cucullata*; shell-stock; shucked meats; D-value; post-harvest technology.

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## 1 Introduction

Oysters (*Saccostrea cucullata*) are bivalve (two-shelled), soft-bodied mollusc, mostly marine or estuarine origin. They can grow in all tropical seas, mainly between tidal or in shallow water levels. Oyster is considered to be a valuable food item as they constitute a rich source of essential macro- and micronutrients for providing a balance diet. The chemical composition of oyster flesh varies over a wide range depending upon the species, the place of origin, and the season. The whole body of oyster is consumed in either cooked (baked, boiled, steamed and fried) or raw. 100 g of uncooked serving contain 7.9 g of protein, 3.5 g of carbohydrates, 2 g of saturated fat, 0.057 g of

cholesterol, and 75 calories. In addition, this serving size supplies 65%. 35%, 100% of the US recommended daily allowance (USRDA) for niacin, iron, vitamin $B_{12}$, and zinc, respectively (Nagabhushanam and Bidarkar, 1978).

The edible oysters are very popular as raw and processed food in the South Indian states, particularly in Goa, in the South East Asian countries, Europe, Australia, USA, etc. Technology of the culturing requires a congenial aquatic environment in terms of salinity, pH, temperature, and microbial load (Bhattacharyya et al., 2010). Naturally, oyster accumulates microorganisms during the process of filter feeding. These shellfish are prone to harsh environmental contamination by fecal pathogens like *Salmonella* sp. *Shigella* sp. and *Escherichia coli* (Musa et al., 2008). Illnesses due to food-borne contamination frequently occur, but rarely documented. *E. coli* and *Salmonella* sp. could extensively spread inside the body of human beings consuming oyster and lead to serious infection to the human and death (Forsythe, 2002). *E. coli* are able to cause erythema nodsum, haemolytic uremic syndrome, and sero-negative arthopathy in humans. Salmonellosis potentially causes aortitis, colitis, enhocarditis, orchitis, meningitis, myocarditis, osteomyelitis, pancreatitis, reiter syndrome, rheumatoid syndrome, septicemia, splenic abscess and thyroiditis (Forsythe, 2002). On the other hand, *Vibrio* sp. may be transmitted to humans by the ingestion of raw seafood. Oysters from these waters are often incriminated in human diseases since the oysters are commonly consumed as raw (Haldy, 1997). Therefore, suitable processing and preservation methods are required to prevent the pathogenic microbes particularly in post-harvesting period of oyster.

In many countries, cold storage temperature is generally considered as useful preservation methods before sell and consumption (Seaman, 1991; Aaraas et al., 2004). As example, Australian Shellfish Quality Assurance Programme (ASQAP) recommended that oyster must be stored at ≤10°C for 24 hrs before consumption (Fernandez-piquer et al., 2012). However, consistence refrigeration is difficult to achieve along the entire oyster supply chain, particularly difficult in the developing countries (Madigan, 2008; Depaola et al., 2010). Under both refrigeration and temperature abuse conditions, certain bacterial species can remain in the tissues of oysters. Therefore, it is important to document how storage temperature affect bacterial community especially, the dominant species those may influence the quality of edible oyster. The aim of the current study was to evaluate the effect of storage temperature and hot water treatment on microbial load in the edible oyster.

## 2 Materials and methods

### *2.1 Sampling protocol*

Mature oysters were collected from Frazergaunge, [21°36’55.72” (N), 88°12’33.15” (E)] which is located in the western part of Indian Sundarbans. Each oyster was scrubbed, rinsed with distilled water (four times) and stored in a container, preserved in crystal ice and brought to the laboratory immediately.

### *2.2 Processing*

The meat was aseptically extracted from healthy oysters (5–8 cm) using a sterile knife and immediately used for the microbial analysis of oyster flesh (APHA, 1970). The whole shell-stock as well as shucked meats were stored at four temperatures (viz., at –10°C, 0°C, 4°C and 25°C) for up to 21 days. On a separate study, shell-stock and shucked meat were submerged in boiled water (~100°C) for 0, 5, 10 and 20 min (Andrews et al., 2000). D-value (time to inactivate 90% of the population) were calculated from the straight portion of the survival curves by plotting log of survival counts vs. their corresponding heating times (Whiting, 1993).

### *2.3 Microbial analysis*

In this experiment, for total aerobic heterotrophic bacteria, fungus (moulds), coliforms, fecal coliforms (FCs), *Streptococcus* sp. *Vibrio* sp. and *Salmonella* sp. were quantified before and after storage at different temperatures.

For bacterial analysis, 10 g oyster tissue (meat) was blended with 90 ml of sterile 0.5% (w/v) peptone buffer (pH –7.0) and different dilutions ($10^{-1}$ to $10^{-2}$) were prepared. For quantification of coliform and FC, the standard MPN (Most Probable Number) procedure was adopted using LTB (lowryl tryptose broth) and EC (*Escherichia coli*) culture broth, respectively (Cleseri et al., 1998). Briefly, 10 ml of $10^{-1}$ tissuedilution was added in test tube containing 10 ml volume of double strength media and 1ml of each homogenate ($10^{-1}$ and $10^{-2}$ dilution) was added separately in test tube containing 10 ml volume of single strength LTB broth with inverted Durham's tubes. The total sets were incubated at 35 ± 0.5°C for 24 hrs and examined for the presence of growth accompanied by gas ($CO_2$) production that will be visible as a bubble in the inverted tubes. Cultures that are not capable of fermenting a carbohydrate substrate, there will not be a concomitant evolution of gas. This is a negative reaction. The density of bacteria was calculated on the basis of positive and negative combination of the tubes. The MPN was calculated and results were expressed as 'presumptive coliform MPN/100 g'. Then the positive cultures were inoculated into brilliant green lactose bile broth and the tubes were incubated at 35 ± 0.5°C for 24 hrs and examined for growth with gas production. The MPN of total coliform (TC) was calculated and results were expressed as 'confirmed coliform MPN/100 g'. To quantify the FC, inoculum from 24 hrs positive presumptive tubes were aseptically transferred to tubes of EC medium. These tubes were incubated at 44.0 ± 0.5°C for 24 hrs and examined for the presence of growth with gas production. Results were expressed as 'FC MPN/100 g'. The quantification of total bacteria (TBC), total fungi, fecal Streptococci, *Vibrio* sp. and *Salmonella* sp in all oysters samples was done by standard plate counting method using tryptose glucose beef extract agar (TGBEA), potato-dextrose agar, Azide-dextrose agar, thiosulfate-citrate-bile-salt agar and xylose lactose dextrose agar media, respectively (Kaper et al., 1977, 1979).

### *2.4 Statistical analysis*

The statistical significance of the results was analysed through Student's t-test using the origin 6.0 software.

**Table 1** Microbial count of shell-stock oyster before and after storage for 21 days

| *Microbial parameters* | *Control (37 ℃)* | *25 ℃* | | | *4℃* | | | *0℃* | | | *–10℃* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *7 days* | *14 days* | *21 days* | *7 days* | *14 days* | *21 days* | *7 days* | *14 days* | *21 days* | *7 days* | *14 days* | *21 days* |
| TBC (Log cfu/g) | $6.72^{j}$ | $5.30^{a}$ | Spoil | Spoil | $5.39^{e}$ | $5.38^{b}$ | $5.30^{a}$ | $5.66^{h}$ | $5.52^{e}$ | $5.43^{d}$ | $5.71^{i}$ | $5.62^{g}$ | $5.58^{f}$ |
| *Streptococcus* sp. (Log cfu/g) | $2.30^{b}$ | $3.0^{a}$ | Spoil | Spoil | $2.0^{d}$ | $2.30^{e}$ | $2.70^{b}$ | $1.78^{e}$ | $2.0^{d}$ | $2.30^{e}$ | $2.0^{d}$ | $2.0^{d}$ | $2.0^{d}$ |
| *Salmonella* sp. (Log cfu/g) | $3.56^{b}$ | $3.76^{a}$ | Spoil | Spoil | $2.30^{f}$ | $2.30^{f}$ | $2.85^{c}$ | $2.57^{f}$ | $2.60^{e}$ | $2.60^{e}$ | $2.60^{e}$ | $2.60^{e}$ | $2.70^{d}$ |
| *Vibrio* sp. (Log cfu/g) | $3.64^{b}$ | $3.73^{a}$ | Spoil | Spoil | $3.34^{e}$ | $3.26^{d}$ | $3.18^{e}$ | $3.0^{f}$ | $2.0^{h}$ | $2.0^{h}$ | $2.85^{g}$ | $2.0^{h}$ | $2.0^{h}$ |
| TC (Log MPN/g) | $3.15^{g}$ | $4.23^{d}$ | Spoil | Spoil | $4.24^{e}$ | $4.73^{b}$ | $4.81^{a}$ | $3.04^{h}$ | $3.15^{g}$ | $3.54^{e}$ | $2.88^{i}$ | $3.04^{h}$ | $3.38^{f}$ |
| FC (Log MPN/g) | $2.64^{i}$ | $4.15^{c}$ | Spoil | Spoil | $3.88^{d}$ | $4.54^{b}$ | $4.65^{a}$ | $2.49^{j}$ | $2.98^{g}$ | $3.45^{e}$ | $2.40^{k}$ | $2.70^{h}$ | $3.34^{f}$ |
| Total fungi (Log cfu/g) | $6.15^{h}$ | $6.45^{a}$ | Spoil | Spoil | $6.15^{h}$ | $6.20^{f}$ | $6.28^{e}$ | $6.15^{h}$ | $6.36^{d}$ | $6.40^{e}$ | $6.18^{g}$ | $6.36^{d}$ | $6.41^{b}$ |

Note: Values followed by different superscript letters differ significantly at $p \leq 0.05$.

**Table 2** Microbial count of shucked meat oyster before and after storage for 21 days

| *Microbial parameters* | *Control (37 ℃)* | *25 ℃* | | | *4 ℃* | | | *0 ℃* | | | *–10 ℃* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *7 days* | *14 days* | *21 days* | *7 days* | *14 days* | *21 days* | *7 days* | *14 days* | *21 days* | *7 days* | *14 days* | *21 days* |
| TBC (Log cfu/g) | $6.72^{k}$ | $5.27^{a}$ | Spoil | Spoil | $5.38^{e}$ | $5.35^{e}$ | $5.28^{b}$ | $5.45^{i}$ | $5.44^{h}$ | $5.43^{g}$ | $5.45^{j}$ | $5.36^{d}$ | $5.42^{f}$ |
| *Streptococcus* sp. (Log cfu/g) | $2.0^{h}$ | $3.18^{a}$ | Spoil | Spoil | $2.11^{f}$ | $2.54^{e}$ | $2.91^{b}$ | $1.95^{i}$ | $2.04^{g}$ | $2.20^{d}$ | $1.95^{i}$ | $2.04^{g}$ | $2.15^{e}$ |
| *Salmonella* sp. (Log cfu/g) | $3.54^{b}$ | $3.76^{a}$ | Spoil | Spoil | $2.48^{i}$ | $2.48^{i}$ | $2.60^{h}$ | $2.67^{f}$ | $2.48^{e}$ | $2.77^{d}$ | $2.67^{f}$ | $2.66^{g}$ | $2.78^{e}$ |
| *Vibrio* sp. (Log cfu/g) | $3.62^{a}$ | $3.53^{b}$ | Spoil | Spoil | $3.11^{d}$ | $3.18^{e}$ | $3.04^{e}$ | $2.78^{f}$ | $2.0^{h}$ | $2.0^{h}$ | $2.60^{g}$ | $2.0^{h}$ | $2.0^{h}$ |
| TC (Log MPN/g) | $3.08^{j}$ | $4.32^{a}$ | Spoil | Spoil | $4.28^{a}$ | $4.15^{e}$ | $4.96^{d}$ | $3.09^{j}$ | $3.23^{h}$ | $3.73^{e}$ | $2.93^{k}$ | $3.11^{g}$ | $3.43^{f}$ |
| FC (Log MPN/g) | $2.60^{i}$ | $2.32^{d}$ | Spoil | Spoil | $3.23^{e}$ | $3.32^{d}$ | $3.73^{a}$ | $2.78^{g}$ | $3.08^{f}$ | $3.54^{b}$ | $2.61^{h}$ | $2.78^{g}$ | $3.38^{c}$ |
| Total fungi (Log cfu/g) | $6.11^{j}$ | $6.45^{c}$ | Spoil | Spoil | $6.18^{g}$ | $6.30^{g}$ | $6.34^{f}$ | $6.15^{i}$ | $6.36^{e}$ | $6.40^{d}$ | $6.15^{i}$ | $6.48^{b}$ | $6.50^{a}$ |

Note: Values followed by different superscript letters differ significantly at $p \leq 0.05$.

## 3 Results and discussion

### *3.1 Effect of storage temperature on the microbial community in shell-stock and shucked meats*

Mean levels of total bacteria was significantly ($p < 0.05$) increased in both shell-stock and shucked meats after seven days of storing at all levels of exposed temperature, i.e., at 25°C, 4°C, 0°C and –10°C (Tables 1 and 2). Whereas, fungal counts of shell-stock and shucked meat were elevated significantly ($p < 0.05$) after 14 days at all storing temperatures except at 25°C. The mean level of coliforms and FCs were also significantly ($p < 0.05$) increased with the duration of storage in all storing temperature, as did the levels of *Salmonella* sp. The quantity of *Vibrio* sp. was decreased in both the samples in a time dependent manner in all the storing temperature except at 25°C. The occurrence or growth of all the studied microbial groups was much higher in sucked meats than its shell-stock state (Tables 1 and 2) because shell-stocks oyster secrets certain antimicrobial substances that may act to prevent bacterial and fungal growth in oyster meats and liquors. Similarly, comparative studies of the storage treatments revealed that shucked meats developed higher microbial loads than shell-stocks because during processing, removes certain antimicrobial factors (Hood et al., 1983). In contrast, *Vibrio* sp. concentration was significantly higher in oysters stored as shell-stocks due to higher volume of stored body fluid in different sacks within the shell. The concentrations of all studied microbial groups were decreased with lowering the storage temperature because low temperature acts as a microbiostasis. The cold storage has proved to be effective in reducing the number of viable bacterial cells and about 80% of the living microbial cells present in shell-stock oyster were killed or inactivated when the temperature was lowered to about 0°C during the period of ice storage (Andrews et al., 2000). In the present work, it was also observed that the mean levels of all bacterial groups were increased with increase of storage time. This may be due to the fact that a new condition created in the oyster during storage including anaerobiosis, accumulation of waste and lowering of body pH. Apart from these, different bacteria may also respond more efficiently to the different storage temperature with more cold-adapted species strongly promoted under refrigeration condition and leads to increase the microbial growth with storage time (Fernandaz-Piquer, 2012). All these factors may contribute separately or concomitantly the growth of indigenous microflora and promoted the spoilage of oyster tissue.

The growth of studied indicator microbial groups was somehow retarded with lowering of storage temperature. But outnumber growth after 21 days of storage at all temperatures are not suitable for its consumption, therefore, a suitable cost effective processing technique is very essential to its preservation.

### *3.2 Effect of boiling on the survival of microbial community in the shell-stock and shucked meats*

The heat treatment was very effective in the reduction of microbial load in the oysters. The present study revealed that the thermal reduction time (D value) for total bacterial count (TBC) in shell-stock and shucked meat was 2.7 min and 2.4 min respectively (Figure 1). An exposure time of 5 min was effective for shell-stock and shucked meat in reducing the number of *Vibrio* sp. by 95% and this organism was totally eradicated after

10 min of boiling. The TC and FC in shucked meat and shell-stock oysters were reduced by 100% after 10 min and 20 min boiling, respectively (Table 3). This may be due to the fact that the heat shock at high temperature for quick time methods can facilitate denaturation of essential proteins and reduce the microbial load. Similarly, it also observed that the numbers of aerobic spoilage bacteria of heat treated shell-stock oyster were reduced by 99.99% (Andrwes et al., 2000; Hesselman et al., 1999). So, this simple and inexpensive method could be applied for preservation of this valuable seafood before storage or packaging. This will facilitate the marketing of oyster in hygienic condition.

**Figure 1** Thermal reduction time (D-value) for shell-stock oyster and shucked oyster (see online version for colours)

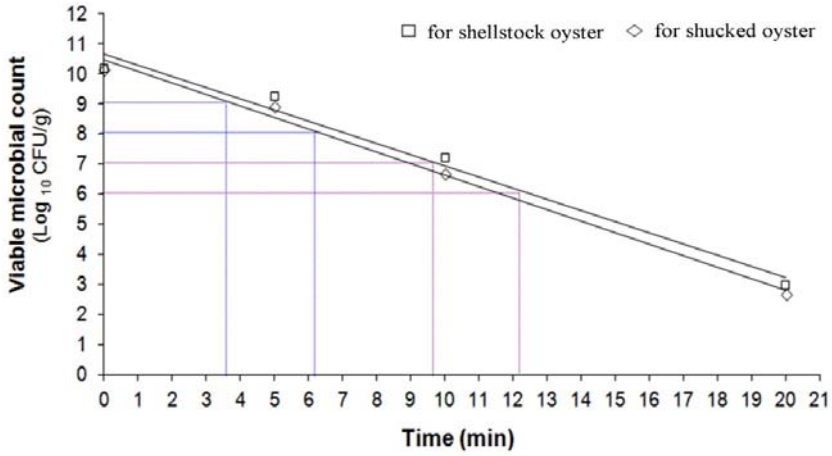


**Table 3** Effect of 100°C heat treatment on the survival of microbial community in the shucked meat and whole shell oyster

| *Microbial parameters* | *Boiling time (min)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Shucked meat oyster* | | | | *Whole shell oyster* | | | |
| | *0* | *5* | *10* | *20* | *0* | *5* | *10* | *20* |
| TBC (Log cfu/g) | $10.16^{c}$ | $8.96^{e}$ | $6.69^{g}$ | $2.68^{a}$ | $10.17^{b}$ | $9.82^{d}$ | $8.93^{f}$ | $2.98^{h}$ |
| *Vibrio* sp. (Log cfu/g) | $2.77^{b}$ | $1.52^{d}$ | 0 | 0 | $2.78^{a}$ | $2.0^{c}$ | 0 | 0 |
| *Salmonella* sp. (Log cfu/g) | $1.00^{a}$ | 0 | 0 | 0 | $1.0^{a}$ | 0 | 0 | 0 |
| Total coliform (Log MPN/100 g) | $3.15^{b}$ | $1.60^{e}$ | 0 | 0 | $3.18^{a}$ | $2.80^{c}$ | $2.32^{d}$ | 0 |
| Fecal coliform (Log MPN/100 g) | $2.48^{a}$ | $1.30^{e}$ | 0 | 0 | $2.49^{b}$ | $2.30^{c}$ | $2.04^{d}$ | 0 |

Note: Values followed by different superscript letters differ significantly at $p \leq 0.05$.

## 4 Conclusions

The bacterial concentration in live rocky oyster (*S. cucullata*) was unexpectedly high and significantly ($p < 0.05$) shifted after post-harvest storage depending on storage temperature and duration of storing. Therefore, oysters might be stored for a limited period as shell-stock and during cooking it should be prepared as shucked meats. The effect of hot water treatment (100°C for 20 min) was very effective in reducing the microbial load to non-detectable levels in shell-stock and shucked meat of the edible oyster (*S. cucullata*). Quick heat shock or washing with boiling water is a simple and inexpensive method for killing of normal microflora and pathogenic contaminant in this useful sea food. This can be applied to large scale processing of oyster before marketing.

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