Microbiological, Chemical, and Sensory Assessment of Pacific Oysters (*Crassostrea gigas*) Stored at Different Temperatures

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Abstract

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The changes were studied in microbiological, chemical, and sensory properties of Pacific oysters stored at 10°C, 5°C, and 0°C. *Pseudomonas* (22%) and *Vibrionaceae* (20%) species were dominant in raw oysters. The dominant bacteria found in the spoiled samples were *Pseudomonas* regardless of the storage temperature. During storage, rapid increases in aerobic plate count (APC) values of the samples stored at 10°C and 5°C were observed, while no obvious lag phases were detected. With the samples stored at 0°C, a decrease in APC value during the first 4 days and a lag phase of about 6 days were observed. The APC values of the samples stored at 10°C, 5°C, and 0°C reached the level of 10^7 CFU/g on day 6, 10, and 18, respectively. All the tested samples stored at different temperatures revealed a slight decrease in pH and a significant increase of total volatile basic nitrogen (TVB-N) during storage. The average TVB-N concentration of about 22.0 mg N/100 g was observed at the end of the shelf-life as determined by APC. Combined with the sensory assessments, the shelf-life of 6–7, 10–11, and 17–18 days for oysters stored at 10°C, 5°C, and 0°C, respectively, was determined.

Keywords: Pacific oyster; microbial flora; quality assessment; shelf-life

Oysters are the most abundant harvested shellfish in the world and are also highly valued. As fresh seafood, oysters are more perishable than other high-protein foods and have a short shelf-life, which causes substantial practical problems for their distribution. In the seafood products, the changes in flavour, odour, texture, and colour reflect the level of freshness or decomposition, caused primarily by microbial activity (Gram & Huss 1996).

The microflora of molluscan shellfish is more variable than that of fish. The microflora of shellfish is closely related to the aquatic habitat and varies with factors such as salinity, environmental conditions, bacterial load in the water, water temperature, diet, methods of catch, and chilling conditions. The dominant groups of bacteria found on fish and shellfish stored under refrigeration are *Pseudomonas*, *Shewanella putrefaciens*, and *Moraxella*/*Acinetobacter* and, to a lesser extent,

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Aeromonas and *Psychrobacter* (Ashie *et al*. 1996; Gram & Huss 2000).

The shelf-life of fresh seafoods depends on many factors, such as storage conditions (for example temperature and atmosphere), the intrinsic factors of the animals (species, age and size, fat composition, feeding and physiological status), and the qualitative and quantitative compositions of the initial microbial flora (Shewan 1971, 1977; Ward & Baj 1988). Microbiological, biochemical, and sensory changes are associated with the deterioration of seafoods quality during handling and storage. Although a variety of biochemical, physical (Gill 1992, 1997), and microbiological methods (Gram & Huss 1996) have been used to assess freshness, the sensory evaluation is still the most satisfactory method to achieve such a goal (Reineccius 1990). Given that specific spoilage-causing microorganisms cannot be detected by organoleptic or chemical testings, it is useful to conduct microbiological, chemical, and organoleptic analyses when assessing the quality of seafood (Ryder *et al*. 1993).

Only limited information is available in the literature on the shelf-life and microbial flora of Pacific oyster (*Crassostrea gigas*) stored at different temperatures. Microbial flora analysis could gain better perception of the spoilage mechanism, which could lead to methods for prolonging the shelf-life and encourage the marketability of fresh oysters. The present paper reports on the microbial flora of raw and spoiled oysters and the shelf-life of samples stored at different temperatures by evaluating the sensory, chemical, and microbiological changes.

MATERIAL AND METHODS

Oyster. Pacific oysters (*Crassostrea gigas*) of commercial size, i.e., measuring 12–14 cm in the shell length, were collected from a culture farm in the Yellow Sea (China) and transferred in ice to the Seafood Health and Safety Laboratory (Ocean University of China) immediately.

Sample preparation. All oysters were purified in cold water (5°C) with 3.5% marine salt for 30 minutes. After cleaning and draining excess solution, oysters were shucked and wrapped individually in plastic bags. All samples were divided into three lots and stored at 0°C, 5°C, and 10°C, respectively. The samples were examined at 24-hours intervals for sensory assessment and at 48-hours intervals for chemical and microbiological analyses. For each of the experimental conditions, six oysters were randomly chosen from each sampling.

Microbiological analysis – *Determination of aerobic plate count.* The oyster samples were taken aseptically into a vertical laminar-flow cabinet and 25 g were transferred to a stomacher bag. 225 ml of 0.1% peptone water with salt (NaCl, 0.85%, w/v) were added and the mixture was homogenised with a stomacher for 60 seconds. Ten-fold serial dilutions were made and the samples (0.1 ml) were then spread on Marine Agar plates (Ortigosa *et al.* 1994). Aerobic plate count (APC) was determined by counting the number of the colony-forming units after incubation at 25°C for 48 hours.

Isolation and identification of oyster bacteria. After counting the number of the colony-forming units, the colony morphology was observed. 30–50 colonies were randomly picked (to pick many different phenotypes). All the colonies were restreaked on Marine Agar plates three times to obtain pure cultures.

The isolated microorganisms were identified using the scheme given by Bage-Ravn *et al*. (2003), and the data by Holt *et al*. (1993) were consulted. The isolated strains were grouped to the genus level. The following tests were conducted (Gerhardt *et al*. 1981): Gram reaction by the Hucker method; cell morphology by phase-contrast microscopy; flagella type by the Nishizawa and Sugawara method; catalase formation by dropping 3% H_2O_2 solution directly onto each colony; oxidase test by the Kovac method; and O/F-test by the Hugh and Leifson method.

Chemical analysis. The pH of oysters was measured using a pH meter (PHS-3C, Shanghai, China) after blending 10 g of homogenised meat with 100 ml of distilled water. Total volatile bases nitrogen (TVB-N) was measured by micro-diffusion analysis using a Conway's unit and extraction with 5% trichloroacetic acid.

Sensory assessment. The sensory properties of oysters were estimted by a panel of 6 trained panelists from the staff of the Department of Food Science & Engineering, Ocean University of China, according to the freshness grade guide for oyster (He & Morrisey 1999) after an appropriate modification (Table 1).

The panelists were asked to evaluate all four parameters on a scale from 0 (extremely undesirable) to 3 (extremely desirable). An overall 'Freshness score' was calculated as the sum of the four parameters scores (from 0 to 12), and the acceptability

Table 1. Freshness grade guide for oysters

Score	Odor	Body color	Fluid	Texture
3	hay	cream white	clear	sirm and elastic
2	stronger sea-weedy	white	clear, with small amount of debris	soft and less elastic
1	spoiled with slight sour smell	tan/beige	clear with large amount of debris	slightly mushy
0	sour and putrid smell	yellow/light brown	cloudy	mushy

The overall "Freshness score" for the oysters (from 0 to 12) is the sum of the scores from the four parameters (from 0 to 3)

was determined as having a score of over 6. The data from independent 6 panelists were pooled, the points representing mean values of six measurements ± standard deviation.

Statistical analysis. The experiments were repeated twice. The measurements were run in triplicates for each replicate ($n = 2 \times 3$). The results were reported as mean values ± standard deviation.

The Student's t-test was employed to find out the significance between different treatments and days of storage. The differences between the means were considered significant when $P < 0.05$. The program used for the statistical evaluation was SYSTAT Software, version 11.

RESULTS AND DISCUSSION

Microbiological analysis

The changes in aerobic plate counts (APC) of oysters during storage at 10°C, 5°C, and 0°C are shown in Figure 1. Although the initial microbial counts of fish and shellfish vary depending on many factors, the values are generally between 10^2 to 10^5 CFU/g. The initial APC of raw oysters was found here to be 3.2×10^3 CFU/g, which was in the range of those found in earlier studies on fresh aquatic products.

Rapid increases were observed in APC values of the samples stored at 10°C and 5°C while no lag phases were detected. Kim *et al.* (2002) suggested a total aerobic plate count of 10^7 CFU/g as an acceptable quality limit of oysters. The samples stored at 10°C and 5°C got close to this value on day 6 and day 10, respectively. With the samples stored at 0°C, a decrease in APC value during the first 4 days of storage and a lag phase of about 6 days were detected. During storage, the APC values in the samples stored at 0°C were always lower than those in the samples stored at 10°C and 5°C ($P < 0.05$) and surpassed the level of 10^7 CFU/g on day 18.

Microbial flora analysis of raw and spoiled oysters

Microbial flora of raw oysters was complicated, a total of 85 strains were isolated and identified as belonging to 13 genera. Of the 85 isolated strains, 23% (14 strains) and 20% (12 strains) were *Pseudomonas* and *Vibrionaceae*, respectively. *Shewanella*, *Alcaligenes*, *Enterobacteriaceae*, *Moraxella*, *Acinetobacter*, *Flavobacterium*, *Corynebacterium*, *Staphylococcus*, *Micrococcus*, Lactic acid bacteria, and *Bacillus* were also detected as minor organisms. Out of those found, Gram-negative bacteria belonging to the genera *Pseudomonas* and *Vibrionaceae* were dominant (Table 2). This result was more of less different from those obtained in previous works (Puchenkova 1991; Ortigosa *et al.* 1994), since the microbial flora of freshly caught oysters reflects the microbial flora in the surrounding environment and can be influenced by many factors such as the seasonal period, gathering method etc.

The microbial flora of the spoiled samples under different storage temperatures was relatively

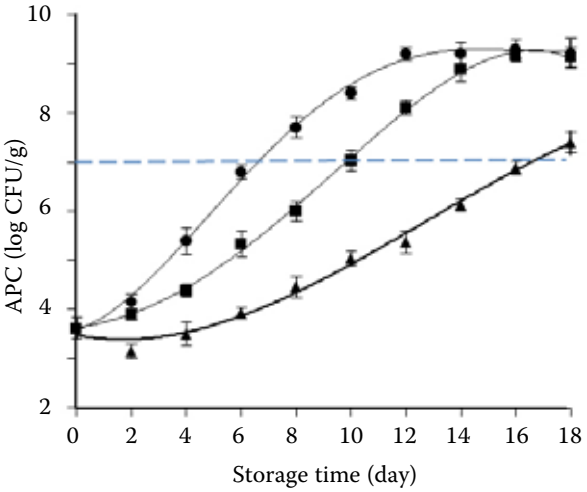

Figure 1. Changes in APC of oysters during storage at 0°C, 5°C, and 10°C

Table 2. Distribution of microbial flora from raw and spoiled Pacific oysters

Bacteria groups	Raw oysters (%)	Spoiled Pacific oysters (%)*		
		10°C	5°C	0°C
Pseudomonas	22	42	60	66
Vibrionaceae	20	18	22	18
Shewanella	5	–	–	–
Alcaligenes	6	2	–	–
Enterobacteriaceae	5	11	1	–
Moraxella	7	6	7	7
Acinetobacter	2	–	–	–
Flavobacterium	8	8	3	5
Total Gram-negative	75	87	93	96
Corynebacterium	3	–	–	–
Staphylococcus	3	–	–	–
Micrococcus	7	5	2	2
Lactic acid bacteria	6	4	1	–
Bacillus	2	2	–	1
Total Gram-positive	21	11	3	3
Unidentified	4	2	4	1
Total	100	100	100	100

*sampling was done when APC reached 10^7 CFU/g; – not detected

simple. The proportion of Gram-negative bacteria was significantly higher than that in raw oysters, *Pseudomonas* amounting to 42% (10°C), 60% (5°C), and 66% (0°C), respectively (Table 2).

Pseudomonas and *Shewanella* have been reported to be the most occurring microorganisms during the ice storage of fish and shellfish. At the end of the shelf-life, no *Shewanella* spp. were detected. The reason may be that *Shewanella* spp. constituted a low initial proportion (5%) and were inhibited by *Pseudomonas* spp. H_2S-producing bacteria, mainly *Shewanella putrefaciens* and *Pseudomonas* spp., are two strongly competitive psychrotrophic microorganisms (Koutsoumanis *et al*. 1999). Gram and Melchiorsen (1996) reported that *Pseudomonas* spp. could inhibit the growth of H_2S-producing bacteria (including *Shewanella putrefaciens*) due to the ability of the former to produce siderophores.

Vibrionaceae occurred in a relatively high proportion during storage and preserved a level of approximately 20% regardless of the temperature. These organisms were generally considered non-spoilers and active strains have been rarely described (Gennari *et al*. 1999). They may show some fermentation activity and converted glycogen to lactic acid, which probably resulted in the slight decrease of pH (Table 3).

Some strains of *Enterobacteriaceae* can grow at 10°C, and their proportion increased to 11% at the end of the storage. The proportion was only 1% at 5°C, possibly because their growth rate was remarkably lower than that of *Pseudomonas* spp. at 0°C, the growth of *Enterobacteriaceae* was completely inhibited. Gennari *et al.* (1999) reported that some strains of *Enterobacteriaceae* were capable of producing putrid odour, but their activity appeared low at low temperatures.

Other Gram-negative bacteria such as *Moraxella* and *Flavobacterium*, and Gram-positive bacteria accounted for small proportions in the spoiled samples and showed no obvious spoilage activity.

The microbiology of fresh, ice-stored, and spoiled fish and shellfish has been intensively studied since the beginning of the nineteenth century. Many reports concerned fish and shellfish from cold and temperate sea waters, and described non-fermenting Gram-negative bacteria as the main part of the microbial flora. In this study, we found that

Table 3. Changes in pH values* of oysters during storage at 0°C, 5°C, and 10°C

Storage time (day)	10°C	5°C	0°C
0	$6.30 \pm 0.12^{a,A}$	$6.30 \pm 0.12^{a,A}$	$6.30 \pm 0.12^{a,A}$
2	$6.22 \pm 0.11^{a,A}$	$6.29 \pm 0.15^{a,A}$	$6.28 \pm 0.11^{a,A}$
4	$6.23 \pm 0.12^{a,A}$	$6.25 \pm 0.10^{a,A}$	$6.26 \pm 0.06^{a,A}$
6	$6.13 \pm 0.07^{ab,A}$	$6.22 \pm 0.08^{a,A}$	$6.27 \pm 0.15^{a,A}$
8	$6.05 \pm 0.16^{abc,A}$	$6.13 \pm 0.08^{ab,A}$	$6.22 \pm 0.08^{a,A}$
10	$6.00 \pm 0.12^{bc,A}$	$6.06 \pm 0.14^{abc,A}$	$6.20 \pm 0.11^{a,A}$
12	$5.95 \pm 0.16^{bc,B}$	$6.00 \pm 0.08^{bc,B}$	$6.17 \pm 0.04^{a,A}$
14	$6.00 \pm 0.08^{bc,AB}$	$5.85 \pm 0.11^{c,B}$	$6.13 \pm 0.14^{a,A}$
16	$5.87 \pm 0.07^{c,A}$	$5.90 \pm 0.13^{bc,A}$	$6.08 \pm 0.12^{a,A}$
17	$5.85 \pm 0.12^{c,A}$	$5.91 \pm 0.14^{bc,A}$	$6.10 \pm 0.14^{a,A}$
18	$5.88 \pm 0.05^{c,B}$	$5.89 \pm 0.13^{bc,AB}$	$6.06 \pm 0.07^{b,A}$

*Points represent mean values of six measurements ± standard deviation ($n = 2 \times 3$)

$^{a–c}$Different letters in the same column indicate significant differences ($P < 0.05$)

^{AB}Different letters in the same row indicate significant differences ($P < 0.05$)

Pseudomonas was the dominant bacteria of oysters during storage. Therefore, controlling the growth of these Gram-negative bacteria may be very important to improve the preservation of oysters.

Chemical analysis

The initial pH value of the control samples was 6.30, which was in agreement with the reports by other authors (Balasundari *et al*. 1997). During storage, the pH values decreased slightly. This decrease might be due to the relative high level of glycogen in oysters and the fact that the spoilage of mollusc shellfish is partly fermentative. At the end of the shelf-life determined by APC, pH values were 6.05 at 10°C (day 6), 6.06 at 5°C (day 10), and 6.06 at 0°C (day 18), respectively. This result was similar to that of Banks *et al*. (1977), who suggested the pH value of about 6.0 as the lower limit of acceptability for oysters.

The changes in the total volatile basic nitrogen (TVB-N) of oysters are shown in Table 4. TVB-N includes the deteminations of TMA (trimethylamine), DMA (dimethylamine), ammonia, and other volatile basic nitrogen compounds, resulting from the degradation of proteins and non-protein nitrogenous compounds, which is chiefly caused by microbial activity (Ruiz-Capillas & Moral 2005). TVB-N was reported as a spoilage compound and proposed as the fish and shellfish spoilage indicator in many studies (Lannelongue *et al*. 1982).

The initial TVB-N value was 4.25 mg N/100 g. During storage, TVB-N values in the samples stored at 10°C and 5°C increased significantly ($P < 0.05$), while in the samples stored at 0°C, TVB-N increased slowlier. A level of 30 mg N/100 g TVB-N is considered to be the upper limit (normally considered as the spoilage level), above which the fishery products are thought to be unfit for human consumption (El-Marrakchi *et al*. 1990; Harpaz *et al*. 2003). In the present study, at the end of the shelf life as determined by APC, all tested samples stored at different temperatures achieved the average TVB-N concentration of about 22.0 mg N/100 g. Molluscs differ in their chemical composition from fish and crustacean shellfish in that they contain significant levels of carbohydrate (glycogen) and a lower total quantity of nitrogen. The reason why TVB-N values of oysters were relatively low at the end of the shelf-life might reside in that the oyster undergoes general acidification as the high glycogen content is converted to lactic acid.

Sensory assessment

The changes in the overall freshness scores of oysters stored at different temperatures are shown in Figure 2. During storage, a gradual decrease

Table 4. Changes in TVB-N* of oysters during storage at 0°C, 5°C and 10°C.

Storage time (day)	10°C	5°C	0°C
0	4.25 ± 0.34^{A}	4.25 ± 0.34^{A}	4.25 ± 0.34^{A}
2	9.03 ± 0.28^{C}	5.89 ± 0.38^{B}	4.14 ± 0.18^{A}
4	13.22 ± 0.56^{C}	9.73 ± 0.45^{B}	5.23 ± 0.54^{A}
6	20.32 ± 0.29^{C}	12.18 ± 0.51^{B}	6.91 ± 0.33^{A}
8	25.69 ± 0.61^{C}	16.66 ± 0.16^{B}	8.47 ± 0.16^{A}
10	34.24 ± 0.31^{C}	20.61 ± 0.78^{B}	10.32 ± 0.31^{A}
12	41.85 ± 0.42^{C}	26.41 ± 0.66^{B}	12.45 ± 0.52^{A}
14	50.67 ± 0.50^{C}	35.34 ± 0.64^{B}	14.44 ± 0.19^{A}
16	60.83 ± 0.25^{C}	45.27 ± 0.39^{B}	18.96 ± 0.22^{A}
18	66.72 ± 0.32^{C}	53.33 ± 0.26^{B}	23.20 ± 0.73^{A}

*Values are means ± standard deviations of data from six independent experiments (n = 2 × 3).
$^{A-C}$ Different letters in the same row indicate significant differences (P < 0.05).

of 'freshness score' was observed. The samples stored at 10°C and at 5°C reached unacceptable freshness scores on day 7 and day 11, respectively, while the samples stored at 0°C remained acceptable up to 17 days.

The shelf-life determined by sensory values given in this experiment was somewhat longer than expected and was inconsistent with the results of APC and TVB-N analysis. The cause might be accounted for by the fact that the sensory evaluation was subjective and could lag behind the biochemical changes and microorganisms propagation. The low storage temperature might exaggerate this phenomenon by delayed external signs of deterioration in oysters while the internal quality changed greatly.

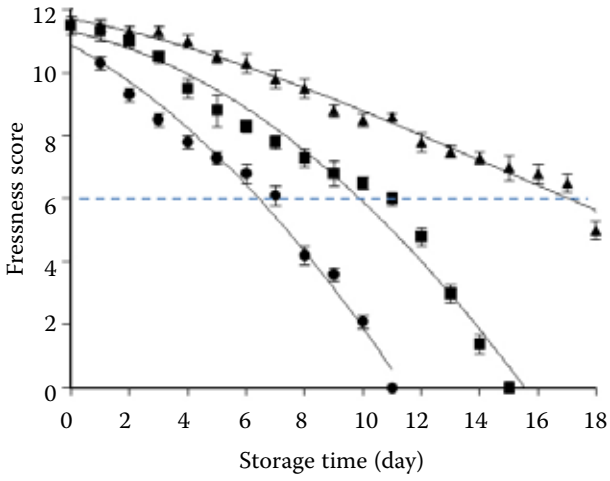

Figure 2. Changes in sensory score of oysters during storage at 0°C, 5°C, and 10°C

In conclusion, when different spoilage parameters were combined together, the shelf-life of oysters stored at 10°C, 5°C. and 0°C was found to be 6–7 days, 10–11 days, and 17–18 days, respectively.

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