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Short communication

Changes in microbial flora of Pacific oysters (*Crassostrea gigas*) during refrigerated storage and its shelf-life extension by chitosan

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ABSTRACT

Changes in microbial flora of Pacific oysters (*Crassostrea gigas*) during storage at 5 ± 1 °C were analyzed and the antimicrobial activity of chitosan was studied to identify its potential in shelf-life extension. The dominant microorganisms were found to be *Pseudomonas* (22%) and *Vibrionaceae* (20%) in raw oysters. During storage, proportion of *Pseudomonas* increased significantly and reached 73% at the end of storage, while *Vibrionaceae* preserved a level of approximate 20%. Wide-spectrum antibacterial property of chitosan against the bacteria isolated from oysters was discovered, and chitosan concentration of 5.0 g/L was eventually determined for application in oyster preservation. Based on microbiological analysis, biochemical indices determination and sensory evaluation, shelf-life of oysters stored at 5 ± 1 °C was determined. Data showed that chitosan treatment extended the shelf-life of oysters from 8–9 days to 14–15 days.

1. Introduction

Oysters are the most abundant harvested shellfish in the world and have a high value. As fresh seafood, oysters have a short shelf-life, which causes substantial practical problems for its distribution. Improvements in the shelf-life of oysters can have an important economic impact by reducing losses and by allowing the products to reach distant and new markets (Rhodehamel, 1992). Delaying or inhibiting growth of spoilage microorganisms in fresh seafood is a major key to improve preservation (Sallam and Samejima, 2004).

Various food preservation techniques have been utilized to improve the microbial safety and extend the shelf-life of seafood in general, including freezing, chemical preservation, salting, and modified atmosphere packaging. Use of preservatives in aquatic products is convenient and universal. Both food processors and consumers have expressed the desire to reduce the use of synthetic chemicals in food preservation, consequently there has been an increased interest in the application of various natural agents as bio-preservative. However, most natural antimicrobials have a limited spectrum of activity and are effective only at very high concentrations. Chitosan is a versatile biopolymer, having a broad range of applications in the food industry (Rudrapatnam and Farooqahmed, 2003). It exhibits antimicrobial activity against a range of food-borne microorganisms and consequently has attracted attention as a potential natural food preservative (Chen et al., 1998; Shahidi et al., 1999). However, to date, there have been very few studies about the use of chitosan for oyster preservation.

The main objective of this study was to analyze the microbial flora changes of Pacific oysters during refrigerated storage and to confirm the potential of chitosan in shelf-life extension by evaluating its antimicrobial function against the bacteria isolated from oysters. Shelf-life of oysters with chitosan treatment was also determined by microbiological analysis, biochemical indices and sensory evaluation in this study.

2. Materials and methods

2.1. Oyster

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

2.2. Chitosan

Chitosan, in powder form, was obtained from Lanzhou WEIRI bioengineering Co. Ltd (China). Moisture content of chitosan was less than 6% and it had a deacetylation range of 80–82%. Chitosan was dissolved in sterile water and the final concentrations were 0.5, 1.0, 5.0 and 10.0 g/L, respectively.

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Table 1
Freshness grade guide for oysters.

Score	Odor	Body color	Fluid	Texture
3[a]	Hay	Cream white	Clear	Firm and elastic
2	Stronger sea-weedy	White	Clear, with small amount of debris	Soft and less elastic
1	Slight sour smell	Tan/beige	Clear with large amount of debris	Slightly mushy
0[b]	Sour and putrid smell	Yellow/light brown	Cloudy	Mushy

[a] Extremely desirable.
[b] Extremely undesirable.

2.3. Preparation of oyster samples

All oysters were scrubbed under running water to remove fouling organisms, mud and other debris adhering to the shell, then purified in cold water with 3.5% marine salt for 30 min. After cleaning and draining excess drip solution, oysters were shucked and separated into two lots. Samples were wrapped in sterile plastic bags and stored at 5 ± 1 °C as the control lot. Preservative treatment was done by immersing oysters in the chitosan solution with a concentration of 5.0 g/L for 10 min in a ratio of 1:2 on w/v basis.

2.4. Isolation and identification of oyster bacteria

Oyster samples were taken aseptically in 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 homogenized for 60 s with a stomacher. Ten-fold serial dilutions were made and samples (0.1 mL) were spread on Marine Agar plates (Ortigosa et al., 1994). Aerobic plate count (APC) was determined by counting the number of colony-forming units after incubation at 25 °C for 48 h.

After counting the number of colony-forming units, the colony morphology was noted. Randomly picked 30–50 colonies (to pick many different phenotypes) were restreaked on Marine Agar plates three times to obtain pure cultures. Isolated microorganisms were identified by using a scheme given by Bagge-Ravn et al. (2003) and data from Holt et al. (1993) was consulted. The scheme used was fit for the identification of bacteria isolated from aquatic products and processing environments. The isolated strains were grouped to genus level.

2.5. Determination of antibacterial activity of chitosan by the paper disc diffusion method

The antibacterial activity of chitosan was determined by using the paper disc diffusion method. In brief, chitosan was diluted in sterile water to give concentrations of 0.5, 1.0, 5.0 and 10.0 g/L, respectively. The isolates from oysters were grown in nutrient broth at 25 °C for 18–24 h and diluted to 10^5–10^6 cfu/mL in molten nutrient agar. The inoculated agar (20 mL) was pipetted into sterile Petri dishes and solidified.

Paper discs (diameter 8.0 mm) were dipped in chitosan solutions with different concentrations for a few seconds, then placed on the surface of an inoculated plate and incubated at 25 °C for 48 h. For the control, sterile water alone was applied to the disc. The diameter (mm) of the growth-inhibited zone minus the disc diameter was recorded. Experiments were replicated twice. Measurements were run in triplicate for each replicate ($n = 2 \times 3$).

2.6. Chemical analysis

The pH of oysters was measured using a pH meter (PHS-3C, Shanghai, China) after blending 10 g of homogenized 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.

2.7. Sensory assessment

The sensory properties of oysters were measured by a panel of 6 trained panelists from the staff of Department of Food Science and Engineering, Ocean University of China according to the freshness grade guide for oyster (He and Morrissey, 1999) after 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 sum of the four parameters scores (from 0 to 12), and acceptability was determined as having a score of over 6. The data from independent 6 panelists were pooled and points represent mean values of six measurements ± standard deviation.

2.8. Statistical analysis

Oysters were examined at 24-hour intervals for sensory assessment and at 48-hour intervals for chemical and APC analysis. Experiments were replicated twice. Measurements were run in triplicate for each replicate ($n = 2 \times 3$). Results were reported as mean values ± standard deviation.

The Student's t-test was employed to find out 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.

3. Results and discussion

3.1. Changes in microbial flora

Changes in microbial flora of oysters during storage at 5 ± 1 °C are shown in Table 2. The initial microbial flora (day 0) of oysters was complicated. All isolated strains were identified to belong to 13 genus. Gram-negative bacteria were dominant, and 22% and 20% 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. From these findings, Gram-negative bacteria belonging to the genera *Pseudomonas* and *Vibrionaceae* were dominant. This result was more or less different with previous studies (Puchenkova, 1991; Ortigosa et al., 1994), since the microbial flora of

Table 2
Distribution of microbial flora of oysters during refrigerated storage.

Bacteria groups	Storage time (day)			
	0 (%)	4 (%)	8 (%)	12 (%)
Pseudomonas	22	50	63	73
Vibrionaceae	20	19	22	19
Shewanella	5	–[a]	–	–
Alcaligenes	6	2	–	–
Enterobacteriaceae	5	2	1	–
Moraxella	7	6	4	1
Acinetobacter	2	–	–	–
Flavobacterium	8	8	3	2
Total Gram-negative	75	87	93	95
Corynebacterium	3	–	–	–
Staphylococcus	3	–	–	–
Micrococcus	7	4	2	2
Lactic acid bacteria	6	3	2	–
Bacillus sp.	2	2	–	1
Total Gram-positive	21	11	4	3
Unidentified	4	2	3	2
Total	100	100	100	100

[a] No detected.