



Characterization of microbial community in high-pressure treated oysters by high-throughput sequencing technology



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ABSTRACT

High-pressure (HP) processing technology has great application potential in bivalve mollusk industry. In this study, the effects of HP treatment on shucking and shelf-life extension of Pacific oysters (*Crassostrea gigas*) during 4 ± 1 °C storage were studied. Culture-independent high-throughput sequencing (HTS) technology was used to investigate the microbiota of oysters treated by HP (300 MPa, 2 min) during storage. Results showed that HP treatment at 275 MPa for 3 min or 300 MPa for 2 min could achieve 100% full release of oyster adductor muscle, pressures higher than 300 MPa caused disadvantageous damage of shells. Therefore, HP treatment at 300 MPa for 2 min was particularly appropriate. Based on organoleptic, biochemical and microbiological analyses, shelf-life of 6–8 days for control and 12 days for HP-treated oysters could be expected. The dominant bacteria in fresh oysters were *Vibrio* (28.3%), *Shewanella* (10.3%) and *Pseudoalteromonas* (7.2%). HP treatment changed the spoilage microbiota of oysters dramatically. *Psychrobacter* was dominant in the HP-treated spoiled oysters and its proportion was 42.3%, while *Pseudoalteromonas* (32.2%) and *Shewanella* (19.5%) were dominant in the spoiled oysters without HP treatment. This study confirmed that HP processing was effective in facilitating the removal of oyster meats from the shells and prolonging their shelf-life. More importantly, HP treatment changed the microbiota of oysters during chilled storage.

Industrial relevance: As seafood, oysters have a short shelf-life. High-pressure (HP) processing technology has showed great application potentials in the bivalve mollusk industry. In this work, we determined the effects of HP treatment on the shucking and shelf-life extension of oysters. We used high-throughput sequencing technology to investigate the microbiota of control and HP-treated oysters during refrigerated storage. We found that HP treatment changed the spoilage microbiota of oysters dramatically. *Psychrobacter* was dominant in the HP-treated spoiled oysters, while *Pseudoalteromonas* and *Shewanella* were dominant in the spoiled oysters without HP treatment. Therefore, controlling the growth of *Psychrobacter* could help to improve the quality of oysters treated by HP during storage.

1. Introduction

Oysters have been widely farmed all over the world, with great values in meeting nutrition needs of human beings and promoting fishery economic development (Humphreys, Herbert, Roberts, & Fletcher, 2014). However, oysters are perishable due to their high water and nutrient content that provide advantageous conditions for the growth and reproduction of diverse microorganisms (Ashie, Smith, & Simpson, 1996). Therefore, oysters have a short shelf-life, which causes practical problems for their distribution and consumption.

A variety of food preservation techniques is being studied to extend the shelf-life of seafood, such as freezing, salting, modified atmosphere packaging, and chemical or biological preservatives (Khan, 2015). In the recent twenty years, high-pressure (HP) processing has attracted

more and more attentions mostly because of its advantage in reducing the bacterial loads of foodstuff without causing significant changes in appearance, flavor, texture, and nutrition properties (Yordanov & Angelova, 2010). HP has been widely applied in the commercial processing of oysters (Murchie et al., 2005). Previous works have shown that the application of HP in oyster processing offers several benefits, including HP-induced shucking (He, Adams, Farkas, & Morrissey, 2002), maintenance of flavor and nutrients (Cruz-Romero, Kelly, & Kerry, 2007; Cruz-Romero, Smiddy, Hill, Kerry, & Kelly, 2004), and improved safety due to inactivation of microorganisms (Murchie et al., 2005).

Microorganisms play a key role in the spoilage of aquatic products. Gram and Dalggaard (2002) proposed that at the point of sensory rejection, the so-called spoilage microbiota is composed of

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microorganisms that have contributed to the spoilage and microorganisms that have grown but not caused unpleasant changes. The former is the so-called specific spoilage organism (SSO) of the product. Scientists have proved that HP could cause injury and even death of microorganisms, thus affecting the spoilage microbiota. Cruz-Romero, Kelly, and Kerry (2008) found that HP treatment clearly influenced the microbiota of oysters compared to untreated oysters. *Pseudomonas* spp. was the major spoilage aerobic bacteria in HP-treated oysters during chilled storage. Prapaiwong, Wallace, and Arias (2009) reported that HP treatment was effective in reducing microbial loads in raw oysters, but no qualitative difference between HP-treated and raw oyster microbiota could be inferred from the experiment data and *Vibrio* and *Shewanella* were the dominant genera. The studies listed above were based on traditional culture method. Since most of the microorganisms could not be cultured, the experiment data obtained from culture method cannot accurately clarify the microbiota of oysters (Cao, Zhang, Meng, Zhao, & Liu, 2016; Caporaso et al., 2012). Molecular biology techniques such as real-time polymerase chain reaction (real-time PCR), denaturing gradient gel electrophoresis (DGGE), and terminal restriction fragment length polymorphism (TRFLP) could not fully examine the microbial species and their proportions in specific samples. Nowadays, the emerging high-throughput sequencing (HTS) technology has attracted wide attentions for its satisfactory efficiency and accuracy in microbiota analyses.

In this study, the effects of HP treatment on the shucking and shelf-life extension of oysters were determined. HTS technology was applied to investigate the microbiota of control and HP-treated oysters during refrigerated storage.

2. Material and methods

2.1. Oyster

Pacific oysters (*Crassostrea gigas*) of commercial size (10–12 cm in shell length) were collected from an aquatic farm (water temperature of 12 °C and salinity of 32‰) in Yellow Sea (China), and transferred in ice to the Food Engineering and Nutrition Laboratory (Yellow Sea Fisheries Research Institute) within 2 h.

2.2. Sample preparation and high-pressure treatment

Fresh oysters were washed under tap water. After cleaning and draining excess drip solution, each oyster was packed in a polypropylene pouch and subjected to a vacuum packer (VOKIN Power Sealer, Shanghai, China) to ensure that no air remained in the pouch, then vacuum packed in a further outer pack to prevent contamination of the oyster in case that the bag was punctured by the shell or pieces of shell during HP treatment.

HP treatments were carried out in a 600 mL capacity high-pressure vessel (HPP, L3, Senmiao, China) at 20 °C. Water was used as pressure transmission medium. Oysters were divided into ten groups: (1) pressure treatment at 100 MPa for 3 min; (2) 200 MPa for 3 min; (3) 250 MPa for 3 min; (4) 275 MPa for 1 min; (5) 275 MPa for 2 min; (6) 275 MPa for 3 min; (7) 300 MPa for 1 min; (8) 300 MPa for 2 min; (9) 300 MPa for 3 min; (10) 350 MPa for 1 min. 20 oysters were used for each treatment. After HP treatment, each oyster was examined and recorded for shucking effects as follows: no release of adductor muscle, partial release of adductor muscle, full release of adductor muscle and excessive release of adductor muscle.

Based on the shucking results, HP (300 MPa, 2 min) treated samples were selected for the subsequent shelf-life and microbiota analyses. After pressure treatment, the oysters were removed from their outer packaging. The oyster meat was removed from the shell aseptically and transferred to sterile 150-mL screw cap polypropylene jars, 6 oysters per container. Hand-shucked oysters were used as control. All the samples were stored at 4 ± 1 °C for a 14-day storage trial.

Table 1
Freshness grade guide for oyster.

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. Shelf-life determination

Oyster samples were enumerated for sensory evaluation, biochemical and microbiological tests on day 0 and at 2-day intervals during storage.

2.3.1. Sensory assessment

The sensory properties of oysters were measured by a panel of 6 trained panelists from the staff of Department of Food Engineering and Nutrition, Yellow Sea Fisheries Research Institute, according to the freshness grade guide for oyster (Sasaki, Iwanaga, & Hamaoka, 1994) after modification (Table 1). Four parameters on a scale from 0 (extremely undesirable) to 3 (extremely desirable) were evaluated. An overall 'sensory score' was calculated as sum of the four parameter scores (from 0 to 12), and acceptability was determined as having a score of over 6. The data from 6 independent panelists were pooled and points represent mean values of six measurements $\pm$ standard deviation.

2.3.2. Biochemical tests

Total volatile bases nitrogen (TVB-N) was measured by micro-diffusion analysis using a Conway's unit.

Lipid oxidation was assessed by measuring thiobarbituric acid reacting substances (TBARS) and was expressed as milligrams of malonaldehyde (MDA) per kilogram of oyster meat.

2.3.3. Microbiological test

Oyster samples were taken aseptically, 5.0 g were transferred to a stomacher bag, and 45.0 mL of 0.1% peptone water with salt (NaCl, 0.85%, w/v) were added. Ten-fold serial dilutions were made if necessary. Samples were homogenized for 60 s and 0.1 mL homogenate solution was spread on marine agar plates (Ortigosa, Garay, & Pujalte, 1995). Aerobic plate count (APC) was determined by counting the number of colony-forming units after incubation at 30 °C for 48 h.

2.4. Odor analysis by electronic nose

Portable Electronic Nose (Air sense Analytics GmbH, Germany) with an array of 10 different metal oxide sensors was used for the odor analysis. Homogenized oyster meat of 5.0 g was placed in a 250 mL conical flask and kept at 20 °C about 30 min for stabilization. A run project was used as follows: reference air, 60 s; sample injection, 5 s; sample measurement, 60 s; wash, 120 s. Data was collected at 1 s intervals, and a 10-s sampling interval near the end of the sampling segment was used.

Statistical analyses were done by the E-nose built-in software (WinMuster, Version 1.6.2, 2014). The data from E-nose was elaborated through principle component analysis (PCA). The data is displayed in a two-dimensional figure, in which the axes correspond to the system default principle components and samples are distributed in this two-dimensional space.

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