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High Pressure Treatment Changes Spoilage Characteristics and Shelf Life of Pacific Oysters (*Crassostrea gigas*) During Refrigerated Storage

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Abstract The effects of high pressure (HP) treatment on spoilage characteristic and shelf life extension of Pacific oysters (*Crassostrea gigas*) during refrigerated storage were studied. Results showed that HP treatment of 275 MPa for 3 min or 300 MPa for 2 min could achieve 100% full release of oyster adductor muscle, pressures higher than 350 MPa caused excessive release as the shells of oysters were broken, thus use of higher pressures should be cautious in oyster processing industry because of its adverse impact on the appearance of shells. HP treatment (300 MPa, 2 min) was proper for the shucking of Pacific oyster (*Crassostrea gigas*) in China. This treatment caused no organoleptic disadvantage. Moreover, HP treatment resulted in obvious differences in biochemical spoilage indicators (pH, TVB-N and TBARS) changes and volatile compounds profile determined by electronic nose during storage. HP treatment (300 MPa, 2 min) also led to a reduction of aerobic bacterial count (APC) by 1.27 log cycles. Furthermore, the APC values of oysters treated by HP were always lower than those of the control samples during storage. Based on the organoleptic, biochemical and microbiological indicators, shelf life of 6–8 d for control and 12 d for HP-treated oysters could be expected. HP treatment showed great potential in oyster processing and preservation.

Key words high pressure processing; oyster; spoilage; E-nose; shelf life

1 Introduction

Oysters have great values in meeting nutrition needs of human beings and promoting fishery economic development. However, oysters are perishable and have a short shelf-life (Ashie *et al*., 1996), which causes practical problems for their distribution and consumption. In China, Pacific oyster (*Crassostrea gigas*) has been one of the most abundantly harvested shellfish. Although Chinese consumers usually eat fully cooked oysters, there is a trend of consuming oysters served as raw or minimally cooked, which has been popular in many countries of the world. New techniques that can prolong the shelf- life of oysters and protect consumer health are strongly demanded.

Various food preservation techniques have been utilized to extend the shelf-life of seafood, such as freezing, salting, modified atmosphere packaging, and chemical or biological preservation (Khan, 2015). Among them, high pressure (HP) processing has attracted a lot of attention mostly because of its advantage in reducing the bacterial loads of food material without causing significant changes in appearance, flavor, texture, and nutrition properties (Yordanov and Angelova, 2010).

HP technique has been increasingly employed in the commercial processing of oysters (Murchie *et al*., 2005). Previous work has shown that the application of HP treatment in oyster processing offers several benefits, including HP-induced shucking, maintenance of flavor and nutrients, and improves safety due to inactivation of microorganisms (He *et al*., 2002; Cruz-Romero *et al*., 2007; Murchie *et al*., 2007).

However, little literature is available about the effect of HP treatment on the spoilage characteristic of Pacific oysters in China. The aim of this study was to investigate the effect of HP treatment on the quality attributes of oysters, such as sensory score, E-nose profile, biochemical index (pH, TVB-N, TBARS) and microbiological index (APC). Shelf-life of oysters with HP treatment was also determined in this study.

2 Materials and Methods

2.1 Oyster

Pacific oysters (*Crassostrea gigas*) with commercial size (10–12 cm in shell length) were collected from a cul-

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ture farm in Yellow sea (China) and transferred in ice to the Food Engineering and Nutrition Laboratory (Yellow Sea Fisheries Research Institute) within two hours.

2.2 Sample Preparation and High Pressure Treatment

Oysters were washed under running water. After cleaning and draining excess drip solution, each oyster was packed in a waterproof bag. The packaged samples were subjected to a vacuum packer to insure that no air remained in the bags.

High pressure treatment was carried out in a 600 mL capacity high pressure vessel (HPP.L3, Senmiao, China). Water was used as pressure transmission medium. Oysters were classified into ten groups: 1) 100 MPa, 3 min; 2) 200 MPa, 3 min; 3) 250 MPa, 3 min; 4) 275 MPa, 3 min; 5) 275 MPa, 2 min; 6) 275 MPa, 1 min; 7) 300 MPa, 3 min; 8) 300 MPa, 2 min; 9) 300 MPa, 1 min; 10) 350 MPa, 3 min. After HP treatment, each sample was examined for shucking effect.

2.3 Sensory Assessment

Based on the shucking results, HP (300 MPa, 2 min) treated samples were selected for spoilage characteristic analysis. Oyster meat was wrapped individually in sterile plastic bag and stored at 4±1℃. Hand-shucked oysters were used as control.

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 *et al*., 1994) after appropriate modification (Table 1).

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

Notes: [a] extremely desirable; [b] extremely undesirable.

Four parameters on a scale from 0 (extremely undesirable) to 3 (extremely desirable) were evaluated. An overall 'freshness 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 ± standard deviation.

2.4 Electronic Nose Analysis

Portable Electronic Nose (Air sense Analytics GmbH, Germany) with an array of 10 different metal oxide sensors was used for the odor analysis. Oyster meat 5.0 g was placed in a 250 mL conical flask and kept at 20℃ about 30 min for stabilization. A run project was used as following: reference air, 60 s; sample injection, 5 s; sample measurement, 60 s; wash, 120 s. Data were 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 first two principle components and samples are distributed in this tow dimensional space.

2.5 Biochemical Tests

The pH values of oysters were measured using a pH meter (PHS-3C, Shanghai, China) after blending 10.0 g of homogenized meat with 90.0 mL of distilled water.

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.

2.6 Microbiological Tests

Oyster samples were taken aseptically, 10.0 g were transferred to a stomacher bag, and 90.0 mL of 0.1% peptone water with salt (NaCl, 0.85%) were added. Other 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 *et al*., 1994). Aerobic plate count (APC) was determined by counting the number of colony-forming units after incubation at 30℃ for 48 h.

2.7 Statistical Analysis

Experiments were replicated twice. Measurements were run in triplicate for each replicate ($n = 2\times3$). Results were reported as mean values ± standard deviation.

The program used for the statistical evaluation was SYSTAT Software, version 11. Mean values were submitted to Student's *t*-test for significant differences ($P < 0.05$) between different treatments and storage periods.

3 Results and Discussion

3.1 Shucking Parameters

Shucking is a very important function of HP treatment in oyster processing. HP can cause disruption of non-covalent interactions in the tertiary protein structures, which results in the denaturation of muscle proteins and

connective tissues and finally causes the release of oyster adductor muscle (Cruz-Romero *et al*., 2004).

The effects of different treatment pressures and times on shucking are showed in Table 2. Pressures lower than 100 MPa had no effect. Higher pressures and longer times were more effective in severing adductor muscle and releasing oyster meats. Treatment at 275 MPa for 3 min or 300 MPa for 2 min could achieve full release. However, 350 MPa for 3 min caused excessive release as the shells of oysters were broken. Use of pressures higher than 350 MPa should be cautious in oyster processing industry because of its adverse impact on the appearance of shells.

These results were similar to those reported by He *et al*. (2002) and Hsu *et al*. (2009). The optimum HP parameters may vary with oyster species and size, and therefore should be adjusted by individual processors.

Table 2 Shucking effect of HP treatments on oysters

Shucking effect† (%)	100 MPa 3 min	200 MPa 3 min	250 MPa 3 min	275 MPa			300 MPa			350MPa 3 min
				1 min	2 min	3 min	1 min	2min	3min	
No release	100	60	0	0	0	0	0	0	0	0
Partial release	0	30	20	20	10	0	15	0	0	0
Full release	0	10	80	80	90	100	85	100	100	85
Excessive release	0	0	0	0	0	0	0	0	0	15

Note: †20 oysters were used for each treatment.

3.2 Sensory Evaluation

No significant differences in sensory score were detected between the control and HP-treated groups before storage ($P > 0.05$), which indicated that no organoleptic disadvantage was engendered by HP treatment. However, the color score of the HP group was a little lower than that of the control group (Fig.1).

During storage, gradual decreases in sensory score were noticed both in the control and HP groups. No significant differences were noticed during the first 2 d. From day 4, a sharp decrease in score was detected in control samples and the score became unacceptable on day 8, while the score of samples treated by HP decreased much slower and remained acceptable up to 12 d.

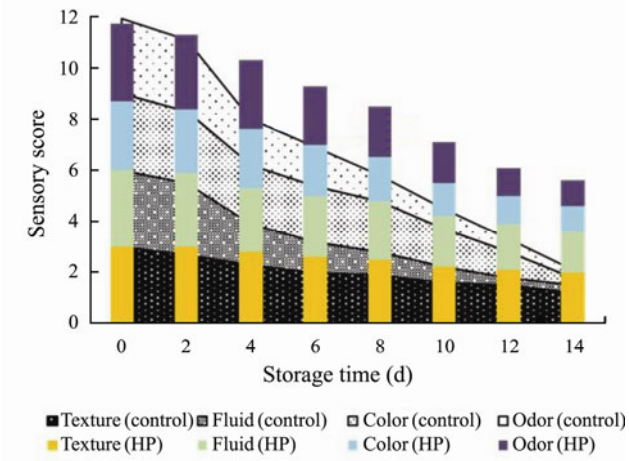

Fig.1 Changes in sensory score of oysters during refrigerated storage.

3.3 Electronic Nose Analysis

Electronic nose is a rapid and non-destructive method for evaluating volatile compounds and has been widely used in food industry. The main applications of electronic nose in sea foods are quality assessment, spoilage identification, off-flavors detection, and bacterial strains classification (Alam *et al*., 2012). Data processing techniques include principal component analysis (PCA), linear discriminate analysis (LDA), partial least squares (PLS), functional discriminate analysis (FDA), cluster analysis (CA), fuzzy logic or artificial neural network (ANN) (Scott *et al*., 2006). Among these techniques, PCA is commonly performed to identify discrimination of individual composition variables among the measured samples. Samples with similar patterns are located close to each other.

The analyses showed that electronic nose discriminated oyster samples with different regions in the two-dimensional plot (Fig.2). The region of control oysters was located away from that of the HP-treated samples before storage (day 0). This departure may due to the relatively higher level of lipid oxidation of the HP group (Table 3). However, these differences in lipid oxidation have little influence on sensory evaluation. For the control oysters, there were considerable distances among the regions with different storage periods. Therefore, electronic nose may be used as a rapid method to evaluate freshness of oysters. For the HP-treated oysters, the regions of day 0 and day 4 were close to each other, as the quality deterioration of oysters in the early period was slowed down by the HP treatment. This result was consistent with the changes of sensory score and APC in the first 4 d storage. The regions of HP-treated oysters with 8 d and 12 d storage periods departed from that of the first 4 d. There were significant differences between the control and HP-treated groups on the same day. It could be presumed that HP treatment changed the odors of oysters and their decay process.

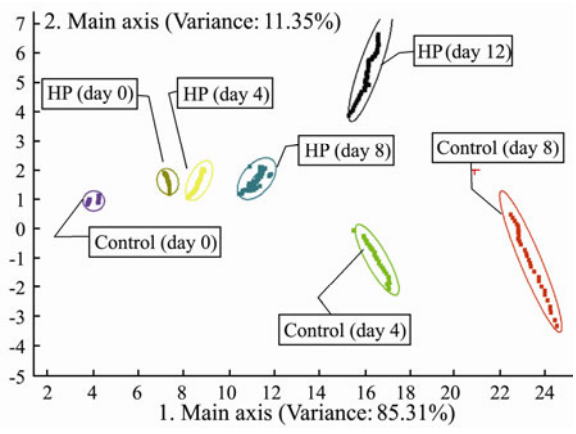

Fig.2 Principal component analyses of oysters by E-nose during refrigerated storage.

Table 3 Changes in pH, TVB-N and TBARS of oysters during refrigerated storage

Storage time (d)	pH		TVB-N (mg N (100g)$^{-1}$)		TBARS (mg kg^{-1})	
	Control	HP (300 MPa, 2 min)	Control	HP (300 MPa, 2 min)	Control	HP (300 MPa, 2 min)
0	$6.37\pm0.04^{a,A}$	$6.42\pm0.02^{a,A}$	$4.32\pm0.09^{a,A}$	$4.19\pm0.12^{a,A}$	$0.42\pm0.03^{a,A}$	$0.69\pm0.07^{a,B}$
2	$6.32\pm0.03^{a,A}$	$6.40\pm0.05^{a,A}$	$7.63\pm0.22^{b,A}$	$4.33\pm0.08^{a,B}$	$0.44\pm0.04^{a,A}$	$0.87\pm0.06^{b,B}$
4	$6.23\pm0.03^{b,A}$	$6.31\pm0.01^{b,B}$	$10.33\pm0.15^{c,A}$	$5.28\pm0.22^{b,B}$	$0.57\pm0.04^{b,A}$	$0.89\pm0.07^{b,B}$
6	$6.13\pm0.02^{c,A}$	$6.26\pm0.03^{b,B}$	$15.51\pm0.13^{d,A}$	$7.78\pm0.23^{c,B}$	$0.61\pm0.02^{b,A}$	$1.04\pm0.07^{bc,B}$
8	$6.05\pm0.04^{d,A}$	$6.18\pm0.02^{c,B}$	$20.66\pm0.17^{e,A}$	$11.05\pm0.16^{d,B}$	$0.66\pm0.06^{bc,A}$	$1.06\pm0.05^{c,B}$
10	$5.84\pm0.06^{e,A}$	$6.11\pm0.02^{d,B}$	$32.73\pm0.28^{f,A}$	$14.42\pm0.25^{e,B}$	$0.70\pm0.04^{c,A}$	$1.13\pm0.08^{c,B}$
12	ND	5.97 ± 0.03^{e}	ND	18.29 ± 0.19^{f}	ND	1.36 ± 0.03^{d}
14	ND	5.91 ± 0.04^{e}	ND	23.16 ± 0.30^{g}	ND	1.58 ± 0.09^{e}

Notes: $^{a-g}$Different letters in the same column indicate significant differences ($P<0.05$); $^{A-B}$Different letters in the same row of each unit indicate significant differences ($P<0.05$); ND, not determined.

3.4 Biochemical Analysis

The initial pH value of oysters was 6.37. This value is consistent with previous studies (Cao *et al*., 2010). HP-treated oysters had a little higher pH than that of the control. During storage, the pH values of the control and HP groups decreased slightly. This decrease may be due to the relatively high level of glycogen in oysters and the fact that spoilage of molluscan shellfish is partly fermentative. At the point of sensory rejection, pH values were 6.05 for the control oysters (day 8) and 5.97 for HP-treated samples (day 12) respectively. Consequently, pH value of 6.0 could be regarded as the boundary of spoilage.

TVB-N includes the measurements of trimethylamine, dimethylamine, ammonia and other volatile basic nitrogen compounds. TVB-N has been reported as fish and shellfish spoilage indicator in many studies (Cheng *et al*., 2014). The initial TVB-N values of the control and HP-treated oysters were 4.32 mg N per 100 g and 4.19 mg N per 100 g, respectively. During storage, TVB-N values of the control oysters increased rapidly, and no lag phase was detected. TVB-N of HP-treated samples increased slower in the first days, and a lag phase of about 2 days was observed. By day 4 and afterwards, however, a sharp rise of TVB-N value was noticed. At the point of sensory rejection, TVB-N values were 20.66 mg N/100g for the control oysters (day 8) and 18.29 for HP-treated samples (day 12) respectively. TVB-N value of 30 mg N/100g is considered to be the spoilage level, above which fish products are considered unfit for human consumption (Harpaz *et al*., 2003). Mollusca contain much more glycogen and lower total quantity of nitrogen than fish and crustacean shellfish. During storage, oyster undergoes general acidification as the high glycogen content is converted to lactic acid. Thus, TVB-N values of oysters were relatively lower when they decayed.

TBARS value represents the degree of lipid oxidation. The initial TBARS value of the control oysters was 0.42 mg kg^{-1}. HP treatment immediately increased the TBARS value to 0.69 mg kg^{-1} (P<0.01). This result is similar to that of Lai *et al*. (2010), who also found that HP could accelerate the oxidation of oyster lipid. Oysters are rich in metal ions, like iron and copper (Bruner *et al*., 2014). HP treatment may promote the release of these metal ions, which accelerate lipid oxidation. During storage, TBARS value of the control oysters increased slowly, and a two days' lag phase was detected. TBARS of HP-treated samples increased rapidly during storage, and no obvious lag phase was noticed. At the point of sensory rejection, TBARS values were 0.66 mg kg^{-1} for the control oysters (day 8) and 1.36 mg kg^{-1} for HP-treated samples (day 12) respectively. TBARS value of 2.0 mg kg^{-1} is normally regarded as the spoilage level, above which fishery materials and products will develop objectionable odor and taste for human consumption. TBARS of both the control and HP-treated groups did not exceed 2.0 mg kg^{-1} during storage period. However, HP treatment accelerated the degradation of lipid, which is a disadvantage in HP technique application.

3.5 Microbiological Analysis

Although the initial microbial counts of fish and shellfish vary depending on many factors, the values are generally between 10^2–10^5 CFU g^{-1}. The initial APC of raw oysters was 3.05×10^3 CFU g^{-1}, which was in the range of those found in earlier studies of fresh aquatic products (Ginson *et al*., 2013; Li *et al*., 2013; Reyes *et al*., 2015). A rapid increase in APC value was observed in the control group, and no lag phase was detected. Kim *et al*. (2002) suggested a total aerobic plate count of 10^7 CFU g^{-1} as an acceptable limit for oysters. Control samples reached this level on day 6. This was different with the sensory evaluation in shelf life determination, which indicated a shelf life of 8 d. The reason of this discrepancy might be that sensory evaluation was subjective and could lag behind microorganism propagation. Low storage temperature might exaggerate this phenomenon by delayed exterior signs of deterioration in oysters while interior quality changed greatly. APC was more sensitive than the sensory testing of the volunteers.

HP treatment showed immediate bactericidal function and APC of HP-treated group was significantly reduced by 1.27 log cycles ($P<0.01$). Furthermore, a lag phase of 4 days was detected. During storage, the APC values of HP-treated oysters were always lower than those of the control samples ($P<0.05$) and surpassed the acceptable limit by day 12. Considering the organoleptic, biochemical and microbiological indicators, it can be inferred that

the shelf life was 6–8 d for control and 12 d for HP-treated oysters (Fig.3).

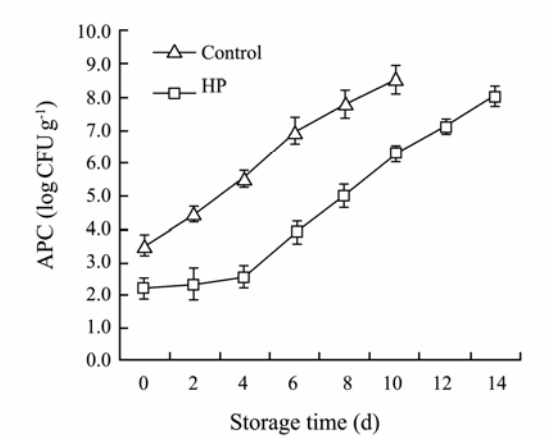

Fig.3 Changes in APC of oysters during refrigerated storage.

4 Conclusions

The optimum HP parameter for Pacific oyster (*Crassostrea gigas*) shucking was found to be 300 MPa for 2 min, which caused obvious differences in biochemical spoilage indicators (pH, TVB-N and TBARS) changes and volatile compounds profile determined by E-nose. HP treatment significantly reduced the microbial load by 1.27 log cycles ($P<0.01$) and slowed down the growth of microorganisms during storage. Therefore, shelf life of oysters treated by HP extended from 6–8 d to 12 d under refrigerated condition.

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