Original paper

Optimization of Hydrostatic Pressure Processing to Extending Shelf-life with Minimal Quality Changes of Refrigerated Abalone

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Received June 11, 2015 ; Accepted March 3, 2016

This study investigated the effects of pressure level, holding time, and storage period on the physicochemical properties of chilled abalone. Pressure level and storage period had a significant effect on the qualities of abalone, while the impact of holding time was negligible compared to those of pressure level and storage period. Pressurization at 400 – 500 MPa had the potential advantage to extend shelf-life of abalone to more than 15 days, while quality modifications were noticeably identified. Moderate pressurization (< 200 MPa) had no effect on the shelf-life of abalone. The optimal processing condition was 200 – 300 MPa at which the shelf-life of abalone could be extended to around 10 days with minimizing quality modification. Although, the mechanisms involved in shelf life extension and quality modification of pressurized abalone were not fully understood, the present study demonstrated the potential application of high pressure in the abalone industry.

Keywords: abalone, high pressure, surface response methodology, physicochemical, shelf-life

Introduction

Abalone has a long history as a major marine source of consumption in East Asia. In recent years, abalone has become a highly valuable sea product worldwide due to its nutritional advantages. Despite the limited harvest of wild abalone, explosive consumer demand triggered abalone production via farming. It was generally reported that abalone has high lethality and mortality, and the shelf life of chilled abalone muscle is very limited (~3 days), which accounted for the majority of economic damage to abalone farming (Chiou *et al.*, 2002; Braid *et al.*, 2005; Jo *et al.*, 2014). Although, freezing or drying techniques have been adopted to extend the shelf life of abalone, these products manifested quality modifications against chilled products. Strategies regarding how to extend the shelf life of abalone under chilled condition are necessary.

Traditionally, freezing and drying have been adopted to preserve abalone for long-term storage. However, these processes also caused textural modifications, which were seen by consumers

as lowering product quality. To extend the shelf life of abalone under chilled state, therefore, application of high pressure processing has been recommended. Briones *et al.* (2010) reported that pressurized abalone at 500 – 550 MPa extended the shelf life of abalone to longer than 65 days. However, the authors also observed the quality modifications of abalone after high pressure processing (Briones-Lebarca *et al.*, 2012).

Based on our previous study (Jo *et al.*, 2014), more than 300 MPa (3 min holding time) was required to achieve the pasteurization effect of chilled abalone, and the shelf-life of abalone could be extended for 10 days via 300 MPa pressurization. Still, protein denaturation was also found the pressurized abalone at higher than 300 MPa. Actually, increasing holding time is an alternate parameter contributing to the shelf-life of abalone in high pressure processing, although the impact of holding time on the shelf life of abalone was not noticeable rather than that of pressure level (Hong *et al.*, 2012). In the current study, therefore, optimization of high pressure processing (pressure level, holding

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time and storage period) to minimize quality deterioration of chilled abalone was conducted using response surface methodology.

Materials and Methods

Materials A total of 150 live abalone (*Haliotis discus hannai*) were purchased from a local market. The abalone was shucked manually and digestive tracts were carefully removed. The abalone muscles were cleaned using running water and each three abalone was vacuum-packaged (total 50 packages) using a poly nylon pouch and kept at 4°C in a refrigerator prior to pressurization (within 4 h). For experimental replications, the abalone was purchased on three different days (n=3). All chemicals used in this study were analytical grade.

High pressure treatment Each of two sample packages (each three abalones) of a treatment was pressurized using a lab-scale high pressure system as described in our previous study (Jo *et al.*, 2014). In brief, the system consisted of a pressure vessel, pressure generator and pressure intensifier. Water was used as a pressure-transmitting medium. Compression and depression rates were controlled to 16 and 40 MPa/s. For each treatment, two sample packages were randomly selected. Once the targeted pressure level was reached, the samples were held under constant pressure level for given holding time as presented in Table 1. After depression, the sample packages were stored at 4°C in a refrigerator for given storage periods (up to 20 days). To monitor the quality changes of unpressurized control, ten packages were also stored at the same storage condition for given days.

Sampling The sampling of abalone was followed based on our previous study (Jo *et al.*, 2014). One package of each treatment was used for determination of total plate count (TPC). A 1 g portion of abalone was aseptically taken from the surface on the abalone foot muscle. The remaining portion of the muscle was used for total volatile basic nitrogen (TVB-N) and thiobarbituric acid-reactive substances (TBARS) determinations. From the other package, sample color was taken from the surface of the muscle, and cylindrical samples were taken using a borer (4 cm in diameter) for mechanical strength determination. The remaining portion of muscle was used to analyze pH and water-holding capacity (WHC). If possible, the same parts of the samples were selected for the same analysis.

Color The color of the abalone surface was determined using a CR-10 color reader (Konica Minolta Sensing Inc., Tokyo, Japan) with 8 mm measuring diameter and 8° illumination angle (CIE standard illuminant D₆₅). Prior to determination, the color reader was calibrated with white standard plate ($L^* = 97.83$, $a^* = -0.43$ and $b^* = +1.98$). The CIE L^* , a^* and b^* values were monitored as indicators of lightness, redness and yellowness, respectively. Total color difference (ΔE) between fresh control and each treatment was numerically calculated as follows:

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad \dots\dots \text{Eq. 1}$$

pH From the three abalone samples, each 5 g muscle was taken, and the muscles were placed into 20 mL distilled water in a bag-filter (Interscience, Saint Nom, France). The sample was mashed using a WS400 stomacher (Shanghai Zhisun Equipment Co. Ltd., Shanghai, China) for 3 min. The pH of the filtrated liquid was determined using a S220 pH meter (Mettler-Toledo GmbH, Greifensee, Switzerland).

Water-holding capacity (WHC) The WHC of the treatment was measured by the centrifugation method (Jo *et al.*, 2014). Each 1 g sample (m_1) from three abalone muscles was taken and placed on gauze in a 15 mL test tube. The tubes were centrifuged at 1,500 ×g for 15 min at 4°C. The pellet was carefully removed from the tube, and the tube along with gauze was weighed (m_2). The tube was dried at 102°C for 24 h and weighed again (m_3). The WHC of abalone was calculated from the following equation:

$$\text{WHC (\%)} = \left(1 - \frac{m_1 - m_2}{m_3}\right) \times 100 \quad \dots\dots \text{Eq. 2}$$

Mechanical strength For mechanical strength determination, each of the three cylinders was cut into four strips (1 × 1 × 2 cm), wrapped to prevent evaporative loss and tempered at ambient temperature (~18°C) for 1 h. The sample strips were sheared using a CT3 texture analyzer (Brookfield Labs Inc., Stoughton, MA, USA) equipped with a TA-SBA knife (Brookfield Labs Inc.) under the conditions of 1.5 mm/s test knife speed and a trigger load of 1 g.

Thiobarbituric acid-reactive substances (TBA-RS) The lipid oxidation of abalone muscle was measured by the method of Hoyland and Taylor (1991). TBA reagent was prepared by dissolving 0.69 g 2-thiobarbituric acid (TBA) in 100 mL distilled water followed by mixing with the same volume of acetic acid. A 20 g abalone muscle from each three abalone samples was blended with 10 mL distilled water and filtered using the bagfilter (Interscience). A 0.75 mL filtrate was mixed with 2.5 mL benzene and 2.5 mL TBA reagent. The mixture was centrifuged at 1,500 ×g for 10 min, and lower phase was filtered using a DISMIC 0.45 µm syringe filter (Advantec MFS, Inc., Tokyo, Japan) and boiled for 30 min. After cooling, absorbance at 530 nm was measured. The TBA-RS was calculated using a calibration curve with tetraethoxypropane as a standard. The TBA-RS was expressed by the amount of malonaldehyde (MA) equivalents (mg MA/kg sample)

Total volatile basic nitrogen (TVB-N) The TVB-N of abalone was determined based on micro-diffusion analysis (Conway, 1950). Conway reagent was prepared by mixing 2.5 mL of 0.066% (w/v) methyl red, 2.5 mL of 0.066% (w/v) bromocresol green, 5 g of H₃BO₃ and 100 mL of ethanol in 350 mL of distilled water. Each 20 g sample from three abalone muscles was blended with 10 mL of distilled water and filtered using the bagfilter (Interscience). A 2.5 mL of filtrate was mixed with 10 mL of 7.5% (w/v)

Table 1. Experimental design and high pressure processing conditions

Treatments	Coded unit			Uncoded value		
	X ₁	X ₂	X ₃	Pressure (MPa)	Time (min)	Storage (day)
1	0	0	0	250	8	10
2	0	0	$-\sqrt{2}$	250	8	0
3	0	0	0	250	8	10
4	0	$\sqrt{2}$	0	250	15	10
5	$\sqrt{2}$	0	0	480	8	10
6	1	1	-1	414	13	3
7	-1	1	1	86	13	17
8	1	-1	1	414	3	17
9	0	0	0	250	8	10
10	0	0	$\sqrt{2}$	250	8	20
11	-1	-1	1	86	3	17
12	0	0	0	250	8	10
13	$-\sqrt{2}$	0	0	20	8	10
14	1	1	1	414	13	17
15	-1	-1	-1	86	3	3
16	0	0	0	250	8	10
17	0	$-\sqrt{2}$	0	250	1	10
18	0	0	0	250	8	10
19	-1	1	-1	86	13	3
20	1	-1	-1	414	3	3

trichloroacetic acid (TCA). In the round of a Conway dish, 1 mL mixture and 1 mL saturated K₂CO₃ (60 g in 50 mL distilled water) were added as a releasing agent. As a trapping reagent, 1 mL of Conway reagent was added into the center of the dish. The dish was incubated at 37°C for 1 h and titrated using 20 mM H₂SO₄ until the color of the Conway reagent changed to red. For blank, 5% (w/v) TCA was used, and the TVB-N was calculated as

$$\text{TVB-N (mg\%)} = 0.28 \times (V_s - V_b) \times F \times D / 100 / S \dots \dots \text{Eq. 3}$$

where V_b , V_s , F , D , and S indicate titration volume (mL) of the blank and sample, titer of 20 mM H₂SO₄, dilution factor, and sample amount, respectively.

Total plate count (TPC) TVC of abalone was measured by the method of Hsu *et al.* (2014). Each 1 g sample taken aseptically from three abalones was blended with 9 mL sterilized saline water (0.9% NaCl) in the bagfilter (Interscience) using the WS400 stomacher (Shanghai Zhisun Equipment Co.). A 1 mL of filtrate was diluted down to 10⁷ using the sterilized saline water and plated on plate count agar (Sigma-Aldrich Co., St. Louis, MO, USA) and incubated at 37°C for 2 days. The plates with visual colonies of 30–300 were counted, and TPC was expressed as the logarithm of the number of colony-forming units (log CFU/g).

Optimization All contour plots were superimposed to find optimal pressure condition for abalone. Shelf-life of abalone was evaluated in the ranges of < 1.0 mg MD/kg TBARS, < 20 mg% TVB-N and < 6 Log CFU/g TPC.

Statistical analysis To compare the data obtained from

untreated control, a randomized complete block design was adopted. The data was analyzed by one-way analysis of variance using SAS statistical program ver. 9.1 (SAS Institute, Cary, NC, USA). When the main effect (storage periods) was significant ($P < 0.05$), the differences among the means were compared using Duncan's multiple range test.

Response surface methodology was adopted to design the mathematical model using SAS 9.1 (SAS Institute). The central composite design was employed to optimize the best conditions of pressure level, holding time and storage period (as a shelf-life) coded as X₁, X₂ and X₃, respectively (Table 1). The processing condition consisted of 20 groups in which the center point had six replications to estimate pure error. Each parameter was represented by a second-order polynomial expression as

$$Y = \beta_0 + \sum_i \beta_i X_i + \sum_i \beta_{ii} X_i^2 + \sum_{i < j} \beta_{ij} X_i X_j \dots \dots \text{Eq. 4}$$

where Y , β_0 , β_i , β_{ii} , and β_{ij} were dependent variable, coefficients from constant, linear, quadratic, and interaction terms of independent variables, respectively. X_i and X_j were the levels of independent variables, and k was the number of independent variables. The significances of all terms in the polynomial were judged statistically by computing the F-value at a probability (P) of 0.001, 0.01 or 0.05 (Lee *et al.*, 2006).

Results and Discussion

Changes of fresh abalone during storage All estimated quality parameters of abalone was affected by chilled storage period (Table

Table 2. Physicochemical properties of abalone during refrigerated storage

Physicochemical properties	Storage (day)				
	0	3	10	17	20
pH	6.30 ± 0.02 ^a	5.89 ± 0.05 ^c	5.95 ± 0.05 ^c	6.22 ± 0.05 ^b	6.33 ± 0.02 ^a
Mechanical strength (Kg)	3.30 ± 0.72 ^a	1.26 ± 0.28 ^b	0.90 ± 0.21 ^c	0.75 ± 0.14 ^c	0.72 ± 0.09 ^c
WHC (%)	89.8 ± 2.82 ^a	86.1 ± 2.20 ^b	80.3 ± 1.38 ^d	84.9 ± 1.93 ^{bc}	81.8 ± 1.36 ^{cd}
<i>L</i> *	68.6 ± 1.62 ^a	67.3 ± 0.83 ^a	63.8 ± 2.57 ^b	59.8 ± 2.60 ^c	60.1 ± 2.73 ^c
<i>a</i> *	0.85 ± 0.39 ^b	1.91 ± 0.74 ^a	0.52 ± 0.58 ^b	-0.53 ± 0.65 ^c	-1.38 ± 0.37 ^d
<i>b</i> *	14.6 ± 1.43 ^b	16.6 ± 3.11 ^a	13.0 ± 1.95 ^b	9.14 ± 1.65 ^c	5.47 ± 0.63 ^d
ΔE	1.89 ± 0.89 ^d	3.49 ± 2.25 ^d	5.66 ± 2.00 ^c	10.5 ± 2.93 ^b	12.8 ± 1.92 ^a
TBARS (mg MD/kg)	0.10 ± 0.27 ^e	0.14 ± 0.22 ^d	0.30 ± 0.24 ^c	0.41 ± 0.26 ^a	0.36 ± 0.25 ^b
TVB-N (mg%)	10.7 ± 0.38 ^d	13.5 ± 0.47 ^d	45.2 ± 3.95 ^c	65.9 ± 4.66 ^b	84.7 ± 10.6 ^a
TPC (Log CFU/g)	3.61 ± 0.57 ^c	3.47 ± 0.55 ^c	4.74 ± 0.21 ^b	4.87 ± 0.29 ^b	6.34 ± 0.09 ^a

^{a-c}Means (±S.D.) with different superscript within same row are significantly different ($P < 0.05$).

2). The pH of abalone showed a drastic decrease down to 5.89 after 3 days of storage ($P < 0.05$), thereafter a gradual increase in pH was found for 10 days of storage. After 17 days of storage, the increase in pH of abalone was significant with the storage period ($P < 0.05$) and reached to 6.33 at the end of storage (20 days). The former decrease in pH of abalone was thought to muscle glycolysis accumulating lactic acid in the muscle tissue. Based on the report of Fluckiger *et al.* (2011), abalone is rich in glycogen, the major energy source of abalone. Post-mortem glycolysis accounted for the decrease in pH of abalone during the initial storage period (Brown *et al.*, 2008). Meanwhile, enzymatic proteolysis would contribute to the increase in pH of abalone muscle during the prolonged storage period (Ashie and Simpson, 1997). The proteolysis of abalone muscle caused tissue degradation as confirmed by significant decrease in the mechanical strength of abalone at 10 days of storage ($P < 0.05$). The WHC of abalone also showed a significant decrease at 10 days of storage ($P < 0.05$), which was likely manifested by protein oxidation. In the present study, TBA-RS of abalone showed a significant increase at 17 days of storage ($P < 0.05$), although the values were lower than 0.5 mg MD/kg throughout the storage periods. The low TBA-RS of abalone reflected the small amount of lipid content in foot muscle of abalone. Nevertheless, Xiong (2000) indicated that the protein oxidation was promoted by lipid oxidation in vice versa, and these phenomena were involved in quality deteriorations such as loss of WHC. All CIE color parameters of abalone decreased during storage period ($P < 0.05$), although *a** and *b** of abalone stored for 3 days were higher than the fresh control ($P < 0.05$). As identified by Tajima *et al.* (1980), β -carotene is the major muscle pigment of abalone. Degradation of the pigment during storage was possibly responsible to the decrease of the color parameters. TVB-N of abalone stored within 3 days exhibited less than 20 mg%, after while TVB-N proportionally increased with the storage periods ($P < 0.05$). In particular, abalone stored for 10 days showed 45.2 mg% of TVB-N, which generated the strong off-flavor. Simultaneously, the total plate count also showed a significant increase after 3 days

of storage ($P < 0.05$). Based on the above results, the shelf-life of abalone was around 3 days under refrigerated storage, while physicochemical changes abalone was already detected at 3 days of storage. To extend shelf-life of abalone in refrigerator, therefore, suppressing activities of proteolytic enzymes and contaminant microorganisms were required.

Statistical analysis of model Table 3 shows the dependent variables estimated as physicochemical qualities of pressurized abalone. The data was fitted by second-order polynomial regression model and the correlation coefficients are shown in Table 4. All estimated regression models well explained the data variation and significantly represented the relationship between independent variables and responses. All models were statistically significant ($P < 0.05$) without lack-of-fit ($P > 0.05$) and showed higher than 0.61 of *R*-square values.

pH of pressurized abalone The main contributor affecting the pH of abalone was pressure level (Fig. 1). At day 5, the estimated pH of abalone was generally proportional to the pressure level. Increasing holding time tended to slightly increase the pH of abalone. At day 10, the pH of abalone was slightly higher than those estimated at day 5, whereas the similarity of the pH pattern of abalone as functions of pressure and time was maintained. Alternately, the pattern of pH changed at day 15 at which point the abalone pressurized at < 200 MPa showed noticeable increase in pH. For regression model, pressure level and holding time had significant quadratic and interaction effects on the pH of abalone ($P < 0.01$). It was believed that the changes in pH of pressurized abalone were related to structural changes in muscle pH.

Immediately after pressurization, Jo *et al.* (2014) reported that the abalone showed lower pH with increasing pressure up to 200 MPa, and thereafter increased again. According to the glycogen content of abalone foot muscle, the former pH decline of abalone would be accounted for the acceleration of post-mortem glycolysis under high pressure (Macfarlane, 1973). Alternately, protein denaturation was favorable at excessive pressure levels (> 300 MPa), which manifested the increase in pH of abalone. During

Table 3. Experimental results of physicochemical properties of pressurized abalone

Treatments	Physicochemical properties						
	pH	Mechanical strength (Kg)	WHC (%)	ΔE	TBA-RS (mg MD/kg)	TVB-N (mg%)	TPC (Log CFU/g)
1	5.67	1.66	81.5	4.07	0.23	18.6	6.25
2	6.31	2.95	89.7	6.42	0.18	11.0	2.00
3	5.74	2.24	80.9	4.19	0.44	24.9	6.42
4	5.97	2.36	81.0	4.85	0.19	18.3	6.16
5	6.55	1.29	83.8	1.55	0.19	21.3	5.17
6	6.59	2.37	86.3	5.88	0.09	19.3	2.15
7	6.32	0.60	87.4	4.11	0.40	60.6	7.13
8	5.99	1.83	84.2	4.58	0.22	20.7	4.15
9	5.72	1.46	78.7	3.05	0.46	21.4	6.03
10	5.97	0.63	82.0	5.99	0.61	38.5	7.59
11	6.18	0.69	85.6	5.70	0.44	60.4	6.31
12	6.10	1.48	80.5	4.05	1.19	25.4	6.56
13	5.87	0.62	81.1	5.08	0.21	32.2	5.67
14	6.36	1.62	84.6	1.78	0.14	21.9	4.08
15	5.92	1.82	87.4	3.77	0.24	16.5	3.71
16	5.78	1.79	82.7	3.43	0.35	22.5	6.42
17	5.75	1.34	80.2	2.90	0.31	17.9	6.58
18	5.79	2.34	78.5	4.85	0.27	17.1	6.55
19	5.93	1.46	88.6	7.44	0.14	13.8	4.07
20	6.39	2.33	84.6	5.05	0.10	13.8	2.51

Table 4. Regression coefficient for seven dependent variables for pressurized abalone

Term ^a	pH	Shear force	WHC	ΔE	TBA-RS	TVB-N	TPC
β_0	6.25	1.59	93.7	2.43	-2.35	9.60	0.21
<i>Linear</i>							
β_1	-0.20×10^{-2}	0.01*	-0.03	0.01	0.02	-0.04	0.01*
β_2	-2.84×10^{-2}	-0.05	-0.24	0.52*	0.59	0.07	0.2
β_3	-4.96×10^{-2}	-0.12	-1.55*	-0.20	0.36	2.72**	0.67***
<i>Quadratic</i>							
β_{11}	7.74×10^{-6} **	-0.01×10^{-3} *	5.86×10^{-5}	-0.11×10^{-4} *	-0.50×10^{-4} *	0.15×10^{-3} *	-0.28×10^{-4} *
β_{22}	1.18×10^{-3}	0.04×10^{-3}	0.03	-0.19×10^{-3}	-0.04	-0.02	-0.01
β_{33}	3.52×10^{-3} **	0.97×10^{-3}	0.07***	0.02**	-0.68×10^{-2}	0.06	-0.02**
<i>Interaction</i>							
β_{12}	6.40×10^{-5}	0.04×10^{-3}	-1.45×10^{-4}	-0.62×10^{-3}	0.90×10^{-4}	0.14×10^{-2}	-0.25×10^{-3}
β_{13}	-1.39×10^{-4} **	0.08×10^{-3}	0.97×10^{-4}	-0.35×10^{-3}	0.32×10^{-3}	-0.01***	-0.23×10^{-3}
β_{23}	1.07×10^{-3}	0.05×10^{-3}	-0.21×10^{-2}	-0.03**	0.11×10^{-3}	-0.01	0.27×10^{-2}
R^2	0.88	0.77	0.79	0.85	0.61	0.95	0.90
P	0.00**	0.02*	0.02*	0.00**	0.05*	0.00***	0.00***

^aSubscription numbers 1-3 represent pressure level, holding time and storage day, respectively.

*Significant at 0.05 level.

**Significant at 0.01 level.

***Significant at 0.001 level.

storage, spoilage of abalone was responsible for the increase in muscle pH. The pH of abalone pressurized at > 300 MPa was maintained stably during storage, while those treated at < 200 MPa showed a great increase in pH after 15 days of storage. Greater than 300 MPa of high pressure effectively deactivated the

microorganisms and proteolytic enzymes, which was confirmed by the lack of change in pH of abalone during the storage period. However, the pressure level of < 200 MPa had no effect on the microbials and enzymes, and probably showed an increase in pH during the storage period. The results suggest that the impact of

Fig. 1. Effects of pressure level and holding time on the pH of abalone estimated in storage period of (a) 5, (b) 10 and (c) 15 days, respectively.

Fig. 2. Effects of pressure level and holding time on the mechanical strength of abalone estimated in storage period of (a) 5, (b) 10 and (c) 15 days, respectively.

high pressure on the abalone quality was dependent on the applied pressure levels.

Mechanical strength of pressurized abalone Compared to 3.30 Kg of fresh abalone, the estimated responses revealed lower than 2.5 Kg after 5 days of storage (Fig. 2). In particular, abalone treated at relatively low pressure levels (< 200 MPa) showed a noticeable decrease in mechanical strength. During storage, the decrease in mechanical strength was still observed. Alternately, 300 – 400 MPa treatment had relatively high mechanical strength during the storage period, although the abalone-treated at these pressure levels also showed the decrease in mechanical strength. The holding time did not generally affect the mechanical strength of the pressurized abalone. Based on the regression model, the pressure level showed a linear and quadratic effect on the mechanical strength of abalone ($P < 0.05$).

The higher mechanical strength of pressurized abalone was also identified by our previous study (Jo *et al.*, 2014). Applied high pressure deactivated the endogenous proteolytic enzymes, thereby showing less degradation of muscle tissue during storage. The maximum mechanical strength of abalone was obtained by 300 – 400 MPa of pressure level, but the mechanical strength was decreased during the storage period from ~2.5 Kg (5 days) to lower

than 2.0 Kg (15 days), reflecting that the enzymes still had an ability to degrade the muscle tissue. Nevertheless, the relatively high mechanical strength of pressurized abalone could be explained by pressure-induced cold-shortening of muscle myofibrils. Jung *et al.* (2000) observed that pressurized myofibrils had a higher dimension than the untreated control. The increase in dimension of myofibrils was evidence of shortening the myofibrils, and this was correlated with tenderness of muscle (Jung *et al.*, 2000). Since the mechanical strength was an important quality indicator of abalone, pressurization showed a potential advantage in delaying the muscle tenderization during storage.

WHC of pressurized abalone The main factor affecting the WHC of pressurized abalone was storage period, which showed significant linear ($P < 0.05$) and quadratic effect ($P < 0.001$) in the model. As depicted in Fig. 3, the pH of abalone decreased with increasing pressure up to 200 – 300 MPa, and thereafter increased again. Holding time also showed a similar pattern of WHC, while the impact of holding time was not as high as shown in pressure level. The WHC of abalone was lower than 89.8% of the fresh control after 5 days of storage, and the decrease in WHC of abalone was greater after 10 days of storage. Meanwhile, the WHC of abalone stored for 15 days was stabilized compared to those for 10

Fig. 3. Effects of pressure level and holding time on the water-holding capacity (WHC) of abalone estimated in storage period of (a) 5, (b) 10 and (c) 15 days, respectively.

Fig. 4. Effects of pressure level and holding time on the total color difference (TCD) of abalone estimated in storage period of (a) 5, (b) 10 and (c) 15 days, respectively.

days of storage.

The WHC of pressurized abalone appeared to be related with pH. The minimal WHC was obtained at the pressure level of around 200 MPa, at which the post-mortem glycolysis was accelerated by accumulating lactic acid in the tissue. As a result, the pH of abalone was low, and this manifested the low WHC of abalone. However, it was unclear why the WHC of abalone pressurized at higher than 300 MPa increased with increasing pressure level. An explanation for this could be found in the myofibrillar protein playing a key role in retaining moisture in the muscle (Lakshmanan *et al.*, 2007), and the protein network induced by pressurization was involved in the improved WHC of the pressurized muscle. Consequently, the results demonstrated that quality modification was inevitable when the pressure higher than 300 MPa was applied to abalone.

Color of pressurized abalone For color estimation, CIE L^* , a^* and b^* parameters were converted to total color difference (ΔE) to simplify the optimization of abalone quality (Fig. 4). Interestingly, the holding time affected the ΔE of abalone in the model where the linear ($P < 0.05$) and interaction effect with the storage period ($P < 0.01$) were estimated significantly. In addition, the quadratic effect of the storage period was also found ($P < 0.001$). At the initial

stage of storage (5 days), increasing pressure level and holding time tended to increase the ΔE of abalone. However, the ΔE pattern of abalone changed with extending the storage period (15 days), and raising the pressure levels and holding times, lowered the ΔE of abalone.

As identified by fresh abalone, the changes in color during storage were characterized by loss of lightness, redness, and yellowness. Meanwhile, pressure-induced color changes of abalone were distinguished from that of fresh abalone. At the early storage (5 days), L^* of abalone was increased with increasing either the pressure level and holding time, while the L^* of pressurized abalone decreased after 10 days of storage, thereafter being stable with the storage period. Linear and quadratic effects ($P < 0.01$) of the storage period was shown in L^* of abalone. In general, the increase in L^* of pressurized muscle was related with muscle protein denaturation, while a^* and b^* were attributed by a balance of chemical redox reaction (Chun *et al.*, 2014). The storage period also showed a significant linear and quadratic effects on a^* and b^* of abalone ($P < 0.001$). For a^* of abalone, interaction term of pressure and holding time was significant ($P < 0.05$). The pattern of a^* and b^* of pressurized abalone was similar to those of L^* value, reflecting an increase with increasing pressure levels and

Fig. 5. Effects of pressure level and holding time on the thiobarbituric acid-reactive substances (TBA-RS) of abalone estimated in storage period of (a) 5, (b) 10 and (c) 15 days, respectively.

Fig. 6. Effects of pressure level and holding time on the total volatile basic nitrogen (TVB-N) of abalone estimated in storage period of (a) 5, (b) 10 and (c) 15 days, respectively.

holding times. Under pressurization, the oxidative enzymatic system was easily inactivated, while the reducing system enhanced its activity by pressurization (Chun *et al.*, 2014). In addition, both values decreased with increasing the storage period. In the present study, ΔE of abalone treated at higher pressure level for longer periods tended to decrease with storage period. It should be noted that the decrease in the ΔE of abalone treated at higher pressure and longer duration in storage did not guarantee the freshness of abalone color because this phenomenon was attributed to decreased L^* , which resulted from the low WHC.

Lipid oxidation of pressurized abalone As indicated by the fresh control, the lipid oxidation did not exceeded 0.5 mg MD/kg throughout the storage period (20 days), hence the abalone is stable against lipid oxidation. For pressurized abalone, TBA-RS showed a dome shape response on the pressure-time plane after 5 days of storage, and the pattern of TBA-RS was maintained during the storage period. An important factor affecting lipid oxidation of pressurized abalone was pressure level, which showed a quadratic effect in regression model ($P < 0.05$). The maximum TBA-RS of pressurized abalone after 15 days of storage was obtained at 200 MPa for 6–9 min condition. Nonetheless, the TBA-RS was lower than 0.5 mg MD/kg.

As shown in our previous study (Jo *et al.*, 2014), abalone foot

muscle was stable against lipid oxidation during long-term storage. According to Dunstan *et al.* (1996), abalone foot muscle contained a minor amount of lipid (0.78–1.33%). In addition, peptides from the abalone muscle hydrolysis, which was evidenced by tissue degradation, had an antioxidant activity (Chiou *et al.*, 2002; Zhou *et al.*, 2012). Eventually, a chilled storage condition might also have an influence on the low TBA-RS of abalone during the storage period.

TVB-N of pressurized abalone The TVB-N of the control was 10.7 mg%, which suggested that the abalone was in a fresh state. As shown in Fig. 6, the TVB-N of pressurized abalone slightly increased after 5 days of storage. In particular, the high increase in TVB-N of abalone was found at relatively low pressure levels (< 100 MPa). During storage, TVB-N of abalone pressurized at lower than 200 MPa showed a drastic increase, whereas the abalone-treated at > 400 MPa did not show an increase of TVB-N. Holding time did affect the TVB-N of pressurized abalone. As shown in the regression coefficient, quadratic pressure effect ($P < 0.001$), linear storage effect ($P < 0.05$), and the interaction effect of pressure and storage ($P < 0.001$) were involved in the TVB-N of pressurized abalone.

TVB-N has been adopted as an indicator of freshness of marine resources. Chiou *et al.* (2002) postulated that the shelf-life of

Fig. 7. Effects of pressure level and holding time on the total plate count (TPC) of abalone estimated in storage period of (a) 5, (b) 10 and (c) 15 days, respectively.

abalone was 3.5 days at 5°C where the TVB-N and free amino acid contents showed a gradual increase during storage. Consequently, the overall shelf-life of abalone could be estimated by TVB-N. According to the Connell (1990), < 20 mg% was maximum limit of TVB-N for freshness of fishes, hence, this study demonstrated that the high pressure was effective technique to extend the shelf-life of abalone. At the pressure level of < 200 MPa, TVB-N sharply increased during storage, indicating that the moderate pressurization had no effect on the shelf-life of abalone. Contrarily, abalone pressurized at > 400 MPa showed low TVB-N during 15 days of storage. This phenomenon is the result of microbial and enzymatic inactivation. Similar results were also obtained by Briones *et al.* (2010) who suggested that pressurization at 550 MPa for 3 and 5 min extended the shelf life of abalone to 65 days. However, the quality modifications such as color and WHC should be also considered, which warranted further exploration.

TPC of pressurized abalone Abalone TPC was closely related with both storage period and pressure level (Fig. 7). During storage period, an increase in abalone TPC was shown, particularly abalone pressurized at < 300 MPa exhibited a proportional increase in TPC during storage, and reached to ~7 log CFU/g after 15 days of storage. Meanwhile, abalone treated at 500 MPa showed a slight increase in TPC during storage, and even had < 4 log CFU/g after 15 days of storage. Holding time hardly contributed to the abalone TPC at a given pressure level.

It is believed that the higher the pressure level, the more effective the microbial inactivation. At least 400 MPa was required to achieve the pasteurization effect of chilled abalone. However, it should be noted that the main quality deterioration of abalone during storage was manifested by protein degradation with a simultaneous increase in TVB-N. Based on the purpose of this study, extending the shelf-life of abalone to 10 days had an advantage in chilled abalone distribution, hence microbial spoilage was not considered as important as TVB-N under refrigerated storage. Consequently, the results indicated that > 400 MPa of pressurization was effective in extending the shelf-life of abalone

for long-term storage (> 15 days). However, pressure-induced quality deteriorations were also found. Moderate pressurization (< 200 MPa) did not contribute to quality modification, but this condition had no preservative effect. By defined the maximum shelf-life of abalone is 10 days, 200 – 300 MPa of pressurization with short processing time (~3 min) was an optimal condition to extend the shelf-life without severe quality loss of abalone.

Conclusion

This study demonstrated the effects of pressure level, holding time, and storage period on the physicochemical properties of abalone. Main factors affecting the physicochemical properties of abalone were pressure level and storage period. Holding time rarely affected the physicochemical properties of abalone, suggesting long processing time was not necessary for high pressure processing. Based on the result, extremely high pressure (> 400 MPa) extended shelf-life to more than 15 days, while quality deterioration such as loss of WHC was involved. Moderate pressure (< 200 MPa) did not extend shelf-life of the abalone. Pressurization at 200 – 300 MPa for few minutes (~3 min) was an optimal processing condition to extend shelf-life of abalone for ~ 10 days, which provided a practical application in the chilled abalone industry.

Acknowledgement This research was a part of the project titled ‘Development of non-thermal processing technology for abalone using high pressure technology’ funded by the Ministry of Oceans and Fisheries, Korea.

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