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Effect of high pressure processing on the quality of squid (*Todarodes pacificus*) during refrigerated storage

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ABSTRACT

The influence of high pressure processing (HPP) on the inhibition of trimethylamine-*N*-oxide demethylase (TMAOase) activity and off-odour production in squid treated at 300 MPa for 20 min was investigated during 12 days of refrigerated storage. TMAOase activity of raw squid (21.5 nkat/g) was significantly decreased to approximately 5 nkat/g after 20 min of HPP. The production of dimethylamine (DMA) in HPP-treated squid for 20 min was significantly decreased to 0.31 μmol/g after 12 days of storage. The decrease in DMA was correlated with the decrease in TMAOase activity. At 300 MPa, the number of total aerobic bacteria in squid was reduced by 1.26 log units after 20 min of HPP. The HPP-treated samples effectively reduced the amount of trimethylamine (TMA). Therefore, the HPP could be used as a promising alternative technology to retard the quality deterioration of squid by inhibiting TMAOase activity and microbial growth.

1. Introduction

Squid (*Todarodes pacificus*), the class of Cephalopod, is one of the most popular seafood products in oriental countries, including China, Japan and Korea. Raw and semi-dried squid products are commonly consumed. However, the squid products are likely to produce various off-odour components during refrigerated and frozen storage. The quality deterioration of squid products is mainly caused by trimethylamine-*N*-oxide (TMAO) reduction and microbial contamination (Chiou, Chang, Lo, Lan, & Shiau, 2000; Fu et al., 2006). TMAO that plays an important role as an osmoprotectant is abundant in fish (Barrett & Kwan, 1985; Fu et al., 2006; Santos, Iobbi-Nivol, Couillault, Giordano, & Mejean, 1998). TMAO is converted to dimethylamine (DMA), formaldehyde (FA), and trimethylamine (TMA), which are responsible for characteristic fishy odours during storage (Benjakul, Visessanguan, & Tanaka, 2004; Kimura & Kimura, 2001; Santos et al., 1998). Two main possible pathways for the degradation of TMAO have been proposed. One is that the enzymatic reaction produces equimolar DMA and FA from TMAO, which is catalysed by trimethylamine-*N*-oxide demethylase (TMAOase) (Fu et al., 2008; Leelapongwattana, Benjakul, Visessanguan, & Howell, 2008b; Nitisewojo & Hultin, 1986; Stanley & Hultin, 1984). The other suggested pathway is a microbial spoilage reaction, resulting in redox-potential decrease, pH increase, and electrical conductance increase, in which TMAO degrades to trimethylamine (TMA) (Ayensa & Gomez-Guillen, 1999; Fu et al., 2008; Krzymien & Elias, 1990; Lundstrom & Racicot, 1983; Nitisewojo & Hultin, 1986; Stanley & Hultin, 1984).

With increasing concern about the quality and safety, the control of off-odours and microbial spoilage during storage has been a major endeavour of the seafood industry, which has led to continuous developments in preservation techniques. Low-temperature storage is mainly used to extend shelf-life and retard microbial growth (Leelapongwattana, Benjakul, Visessanguan, & Howell, 2008a; Wang, Liceaga-Gesualdo, & Li-Chan, 2003). However, the enzymatic and microbial reduction of TMAO could occur during prolonged low-temperature storage of seafood products, leading to quality deterioration (Krzymien & Elias, 1990; Lundstrom, Correia, & Wilhelm, 1982; Matser, Stegeman, Kals, & Bartels, 2000; Nielsen & Jorgensen, 2004). Chemical preservatives such as sodium citrate, pyrophosphate, hydrogen peroxide, sodium alginate, sucrose, and sorbitol have also been employed as chelating agents and cryoprotectants in seafood products to decrease the TMAOase activity (Leelapongwattana et al., 2008b; Parkin & Hultin, 1982). Recently, high pressure processing (HPP) has attracted great attention because it can effectively inhibit pathogenic and spoilage bacteria with less adverse effects on the nutritional quality and organoleptic properties (Amanatidou et al., 2000; Diez et al., 2008). HPP can control the fish enzymes, including cathepsin C, collagenase, chymotrypsin, and trypsin-like enzymes, for preserving fresh-like texture (Ashie & Simpson, 1996;

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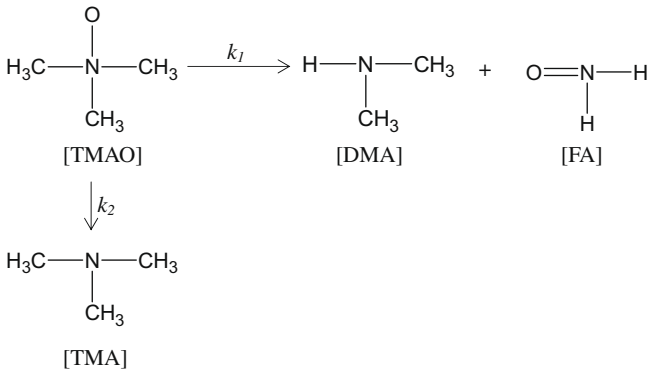

Fig. 1. Postulated reactions for DMA, FA, and TMA formation. k_1 and k_2 represent the rate constants catalysed by TMAOase and microbial growth, respectively.

Cheret, Hernandez-Andres, Delbarre-Ladrat, de Lamballerie, & Verrez-Bagnis, 2006). However, relatively few studies have focused on the effect of HPP on the reduction of unpleasant fish off-odours produced in the postulated reactions (Fig. 1). Therefore, the objective of this study was to investigate the potential of using HPP to reduce the formation of DMA and TMA in squid as measured by TMAOase activity and microbial growth during refrigerated storage. To our knowledge, the present study is the first application of high pressure processing to retard the off-odour development in squid.

2. Materials and methods

2.1. Chemicals

TMAO, DMA, TMA, and FA were purchased from Sigma–Aldrich Chemicals Inc. (St. Louis, MO, USA). Solvents for the extraction of TMAOase, DMA, and TMA were obtained from Fisher Scientific Inc. (Fair Lawn, NJ, USA). Ascorbic acid and L-cysteine were acquired from Fluka Chemical Co. (Milwaukee, WI, USA) and Tris was purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA).

2.2. Sample preparation

Squids (*T. pacificus*) were purchased from the local fisheries market (National Federation of Fisheries Cooperatives, Chuncheon, Gangwon, Korea) within 48 h of capture (February 2009) and transported in ice to the laboratory. The average weight of squids was 0.7 kg (approx. 35 cm length). The squid samples were eviscerated, and each squid sample (25 g of mantle) was vacuum-packaged under a vacuum setting of 20 kPa and sealing time of 0.1 s (Innova, Nozzle Vacuum Sealer, Gasungpak Co., Ltd., Gwanju, Gyeonggi, Korea). Sample pouches (5 × 3 cm, 0.0762 mm thickness) were made from sterile polyethylene filter bags (01-002-57; Fisher Scientific).

2.3. High pressure treatment

The packaged samples were loaded into a high pressure processor (QFP-6, Flow Autoclave Systems, Columbus, OH, USA) and subjected to 300 MPa at 20 °C for 0, 5, 10, and 20 min. The high pressure-treated samples were stored at 4 °C for 0, 4, 8, and 12 days. The pressurisation rate was approximately 5.4 MPa/s, and the depressurisation occurred in less than 10 s. The process hold time did not include the pressure come-up or the de-pressurisation times. The samples without high pressure treatment (0.1 MPa) were used as the control.

2.4. pH measurement

Each sample (25 g) was thoroughly mixed with 50 ml of distilled water for 3 min in a high speed mixer (SFM-555SP; Shinil Industrial Co., Ltd., Seoul, Korea). pH values were measured for all treatments on day 0, 4, 8, and 12 by using a pH metre (Mettler–Toledo International Inc., Toledo, OH, USA).

2.5. TMAOase extraction

The TMAOase extract was according to the methods of Benjakul et al. (2004) with slight modifications. The treated samples (25 g each) were mixed with 50 ml 20 mM Tris–acetate buffer (TA; pH 7.0) containing 0.1 M NaCl and 0.1% Triton X-100. The mixtures were thoroughly blended for 3 min using a Kitchen Aid mixer and centrifuged at 38,500*g* for 30 min at 4 °C (Supra 22 K Plus, High Speed Refrigerated Centrifuge, Hanll Science Industrial Co., Ltd). The supernatants were collected and used as TMAOase crude extract.

2.6. TMAOase activity assay

The TMAOase activity was measured using TMAO as a substrate in the presence of cofactors (cysteine, ascorbate, and $FeCl_2$). The enzymatic reaction of the crude extract (0.5 ml) was initiated by the addition of 24 mM TA (2.5 ml) containing 24 mM TMAO, 2.4 mM cysteine, 2.4 mM ascorbate, and 0.24 mM $FeCl_2$ (pH 7.0). The mixtures were incubated in a water bath (Fisher Scientific) at 25 °C for 20 min. The reactions were terminated by adding 30% trichloroacetic acid (TCA). The reaction mixtures were immediately cooled in an ice-bath to avoid further reactions and centrifuged at 8000*g* for 15 min. The collected supernatants were used for DMA determination. The blank without enzyme extract was run to estimate the nonenzymatical production of DMA, which was subtracted from the total production of DMA. TMAOase activity was expressed as 1 nmol of DMA production under the incubation with an enzyme for 1 s (nkat). The 'katal' is defined as the catalytic amount that produces 1 mol of DMA per second.

2.7. DMA and TMA determination

Samples (25 g) were homogenised with 50 ml of 7.5% cold TCA solution using a high speed mixer (SFM-555SP; Shinil Industrial Co., Ltd.) for 3 min. The homogenates were centrifuged at 3000*g* for 15 min, and the supernatants were neutralised with 1 M NaOH and used for the analyses of DMA and TMA.

2.7.1. DMA analysis

The copper-dithiocarbamate method of Dyer and Mounsey (1945) with slight modifications was used to determine DMA in the samples. The neutralised supernatant extract (2 ml) was thoroughly mixed for 2 min with 5 ml of 5% CS_2 in chloroform and 0.2 ml of alkaline solution containing 40% NaOH and NH_4OH (1:1), followed by the addition of 1 ml of copper–ammonia reagent and 1 ml of 30% acetic acid. The mixture was allowed to stand at 25 °C for 10 min. The chloroform layer was transferred into a screw-capped-test tube and mixed with 0.2 g of anhydrous sodium sulphate. The absorbance was measured at 440 nm using a Spectro UV–Vis Dual Beam Scanning Spectrophotometer. A standard curve for DMA was prepared at 0, 4, 8, 12, 16, and 20 mg/ml in the same manner as described above.

2.7.2. TMA analysis

TMA was measured using the colourimetric method described by Conway and Byrne (1936) with minor modifications. The sample extract (2 ml) was mixed with 1 ml of 20% FA (pH 7.0) to reduce

interference with the primary and secondary amines, 5 ml of anhydrous toluene, and 3 ml of saturated potassium carbonate in a glass test tube. The mixture was screw-capped, vortexed vigourously, and allowed to stand at 30 °C for 10 min. The toluene phase was mixed with 0.2 g of anhydrous sodium sulphate (Na_2SO_4) to remove traces of water. The water-free toluene was mixed with 5 ml of 0.02% picric acid. The absorbance was measured at 410 nm using a Spectro UV–Vis Dual Beam Scanning Spectrophotometer (Labomed, Inc., Culver City, CA, USA). A standard curve for TMA was prepared at 0, 2.5, 5.0, 7.5, 10.0, and 12.5 mg/ml in the same manner as described for the sample.

2.8. SDS–PAGE

The change in protein profiles during refrigerated storage was examined by using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) (Lakshmanan, Patterson, & Piggott, 2005). Each sample (25 g) was homogenised with 50 ml distilled water. The homogenate was centrifuged at 3000*g* for 15 min. The supernatant (0.2 ml) was mixed with an equal amount of denaturing Tris buffer (pH 6.7) containing 10% SDS, 0.002% 2-mercaptoethalnol, and 0.002% bromophenol blue. The mixture was boiled for 10 min and cooled immediately in an ice-bath. For SDS–PAGE analysis of proteins, 5% stacking gel and 8% separation gel were prepared. The denatured sample (10 μl) was loaded into a separate well of polyacrylamide gel at 130 V and 30–40 mA using the mini-PROTEAN 3 cell (Bio-Rad, Hercules, CA, USA). The gels were stained with Coomassie brilliant blue, and the molecular weights of the bands were estimated by the comparison with known protein molecular weight standards (Sigma–Aldrich Chemicals Inc.). The protein markers consist of myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), bovine serum albumin (66 kDa), glutamate dehydrogenase (55 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), and trypsin inhibitor (20 kDa).

2.9. Microbiological analysis

Duplicate samples (25 g) were aseptically mixed with 50 ml of 0.1% peptone water in sterile Whirl-Pak bags (Fisher Scientific, St. Louis, MO, USA). The mixtures were blended for 2 min in a Tekmar Stomacher® laboratory mixer (Stomacher Model 400, Tekmar Co., Cincinnati, OH). The numbers of total aerobic bacteria were directly determined by the pour plating method according to the US FDA Bacteriological Analytical Manual (BAM). Each treated sample was serially (1:10) diluted with peptone water and a proper dilution (1 ml) was pour-plated on trypticase soy agar (TSA). The agar plates were incubated at 20 °C for 48 h to determine the population of total aerobic bacteria.

2.10. Statistical analysis

All experiments were analysed in duplicate with three replicates. Data were analysed by the generalised linear model (GLM) procedure of the Statistical Analysis System (SAS). Least Significant Difference (LSD) procedure was used to determine significant differences among high pressure treatments at the 5% significance level.

3. Results and discussion

3.1. Effects of HPP on the inactivation of TMAOase and the formation of DMA during the refrigerated storage of squid

TMAOase activities in squid treated at 300 MPa for different pressure hold times are shown in Fig. 2. When compared to the control, TMAOase activities in the HPP-treated squid samples were significantly decreased ($p < 0.05$). The highest activity of TMAOase (22 nkat/g of squid) was observed in the control, whilst the HPP-treated samples after 5 min of pressure hold time showed less than 10 nkat/g, indicating that high pressure effectively inactivated the TMAOase. However, TMAOase is heat stable and more active after heating at 80 °C (Kimura, Kimura, & Seki, 2003). Thus, it is most likely responsible for quality loss of seafood through the enzymatic pathway. TMAOase activity is directly related to the formation of DMA and FA, which are equimolarly produced (Benjakul et al., 2004). FA is not easily extracted due to its tissue-binding property. In this study, the production of FA can be estimated by that of DMA. FA is responsible for the loss of functionality and texture as results of denaturation and aggregation of proteins (Fu et al., 2007; Leelapongwattana, Benjakul, Visessanguan, & Howell, 2005). Because the production of DMA is directly related to the poor quality of frozen seafood products, it is used as an indicator of quality loss during storage (Lundstrom et al., 1982). The changes in DMA content were observed in squid treated at 300 MPa for different pressure hold times (Table 1). On day 0, the amounts of DMA (0.21–0.34 μmol/g) were not significantly different among all treatments. The formation of DMA in the control rapidly was increased up to 8 days of refrigerated storage. This result might be attributed to TMAOase activity under refrigerated storage in the absence of oxygen (Lundstrom et al., 1982). The DMA contents

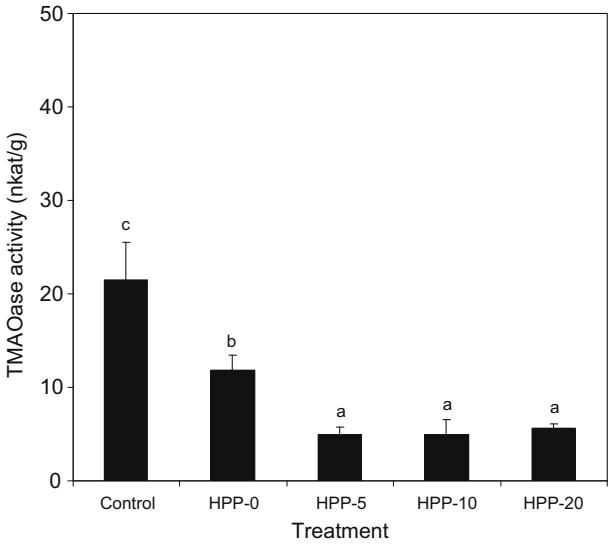

Fig. 2. TMAOase activities of the control and the squid samples treated at 300 MPa for 0, 5, 10, and 20 min. (0 min, HPP-0; 5 min, HPP-5; 10 min, HPP-10; 20 min, HPP-20).

Table 1
DMA content (μmol/g) of squid treated at 300 MPa for different pressure hold times during refrigerated storage.

Treatment[a]	Storage time (day)			
	0	4	8	12
Control	0.34 ± 0.06b,A[b]	1.45 ± 0.10b,B	3.62 ± 0.49b,C	3.69 ± 0.17d,C
HPP-0	0.21 ± 0.03a,A	0.42 ± 0.03a,B	0.74 ± 0.05a,C	1.14 ± 0.06c,D
HPP-5	0.26 ± 0.04ab,A	0.35 ± 0.02a,A	0.47 ± 0.03a,B	0.71 ± 0.04b,C
HPP-10	0.25 ± 0.03ab,A	0.30 ± 0.02a,A	0.40 ± 0.03a,B	0.44 ± 0.02a,B
HPP-20	0.28 ± 0.02ab,A	0.32 ± 0.05a,A	0.37 ± 0.07a,A	0.31 ± 0.03a,A

[a] HPP-0; HPP-5; HPP-10; and HPP-20 represent 0, 5, 10, and 20 min of pressure hold times.
[b] Means with different letters (A–D) within a row are significantly different at $p < 0.05$ and means with different letters (a–d) within a column are significantly different at $p < 0.05$.

Table 2
Changes in microbial population (log cfu/g) on squid treated at 300 MPa for different pressure hold times during refrigerated storage.

Treatment[a]	Storage time (day)			
	0	4	8	12
Control	3.91 ± 0.25c,A[b]	4.85 ± 0.15d,B	6.35 ± 0.10e,C	7.28 ± 0.12e,D
HPP-0	3.64 ± 0.17c,A	4.50 ± 0.10d,B	4.92 ± 0.13d,B	4.98 ± 0.26d,B
HPP-5	3.14 ± 0.11b,A	3.69 ± 0.14c,B	3.68 ± 0.11c,B	3.69 ± 0.20c,B
HPP-10	2.96 ± 0.18ab,A	3.25 ± 0.16b,AB	3.12 ± 0.12b,A	3.56 ± 0.11b,B
HPP-20	2.65 ± 0.15a,A	2.66 ± 0.17a,A	2.37 ± 0.06a,A	2.70 ± 0.09a,A

[a] HPP-0; HPP-5; HPP-10; and HPP-20 represent 0, 5, 10, and 20 min of pressure hold times.
[b] Means with different letters (A–D) within a row are significantly different at $p < 0.05$ and means with different letters (a–e) within a column are significantly different at $p < 0.05$.

Table 3
Changes in pH on squid treated at 300 MPa for different pressure hold times during refrigerated storage.

Treatment[a]	Storage time (day)			
	0	4	8	12
Control	6.61 ± 0.10a,A[b]	7.23 ± 0.12b,B	7.55 ± 0.15b,AB	7.81 ± 0.16b,C
HPP-0	6.62 ± 0.08a,A	6.70 ± 0.09a,A	6.79 ± 0.14a,A	6.84 ± 0.12a,A
HPP-5	6.69 ± 0.16a,A	6.60 ± 0.12a,A	6.69 ± 0.10a,A	6.66 ± 0.15a,A
HPP-10	6.64 ± 0.11a,A	6.57 ± 0.15a,A	6.69 ± 0.16a,A	6.63 ± 0.18a,A
HPP-20	6.61 ± 0.05a,A	6.53 ± 0.17a,A	6.60 ± 0.11a,A	6.59 ± 0.15a,A

[a] HPP-0; HPP-5; HPP-10; and HPP-20 represent 0, 5, 10, and 20 min of pressure hold times.
[b] Means with different letters (A–C) within a row are significantly different at $p < 0.05$ and means with different letters (a–b) within a column are significantly different at $p < 0.05$.

were increased with increasing storage time, except for HPP-20. On day 12, the DMA contents were significantly decreased with increasing pressure hold time. This result indicates that the DMA formation was retarded by HPP treatment.

3.2. Effects of HPP on the reduction of microbial growth and the formation of TMA during the refrigerated storage of squid

The number of total aerobic bacteria present in squid was approximately 3.91 log cfu/g as shown in Table 2. During the HPP treatment, the numbers of microorganisms were reduced by 0.27, 0.77, 0.95 and 1.26 log cfu/g after 0, 5, 10 and 20 min of pressure holding times, respectively. The initial population in the control significantly increased up to 7.28 log cfu/g after 12 days of the refrigerated storage. The numbers of total aerobic bacteria in the HPP-20 treated samples were not significantly increased throughout the storage period, mostly maintaining less than 3 log cfu/g. This observation suggests that HPP treatment led to potentially lethal damage, resulting in loss of proliferation of injured microorganisms. This confirms the physical mechanism that a bacterial cell volume is decreased and then an irreversible mass transfer is induced during HPP treatment (Palou, López-Malo, Barbosa-Cánovas, Welti-Chanes, & Swanson, 1997). No significant differences in pH were observed with the HPP treatments throughout the refrigerated storage period (Table 3). However, the pH values of the control were significantly increased from 6.61 to 7.81 after 12 days of storage ($p < 0.05$). The pH increase in squid may result from the formation of TMA and other volatile compounds. The production of TMA with the control was rapidly increased after 4 days of storage time, whilst that at HPP-10 and HPP-20 was not significantly increased throughout the refrigerated storage (Table 4). The production of TMA in squid is related to the microbial growth, which is commonly used as an indicator of quality deterioration of fish during storage (Baixas-Nogueras, Bover-Cid, Vidal-Carou, Veciana-Nogues, & Marine-Font, 2001; Krzymien & Elias, 1990; Paarup, Sanchez, Pelaez, & Moral, 2002; Vaz-Pires et al., 2008). However, the microbial counts did not precisely reflect the amount of TMA produced as shown in Tables 2 and 4. This observation confirms that the TMA production can be a reliable indicator of bacteria spoilage for only certain species such as *Photobacterium*, *Shewanella*, *Vibrio*, *Rhodobacter*, and enterobacteria (Barrett & Kwan, 1985; Boskou & Debevere, 1997; Dalgaard, 1995; Santos et al., 1998). TMAO, a substrate for TMAOase, acts as an electronic acceptor and is usually reduced to TMA under anaerobic condition (Barrett & Kwan, 1985).

3.3. Change in protein pattern of HPP-treated squid during refrigerated storage

The electrophoresis profiles of water-soluble proteins extracted from the control and HPP-treated squid samples were observed during the refrigerated storage (Fig. 3). Molecular weights of major proteins in raw squid were 100, 59, 50, 42, 38 and 32 kDa. Significant differences in protein patterns were observed between days of refrigerated storage and among treatments. At day 0, the proteolytic changes causing breakdown of myofibrillar proteins were observed at the HPP-treated samples rather than the control (Fig. 3a). The results indicate that pressure can enhance the catalytic activity of endogenous enzymes (Lakshmanan et al., 2005). The low-molecular-weight proteins observed may result from an increased activity of proteolytic enzymes, including endogenous and microbial proteinases (Lakshmanan et al., 2005). Cathepsin B and L present in squid (*Todaropsis eblanae*) were activated under the HPP treatment (Ayensa & Gomez-Guillen, 1999; Lakshmanan et al., 2005). The catalytic activities of enzymes were increased with the control and HPP-treated samples with increasing storage time (Fig. 3b), causing the degradation of myofibrillar proteins. At day 12, the control showed more noticeable bands than the HPP-treated sample, indicating a considerable increase in catalytic activity. This observation suggests that myofibrillar proteins such as paramyosin (97 kDa), actin (42 kDa), and tropomyosin (38 kDa) became water-soluble. The increased enzyme activities in the mantle of squid caused loss of gel formation (Kunihiko, Cho, Takeya, Park, & Nobuo, 2003). The denatured and cross-linked proteins resulted in the formation of FA (Careche & Li-Chen, 1997; Nielsen & Jorgensen, 2004). This observation is in good agreement with previous report in which pressure treatment induces only conformational changes (Yaldagard, Mortazavi, & Tabatabaie, 2008). The results suggest that the effect of HPP on the enzyme

Table 4
TMA content (μmol/g) of squid treated at 300 MPa for different pressure hold times during refrigerated storage.

Treatment[a]	Storage time (day)			
	0	4	8	12
Control	0.12 ± 0.02a,A[b]	1.29 ± 0.16b,B	1.16 ± 0.16b,B	1.19 ± 0.16b,B
HPP-0	0.13 ± 0.01a,A	0.16 ± 0.02a,A	1.06 ± 0.15b,B	1.17 ± 0.16b,B
HPP-5	0.12 ± 0.03a,A	0.20 ± 0.02a,AB	0.19 ± 0.03a,AB	0.23 ± 0.03a,B
HPP-10	0.14 ± 0.02a,A	0.18 ± 0.03a,A	0.19 ± 0.03a,A	0.19 ± 0.03a,A
HPP-20	0.13 ± 0.03a,A	0.16 ± 0.03a,A	0.18 ± 0.02a,A	0.12 ± 0.02a,A

[a] HPP-0; HPP-5; HPP-10; and HPP-20 represent 0, 5, 10, and 20 min of pressure hold times.
[b] Means with different letters (A–B) within a row are significantly different at $p < 0.05$ and means with different letters (a–b) within a column are significantly different at $p < 0.05$.

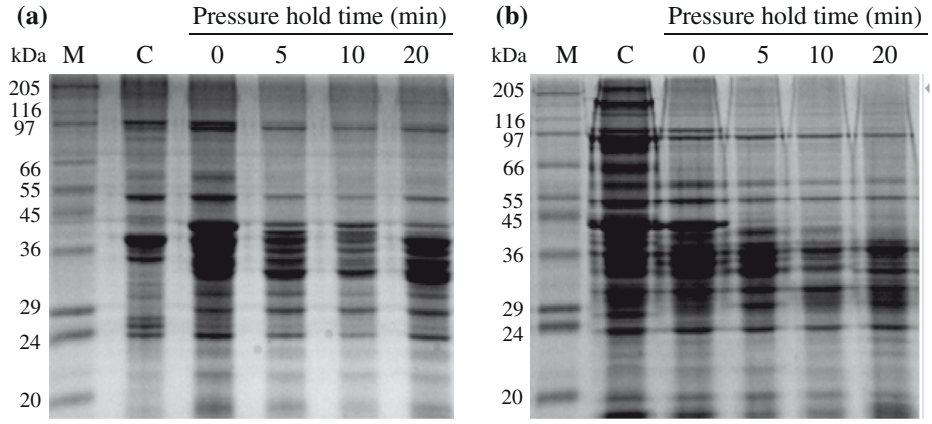

Fig. 3. SDS–PAGE of the water-soluble proteins from the control and the squid samples treated at 300 MPa for 0, 5, 10 and 20 min after 0 (a) and 12 (b) days of refrigerated storage. M, broad range protein marker; C, control; kDa, molecular weight of proteins.

activity may depend on the type of endogenous enzymes and the duration of pressure holding time.

4. Conclusions

High pressure processing (HPP) is of great interest as a potential alternative to traditional thermal processing and chemical preservatives. In recent years, HPP is considered a promising emerging technology for ensuring food quality and safety. In this study, the HPP retarded the formation of DMA and TMA in squid by effectively inactivating TMAOase and inhibiting microbial growth during refrigerated storage. Therefore, the application of HPP can extend the shelf-life and improve the safety and quality of seafood products. Because long-term exposure to DMA, FA, TMA, and other amines can be detrimental to human health by inducing stomach cancer and gastrointestinal tumours, the HPP would be an effective tool for designing a seafood processing system to minimise off-odours.

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