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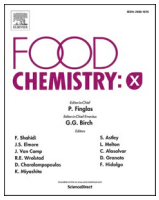

Effect of ultra-high pressure on the relationship between endogenous proteases and protein degradation of Yesso scallop (*Mizuhopecten yessoensis*) adductor muscle during iced storage

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ABSTRACT

This study aimed to explore the effect of ultra-high pressure (UHP) treatment (100–500 MPa, 5 min, 15 ± 1 ℃) on the relationship between endogenous proteases and protein degradation of Yesso scallop (*Mizuhopecten yessoensis*) adductor muscle during iced storage for 28 days. Our findings showed that the UHP treatment kept the water holding capacity stable, increased the hardness and decreased the springiness of scallop adductor muscle during iced storage. 400 and 500 MPa UHP treatments caused protein denaturation and oxidation significantly, decreased protein degradation rate and inhibited the activities of endogenous proteases. According to the correlation analysis, the activities of cathepsin B, D, H, L, calpain and serine protease were positively correlated with TCA-soluble peptides. The activities of endogenous proteases were significantly correlated with protein degradation. Therefore, the effect of UHP on endogenous protease caused the protein degradation rate to slow down and prevented the texture deterioration in scallops.

Introduction

Nowadays, bivalve mollusks are the primary aquaculture source, with China being the largest supplier (FAO, 2020). From a consumer's point of view, the demand for bivalves, such as mussels, clams, scallops and oysters, has increased significantly over time (FAO, 2020). So far, the yesso scallop (*Mizuhopecten yessoensis*) has become an indispensable economic bivalve in the northern coastal areas of China and has brought substantial economic benefits to aquaculture practitioners (Yi et al., 2013; Yu, Liu, Wang, Xue, & Song, 2019). Iced storage is a common method for yesso scallops, but the quality of scallops during storage quickly deteriorates, harming their economic value (Pacheco-Aguilar et al., 2008). Therefore, it is necessary to find new strategies to improve the quality of scallops during iced storage.

Protein degradation often occurs in aquatic products during fishing, processing, transportation and storage (Li, Hu, Li, Li, & Chen, 2014). Protein degradation is the most critical factor that affects the texture of scallops. Previous studies suggested that softening of fish was caused by the degradation of myofibrillar proteins (MPs) (Xue et al., 2021). Furthermore, the enzymes and/or microorganisms caused the protein degradation and then changed the texture (Ma, Wang, & Wei, 2021). Endogenous proteases (cathepsins, calpains and serine proteases) degraded the structural proteins, such as MPs, and led to the texture deterioration of scallop (Liu et al., 2020). Hence, delaying protein degradation may improve the quality of scallops during storage.

Ultra-high pressure (UHP) is one of the most successful non-thermal processing technologies. Various studies have been performed to determine the effect of UHP on the texture of aquacultures (Ginson, Panda, Bindu, Kamalakanth, & Gopal, 2015; Dang et al., 2020; Truong, Buckow, Nguyen, & Stathopoulos, 2016). Previous studies showed that 200 MPa UHP treatment for 10 min inhibited protein degradation in olive flounder fillets during 4℃ storage (Xu et al., 2020). Besides, 400–600 MPa UHP treatment for 15 min inhibited the activities of cathepsin B, D and collagenase in grass carp fillets (Yu et al., 2018). The calpain was no longer active after 300 MPa UHP treatment for 5 min (Chéret, Delbarre-Ladrat, Verrez-Bagnis, & De Lamballerie, 2007). Our previous study also found that 500 MPa UHP treatment reduced the activities of cathepsin B, H and L (Chen, Jiao, Liu, et al., 2022).

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However, limited studies have addressed the effect of UHP treatment on the relationship between protein degradation and endogenous proteases of scallop adductor muscle during iced storage. Therefore, in the present study, scallop adductor muscle were treated with different pressure (100–500 MPa, 5 min, 15 ± 1 ℃) and iced storage for 28 days. Then, MFI, surface hydrophobicity, TCA, free sulfhydryl groups, disulfide bonds, carbonyl content and dityrosine content were determined to evaluate the influence of the UHP treatment on MPs. Subsequently, the activities of cathepsins B, D, H, L, calpains and serine proteases, water holding capacity (WHC) and texture properties of adductor muscles were analyzed. Finally, correlation analysis was performed to find the connection between endogenous proteases, protein degradation and texture characters.

Materials and methods

Sample preparation

Fresh yesso scallops (*Mizuhopecten yessounsis*) were purchased from the local market in Changchun, China, in October 2020, with a size grade of 10 pieces/kg. Scallops were stored in a foam box filled with ice and transferred to the laboratory within 12 h. Scallops were washed immediately after arriving. The adductor muscles (12 ± 2 g and 3 ± 1 cm) were separated and placed into a polyethylene bag, vacuum packed.

UHP treatment

Scallops were divided into six groups and put into the UHP equipment (UHP 600 MPa/30L, Baotou Kefa High-Pressure Technology Company, Baotou, China). The parameters were modified based on our previous study (Chen, Jiao, Yu, et al., 2022), set at 100, 200, 300, 400, and 500 MPa, respectively, holding for 5 min. Table S1 showed the detailed parameters of the UHP treatment. Samples without UHP treatment were used as control. After pressurized, samples were kept in the styrofoam boxes, and stored at 2 ℃ ± 2 ℃ with a scallops-to-ice ratio of 1:2 (w/w) for 28 days.

Water hold capacity (WHC)

2 g of adductor muscles were taken and recorded the weight as W_1. Stuffed the sample into a centrifuge tube after wrapping it with half a piece of 11 cm filter paper. And then, samples were centrifuged at 3000 × *g* for 15 min at 4 ℃ (Allegra X-30R, Beckman Coulter, U.S.A.). After centrifugation, recorded the weight as W_2 (Honikel and Hamm, 1994). The calculation formula was expressed as follows:

$$Water\ holding\ capacity\ (\%) = (1 - \frac{W1 - W2}{W1}) \times 100\% \quad (1)$$

Determination of texture

Texture analyzer (TA.XT.Plus, Stable micro systems, UK) was used to evaluate the textual profile, and the parameters were set as follows: probe descending speed was 1 mm/s before measurement; probe return speed was 1 mm/s after measurement; test speed was 0.5 mm/s; trigger force was 2 g; compression ratio was 50 %.

Shear force is measured perpendicular to the muscle fiber axis and the parameters were set as follows: probe descending speed was 1 mm/s before measurement; test speed was 0.5 mm/s; the initial height was 15 cm, and the compression distance was 13 cm. The data picked the peak value of the shear force profile.

Extraction of MPs

The MPs of scallop adduct muscle were extracted according to the previously described method (Fu et al., 2019). Mixed 2 g of scallop adduct muscle with 20 mL of phosphate buffer (pH 7.0), homogenized (T18, IKA, Germany) the mixture at 10,000 × g for 1 min. Centrifuged at 4 ℃ at 3,000 × *g* for 15 min (Allegra x-30r, Beckman Coulter, USA) and collected the pellet. Then the pellet was homogenized in 20 mL of 0.1 M NaCl and centrifuged (3000 × *g* for 15 min at 4 ℃), repeated the above steps 3 times. Dissolved the precipitates in 0.6 M NaCl-20 mM phosphate buffer (pH 7.0). Used the Biuret method (Gornall, Bardawill, & David, 1949) to determine the concentration of MPs.

Myofibril fragmentation index (MFI)

The method of Olson et al. (OLSON & Stromer, 1976) was used to determine the MFI. Adjusted the concentration of MP to 0.4 ± 0.05 mg/mL. Measured the average of the three aggregate records by the microplate analyzer (FLUOSTAR Omega, BMG LABTECH, Germany), multiplying 200 to obtain the MFI.

Surface hydrophobicity

The method of Fu et al. (Fu et al., 2019) was used to determine the surface hydrophobicity. Mixed 2 mL of 4 mg/mL MP solution with 40 μL of 1 mg/mL bromophenol blue. 1 mL of 0.6 M NaCl-20 mM phosphate buffer was used for the blank sample. Then, the mixture was oscillated at room temperature for 10 min and centrifuged at 10,000 × *g* at 4 ℃ for 5 min. Measured the absorbance at 595 nm. The calculation formula was expressed as follows:

$$BPB\ bound\ (\mu g) = 40\ \mu g \times (A\ control - A\ sample)/A\ control \quad (2)$$

TCA-soluble peptide

The method of Scricket et al. (Sriket, Benjakul, Visessanguan, Hara, & Yoshida, 2012) was used to determine the content of TCA soluble peptides. Mixed 3 g of adductor muscles with 27 mL of 5 % TCA solution. The mixture was homogenized at 10,000 × g, 4 ℃ for 10 min. Collected the supernatant and used BCA protein quantitative kit (Beyotime Biotechnology, China) to determine the content of TCA soluble peptide, expressed as μM tyrosine/g adductor muscle.

Free sulfhydryl and disulfide bond

The method of Chen et al. (Chen, Jiao, Liu, et al., 2022) was used to determine the free sulfhydryl groups and disulfide bonds. The calculation formula was expressed as follows:

$$M\left(\frac{nM}{mg}protein\right) = \frac{\Delta A_{412}}{b \times E \times C} \times \frac{1.1mL}{0.1mL} \times 10^6 \quad (3)$$

The b is the light transmission distance of the microplate reader with a sample volume of 200 μL per well, which is 0.588 cm; The E is the molar extinction coefficient ($M^{-1}cm^{-1}$), the free sulfhydryl is 14,150, and the disulfide bond is 13,600; The C is the protein concentration (mg/mL).

Carbonyl content

The purified MPs were dissolved in 20 mM phosphate buffer (pH 6.8) containing 0.6 M NaCl, and used the Biuret method (Gornall et al., 1949) to measure the protein concentration, then adjusted to 8 mg/mL. The determination of carbonyl content was slightly modified based on the method of Oliver et al. (Oliver, Ahn, Moerman, Goldstein, & Stadtman, 1987). 0.5 mL protein solution was dispensed into two centrifuge tubes, and 2 mL 2,4-dinitrophenylhydrazine (DNPH) solution (10 mM, dissolved with 2 M HCl) was added into one tube, 2 mL 2 M HCl solution was added to the other tube as the control group. Samples were protected from light for 1 h (vortex every 10 min). Added 2.5 mL 20 % trichloroacetic acid to precipitate the protein and then centrifuged at

11,000 × *g* for 3 min. Used 2 mL ethyl acetate/absolute ethanol (1:1, v/v) to wash and collect the residue. Then 6 mL of 6 M guanidine hydrochloride was added to dissolve the residue and centrifuged at 11,000 × g for 3 min. Used a UV spectrophotometer to measure the absorbance of the supernatant at a wavelength of 370 nm. Finally, the molecular absorption coefficient of 22,000 L·(mol·cm)$^{-1}$ was used to calculate the carbonyl content in protein (nM/mg protein).

Dityrosine content

The dityrosine content was determined according to the method of the Davies' with slight modifications (Davies, 1987). Dissolved 10 mg of the sample in 10 mL of 20 mM phosphate buffer (containing 0.6 M potassium chloride) with a pH of 6.0. The insoluble matter in the solution was removed by filtration several times, then the method of Biuret (Gornall et al., 1949) was used to measure the dissolved protein concentration. The tyrosine content was measured with the fluorescence spectrophotometer (Lumina Fluorescence Spectrometer Thermo, USA). The excitation wavelength was 325 nm, the emission wavelength was 420 nm, and the slit width was 10 nm. The tyrosine absorbance divided by the protein concentration was the relative fluorescence value, and the unit was arbitrary units (abbreviated AU).

Endogenous proteases activities

The method of Hultmann et al. (Hultmann, Phu, Tobiassen, Aas-Hansen, & Rustad, 2012) with slight modifications was used to determine the activities of cathepsin B, H, L, and calpain. 3 g of scallop adductor muscle was taken and mixed with a triple volume of phosphate buffer (pH 7.6), homogenized, centrifuged at 10,000 × *g* at 4 ℃ for 30 min and collected the supernatant. Cathepsin B, H, L, and calpain were added with different substrates, reaction buffers, and pH values, as shown in Table S2.

The cathepsin D activity was determined according to the method of Hagen et al. with slight modifications (Hagen, Solberg, & Johnston, 2008). Mixed 0.6 mL 0.2 M citric acid buffer (pH 6) and 0.2 mL crude enzyme solution and preheated for 10 min, then 0.2 mL 30 mg/mL bovine hemoglobin (37℃) was added and incubated for 1 h, and used 1 mL 10 % (w/V) TCA solution to stop the reaction. The UV absorbance value of the filtered supernatant was determined at 280 nm. The enzyme unit (U) is defined as the absorbance value increased by 0.001 per gram of crude enzyme solution per minute at 37 ℃.

Scallop adductor muscle was mixed with 4 times volume (w/v) of 20 mM Tris-HCl (pH 7.5) and homogenized at 10,000 × g for 0.5 min. The connective tissues and large fat particles were filtered out by gauze, and the rest was the crude enzyme solution.

Added 50 μL of buffer (150 mM Bis-Tris, pH 8.0) to 50 μL of the crude enzyme solution and pre-warmed at 30 ℃ for 10 min. Added 50 μL of 0.09 mM fluorescent substrate (Boc-Phe-Ser-Arg-AMC) to initiate the reaction at 30℃ for 15 min, then added 1.5 mL of termination solution (1 % (w/v) SDS, 50 mM Bis-Tris, pH 7.0). The fluorescence excitation was 360 nm, emission wavelengths were 460 nm, and slit widths were 10 nm. 50 μL of crude enzyme solution was replaced by 50 μL of buffer (150 mM Bis-Tris, pH 8.0) for the blank. The enzyme required to hydrolyze the substrate and release 1 nM of AMC product per minute at the corresponding temperature (1 nM/min) was defined as an enzyme unit (U).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

The MPs (4 mg/mL, 5 μL) were mixed with 2 × buffer (5 μL), heated at 95℃ for 5 min, and loaded onto a 10 % polyacrylamide gel electrophoresis gel (Dingguo Changsheng, Beijing, China). The Coomassie Brilliant Blue R-250 was used to stain the protein band. A Luminescent image analyzer (ImageQuant LAS 500, Shanghai, China) was used to scan the bands.

Statistical analysis

Performed at least three replicates for each experiment. Prism 9 was used for statistical analysis. The origin 2021b was used to draw the correlation analysis diagram. Data were presented as the mean ± standard deviation (SD) for all the results. Comparisons of statistical significance between groups were determined using Student's *t*-test or one-way analysis of variance (ANOVA) followed by Duncan's multiple tests. A p-value < 0.05 was considered statistically significant.

Results

Changes in WHC and texture

The WHC of muscle affects the drip loss of aquatic products. As shown in Fig. 1, the WHC of the 500 MPa pressure-treated scallop adductor muscles was significantly reduced. However, after iced storage for 28 days, each group's centrifugal WHC decreased by 33.31 %, 15.46 %, 6.04 %, 8.18 %, 6.93 % and 3.14 %, respectively. Compared with the control group, the centrifugal WHC of the UHP-treated adductor muscle was more stable during iced storage. Moreover, the texture of scallop adductor muscle was altered under different UHP treatments. Compared with the control group, UHP-treated groups had higher hardness, shear force, and lower springiness (Fig.S1A-D).

Changes in protein properties

The protein in scallop adductor muscle is mainly composed of MP (60 %-70 %), sarcoplasmic protein (20 %-35 %) and muscle matrix protein (2 %-5%) (Pariser, 1973) To further explore the protein denaturation during iced storage, MFI, surface hydrophobicity and TCA soluble peptides were determined in the present study.

The MFI, surface hydrophobicity and TCA-soluble peptides content of the control group were 29.93 ± 2.54, 13.59 ± 0.43 μg and 1.14 ± 0.04 mg/g, respectively. Moreover, the values of MFI and surface hydrophobicity increased significantly ($p < 0.05$) in the 400 and 500 MPa UHP treated groups (Fig. 2A-B). A two-way ANOVA revealed that the UHP treatment and the storage time significantly affected MFI and surface hydrophobicity ($p < 0.05$). With the increasing pressure and storage time, all groups maintained an increasing trend (most but not all). Meanwhile, there was no significant effect on the change of TCA-soluble peptides ($p > 0.05$).

Changes in protein oxidation

In this study, free sulfhydryl, disulfide bond, carbonyl and dityrosine

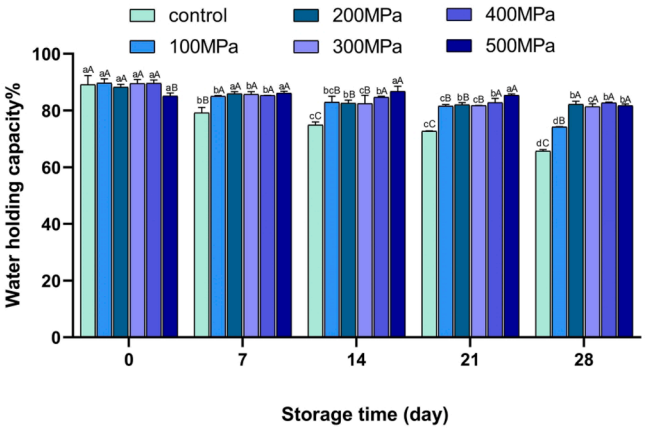

Fig. 1. Changes in water hold capacity of scallops with different treatments during iced storage. ($p < 0.05$) ($n = 3$).

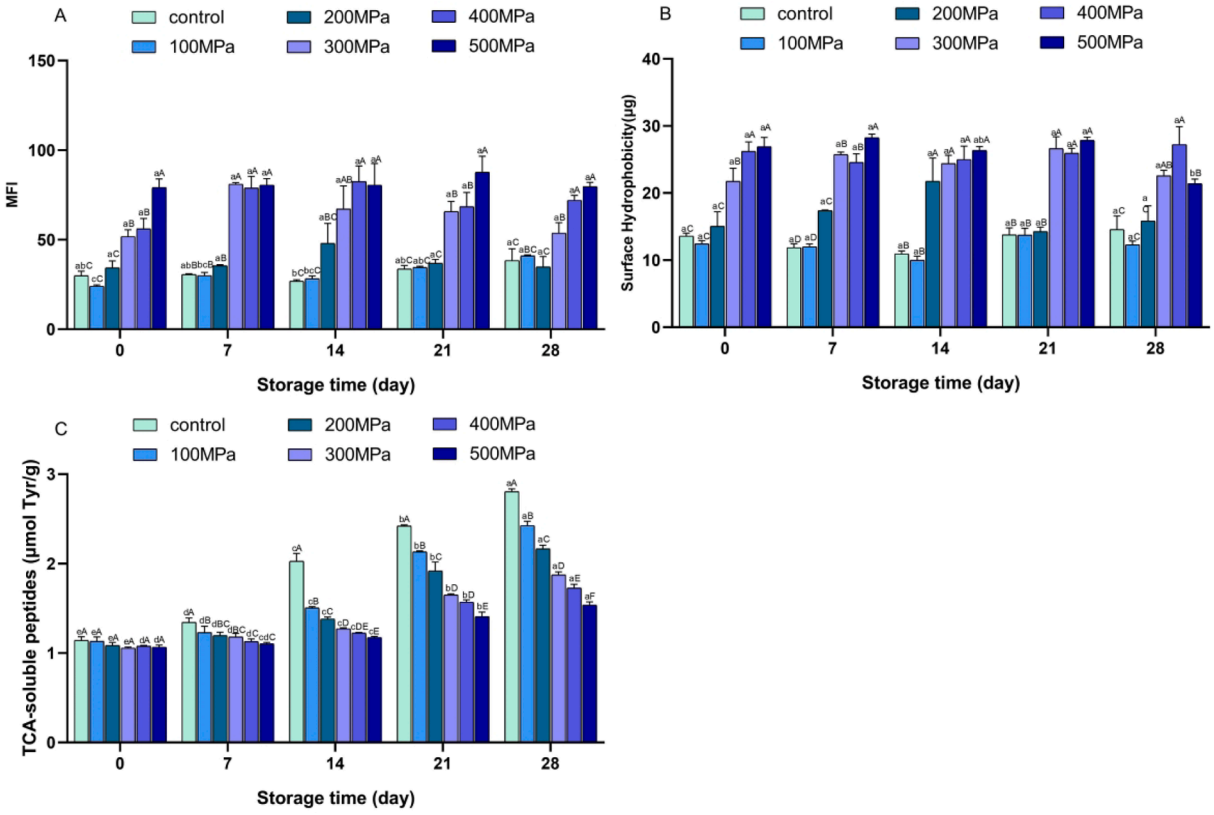

Fig. 2. Changes in protein properties of scallops with different treatments during iced storage. (A) myofibrillar fragmenting index, (B) surface hydrophobicity, (C) TCA-soluble peptide of ultra-high pressure-treated scallops during iced storage. ($p < 0.05$) ($n = 3$).

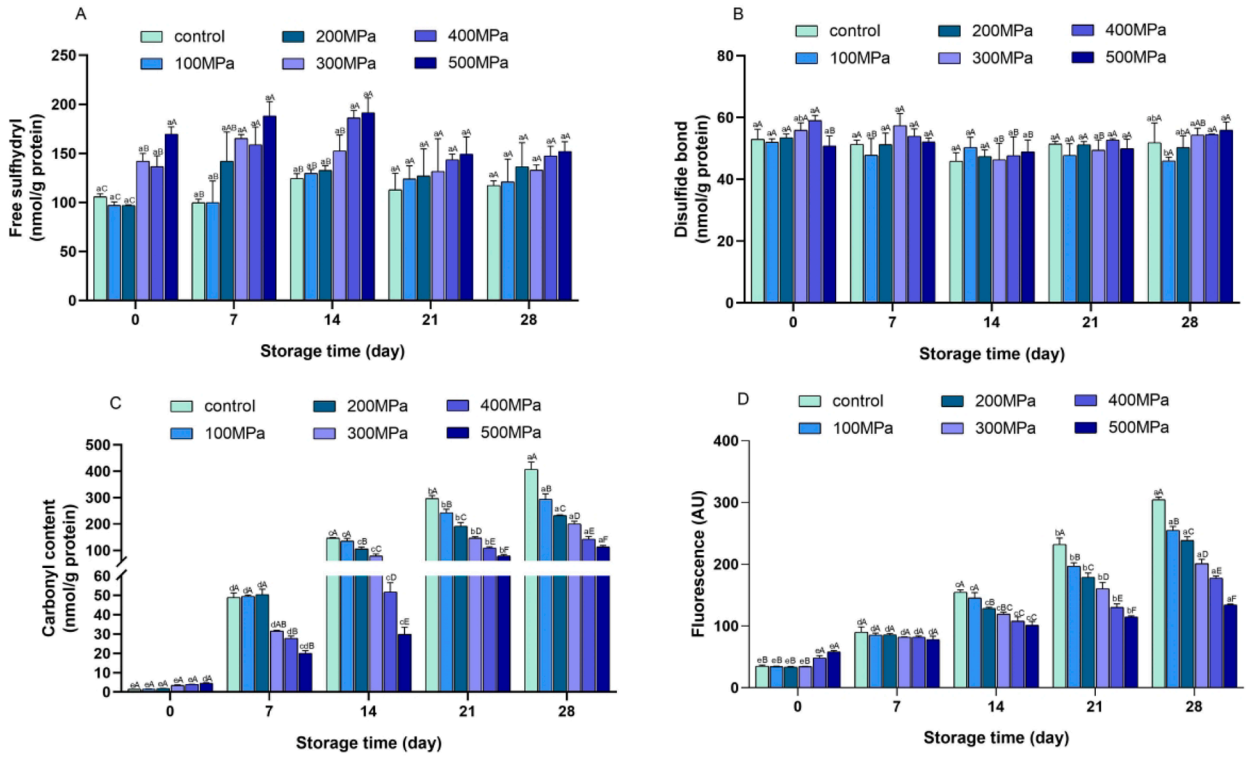

Fig. 3. Changes in Protein oxidation of scallops with different treatments during iced storage. (A) free sulfhydryl content, (B) disulfide bond, (C) carbonyl content, (D) dityrosine content of UHP treated scallops during iced storage. ($p < 0.05$) ($n = 3$).

content were used as indicators for protein oxidation. As shown in Fig. 3, the initial free sulfhydryl, disulfide bond, carbonyl content and dityrosine content of fresh scallops were 105.66 ± 3.05 nM/g, 52.97 ± 3.27 nM/g, 1.42 ± 0.02 nM/g and 34.98 ± 1.63 AU, respectively. The values of free sulfhydryl and dityrosine content increased significantly after 500 MPa UHP treatment ($p < 0.05$) (Fig. 3A and D). Besides, the UHP treatment had no significant effect on disulfide bonds and the carbonyl content (Fig. 3B and C). With the increase in storage time, the content of free sulfhydryl groups is increased and then decreased (most but not all), while the carbonyl and dimer tyrosine content maintained an increasing trend (most but not all).

Changes in endogenous proteases

The activities of cathepsin B, D, H, L, calpain and serine protease in fresh scallops were 0.12 ± 0.006 U/g, 0.11 ± 0.003 U/g, 0.11 ± 0.005 U/g, 0.1 ± 0.006 U/g, 2.19 ± 0.07 U/g, and 1.37 ± 0.052 U/g respectively. Simultaneously, the activities of endogenous proteases were significantly increased in the 100, 200, and 300 MPa UHP treated groups ($p < 0.05$), while the activities were significantly reduced in the 400 MPa and 500 MPa groups ($p < 0.05$) (Fig. 4A-F). A two-way ANOVA revealed that the UHP treatment and the storage time significantly affected the endogenous proteases.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

MP is mainly composed of thick and thin filaments. The main protein of thin filaments is actin (42 kDa), and the main protein of thick filaments is myosin. According to the molecular weight, myosin can be divided into a myosin heavy chain (245 kDa) and a myosin light chain (17 kDa).

The SDS-PAGE electrophoresis chart of the MP of pressure-treated

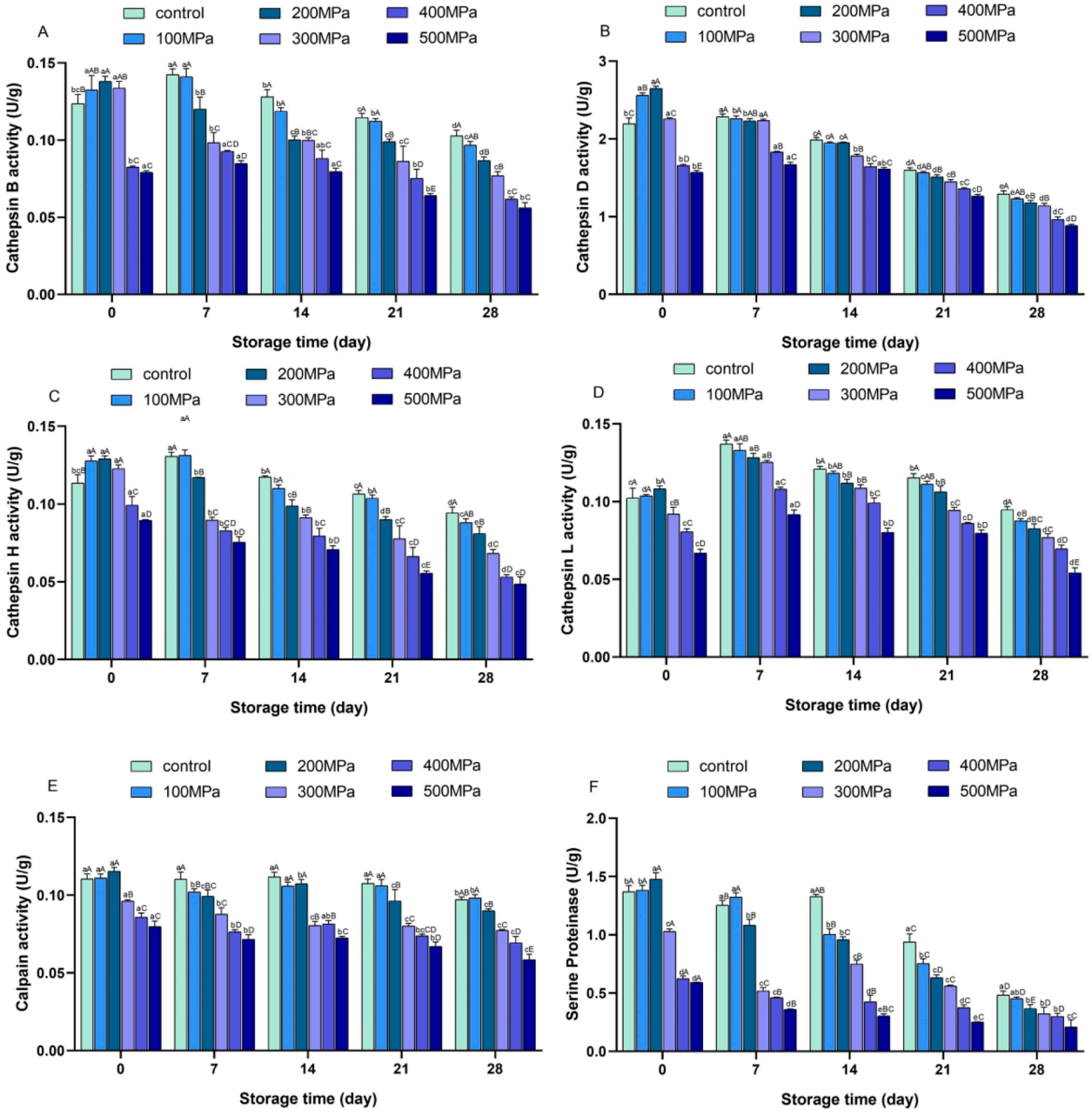

Fig. 4. Changes in endogenous enzyme activities of scallops treated with different ultra-high pressures during iced storage. (A) cathepsin B, (B) cathepsin D, (C) cathepsin H, (D) cathepsin L, (E) calpain and (F) serine protease. ($p < 0.05$) ($n = 3$).

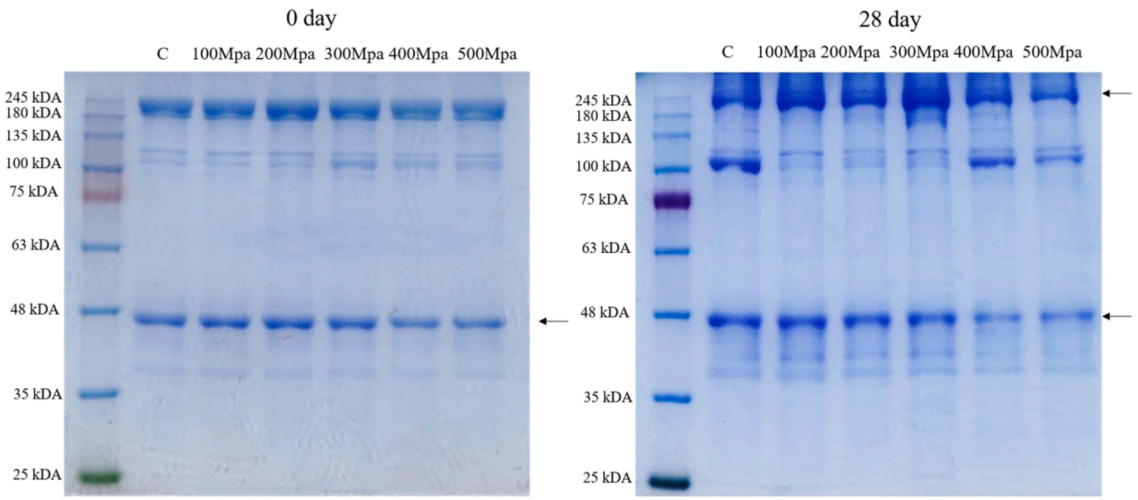

Fig. 5. Changes in SDS-PAGE of myofibrillar protein under different ultra-high pressure treatments during iced storage.

scallops on days 0 and 28 is presented in Fig. 5. After UHP treatment above 400 MPa, the protein bands of MP below 48 kDa became shallow, due to the UHP treatment destroying the natural conformation of actin and small molecule proteins. After 28 days of iced storage, in contrast with other groups that the protein bands below 48 kDa of the 400 and 500 MPa pressure groups were significantly light. The specific manifestations were that the myosin heavy chain (MHC) and myosin bands were degraded(48–35 kDa).

Correlation analysis

As shown in Fig. 6, the correlation analysis showed that the activities of cathepsin B, D, H, L, calpain and serine protease were positively correlated with TCA-soluble peptide. The activities of cathepsin B, D, H, L, calpain and serine protease were significantly ($p < 0.05$) correlated with TCA-soluble peptides (r^2 = 0.75, 0.73, 0.75, 1, 0.71, 0.69, respectively). In addition, the activities of cathepsin B, H, calpain and serine protease were significantly ($p < 0.05$) correlated with MFI (r^2 = -0.76, −0.77, −0.89, −0.75, respectively), surface hydrophobicity (r^2 = -0.69, −0.69, −0.83, −0.67, respectively), hardness (r^2 = -0.55, −0.56, −0.77, −0.61, respectively), and shearforce (r^2 = -0.55, −0.56, −0.73, −0.55, respectively). The springiness was significantly ($p < 0.05$) correlated with the activity of cathepsin L (r^2 = 0.52) and TCA-soluble peptide (r^2 = 0.52).

Discussion

Proteins are critical molecules modified by UHP because they are compressible, and their spatial arrangement has internal empty spaces (Truong et al., 2016). Pressure promotes changes in proteins' secondary,

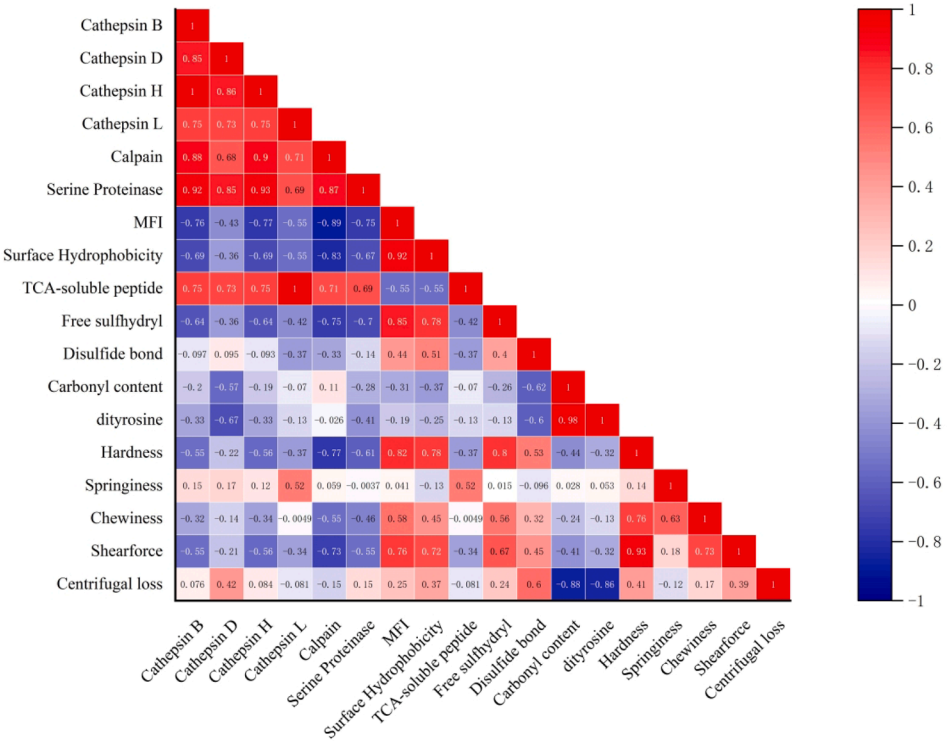

Fig. 6. Correlation analysis between endogenous enzyme activities, protein and quality of scallops treated with UHP during iced storage.

tertiary and quaternary structures, leading to varying degrees of dissociation, unfolding, denaturation and aggregation (Lullien-Pellerin & Balny, 2002). Therefore, the UHP treatment may be directly caused the protein denaturation. The MFI stood for the structural integrity of myofibrils (OLSON & Stromer, 1976). Many researchers have used surface hydrophobicity to monitor the conformational changes of MPs. Traditionally, the protein degradation in fish has been assessed by measuring the concentration of TCA-soluble peptides. In the study, 300–500 MPa UHP treatment significantly increased the value of MFI and surface hydrophobicity, but no significant effect on TCA-soluble peptides (Fig. 2). However, during the iced storage, 400 and 500 MPa UHP treatments retarded the TCA-soluble peptides increase. This indicated that the UHP treatment caused the protein denaturation and decreased the protein degradation rate.

The UHP treatment also caused the protein oxidation. Sulfhydryl (SH) is an integral part of protein functional properties. Traditionally, the protein oxidation-related phenomena has been assessed by measuring the conversion of SH to disulfide bonds (Xiong, Blanchard, Ooizumi, & Ma, 2010). But as shown in Fig. 3B, there was no significant effect on disulfide bonds. Therefore, the change of the SH might not caused by the protein oxidation. The process of protein oxidation is very complicated, and many oxidation products are generated. The significant changes in the carbonyl group occur during muscle protein oxidation. When the protein is oxidized, the side chain of the amino acid residues will undergo a covalent cross-linking reaction with the adjacent active amino acid residues, and the two tyrosine residues will form dimeric tyrosine through complexation. The values of dityrosine content increased significantly in the 400 and 500 UHP treated groups. The protein in the scallop adductor muscle was significantly oxidated. Compared with control group, the values of carbonyl and dityrosine content of 400 and 500 MPa UHP treated groups were decreased during iced storage. This indicated the UHP treatment could retard the protein oxidation of scallop adductor muscle during iced storage.

In the study, we found that the UHP treatment changed the activities of endogenous protease. Consistent with previous studies, the activities of cathepsin B and D increased after 150 and 300 MPa UHP treated 5 min and decreased after 450 MPa UHP treated 5 min in Atlantic mackerel during frozen storage (Fidalgo, Saraiva, Aubourg, Vázquez, & Torres, 2014). This might be due to lower pressure levels caused lysosome rupture and higher pressure levels led to more enzyme inactivation. Besides, the correlation analysis showed that the activities of cathepsin B, H, calpain and serine protease were not significantly correlated with the value of carbonyl content and dityrosine, but significantly correlated with the value of MFI and surface hydrophobicity. Therefore, protein denaturation induced by the UHP treatment may be one of the reasons for the decrease in endogenous protease activities.

The activity of cathepsin was related to protein degradation (Yamashita & Konagaya, 1990). Calpain is a cysteine endopeptidase activated by neutral Ca^{2+} in the sarcoplasm, and triggers the proteolysis of myofibrils to produce new fragments. The role of serine protease is to break the peptide bonds in the large-molecule protein, turning it into a small-molecule protein. Besides, cathepsins B, D, H and L are well-known to cause structural protein degradation in the muscle (Barrett & Kirschke, 1981; Schwartz & Bird, 1977; Yang et al., 2019; Zeinab, Osaana, Wayne, Street, & Vasiljevic, 2015). Our study found that in the 400 and 500 MPa UHP treated groups, the activities of cathepsin B, D, H, L, calpain and serine protease and the protein degradation rate were significantly decreased. The Fig. 6 showed that the activities of cathepsin B, D, H, L, calpain and serine protease were significantly correlated with TCA-soluble peptides. Consistently, previous study used di- and tri-carboxylic acids caused cathepsins activity reduction by 20 %–90 % and then reduced the protein degradation rate (Ge et al., 2022). Therefore, the decrease of protein degradation rate might be caused by the UHP treatment decreased the activities of endogenous protease.

The activities of endogenous protease in MPs caused the changes in texture. The occurrence of muscle softening was caused by cathepsins (mainly cysteine proteases and aspartic proteases), calpains (cysteine proteases) and matrix metalloproteinases (metalloproteinases) (Sentandreu, Coulis, & Ouali, 2002). Our results indicated that the activities of cathepsin B, H, calpain and serine protease were negatively correlated with hardness, chewiness and shearforce, and the activity of cathepsin L was positively correlated with the springiness. The activities of endogenous protease were significantly correlated with the texture. However, the previous studies showed that 400 MPa UHP treatment for 5 min or 450 MPa UHP treatment for 3 min led to Salmonella spp and S. putrefaciens total inactivation and reduced initial loads of spoilage microflora (Cap et al., 2020; Reyes, Tabilo-Munizaga, Pérez-Won, Maluenda, & Roco, 2015). 100–300 MPa UHP treatment induced incomplete inactivation of microorganisms. Microbial growth was one of the reasons of texture deterioration. Therefore, incomplete inactivation of microorganisms might be one of the reasons for the effect of the UHP treatment below 300 MPa on scallop texture. After 300–500 MPa UHP treatment, microorganisms decreased significantly. Therefore, the significant decrease in the activities of endogenous proteases might be the mainly reason for the improvement of texture.

Conclusion

The UHP treatment kept the water holding capacity stable and improved the texture of scallop adductor muscle during iced storage. 400 and 500 MPa UHP treatments for 5 min significantly decreased the activities of endogenous proteases and the protein degradation rate of the scallop adductor muscle. The correlation analysis showed that the activities of cathepsin B, D, H, L, calpain and serine protease were significantly correlated with TCA-soluble peptides. The activities of endogenous proteases were significantly related to protein degradation after the UHP treatment. This study preliminarily proved that the UHP treatment could decrease the protein degradation rate and improve the texture of scallop adductor muscle by inhibiting the activities of endogenous proteases. But in the future, inhibitors and proteomics should be further studied to reveal the molecular mechanism about the relationship between the endogenous proteases and protein degradation under the UHP treatment.

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CRediT authorship contribution statement

Xinyao Zeng: Formal analysis, Investigation, Writing – original draft. **Dexin Jiao:** Conceptualization, Data curation, Investigation, Methodology, Validation, Writing – original draft. **Xiaona Yu:** Formal analysis, Investigation, Methodology. **Lihang Chen:** Investigation. **Ying Sun:** Investigation. **Aoran Guo:** Investigation. **Chen Zhu:** Investigation. **Jinshan Wu:** . **Jingsheng Liu:** Supervision. **Huimin Liu:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100438.

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