Relationship between quality changes of post-rigor tan mutton and myofibrillar protein following high-pressure treatment

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ABSTRACT

Microbial load, protein oxidation, myofibrillar protein changes and quality characteristics (meat color and water retentiveness including centrifugal loss (Cel) and water-holding capacity (WHC)) of post-rigor tan mutton exposed to different high-pressure treatments (200 MPa/500 MPa for 15 min at 18°C) during chilled storage for 7 days (4°C) were evaluated. High-pressure applications of 200 MPa and 500 MPa significantly reduced the number of Total Viable Counts (TVC) during storage (P < 0.05), but excessive pressure treatment (500 MPa) also resulted in a significant increase in protein oxidation and decrease in water retentiveness (P < 0.05), accompanied by deterioration of meat color. 200 MPa treatment also caused adverse effects on the quality of processed meat at the later stage of storage (3–7 d) similar to 500 MPa high pressure, but the initial application of 200 MPa pressure resulted in no significant differences in meat color and retentiveness from the untreated (P > 0.05) and better texture characteristics than untreated samples. ANOVA with Partial Least Squares Regression (APLSR) confirmed the relationship between these variations in quality characteristics induced by high-pressure treatments and protein oxidation and key myofibrillar skeleton proteins.

1. Introduction

Tan sheep with tenderness, high protein content, low cholesterol and rich nutrition is a characteristic dominant animal breed in Ningxia, China, and more than 90% of lamb is sold as cold fresh and frozen meat in the northwest region of China (Li et al., 2019).

The tenderness, colour and water retentiveness are usually deemed as the important quality criteria for evaluating meat for sale since it can be stated directly through appearance, texture and touch. However, these critical characteristics of meat quality would be inevitably deteriorated during postmortem storage owing to the action of endogenous enzymes, leading to prime quality loss (De Oliveira et al., 2019). Also, the onset and promotion of protein degradation during postmortem storage triggered by enzymatic action also create ideal conditions for the growth of microorganisms that contaminate muscle and lead to being susceptible to spoilage (Jo et al., 2014). High-pressure processing (HPP) as a non-thermal sterilization technology has been proved to have superior capacities to diminish microbial loads while retaining most of the sensory, nutrient and functional properties of processed food products (Chouhan et al., 2015). However, the application of HPP to raw meat could induce alterations of meat quality traits in texture, color and water-retaining properties, which depends upon protein susceptibility, the applied pressure and temperature, and the duration of the pressure treatment (Sun & Holley, 2020).
2010), and are related to the conformation state, denaturation, aggregation and gel properties of meat proteins after HPP (Roco et al., 2018). The primary proteins in muscle myofibrils as myosin, actin, troponymosin and troponin-T play an important role in maintaining muscle structural integrity and fluid retention by myofibrils, and high pressure can induce these cytoskeleton proteins to change and thereby affect meat quality (Campus et al., 2010). Jo et al. (2014) pointed out that the unfolding and denaturation of muscle proteins after high-pressure treatments of 400 MPa and 500 MPa for 3 min contributed to the WHC and textural properties of the muscle, and pressurization from 300 MPa to 500 MPa for 3 min could lead to protein unfolding and structural changes in the muscle and compensate for the decline in pH and shear force caused by the acceleration of postmortem glycolysis, thereby resulting in an increase in pH of pressurized post-rigor abalone muscle. Zhang et al. (2015) indicated pressures at <200 MPa induced gel formation of myofibrillar proteins which increased WHC, whereas higher pressures resulted in protein denaturation and reduced WHC. In addition, denaturation of myofibrillar structural proteins such as myosin and actin also affects meat surface and light reflectance, leading to changes in meat color (Grossi et al., 2016).

The traditional texture tests as texture profile analysis (TPA), Kramer test and puncture test are frequently used to assess the textural changes in high-pressure-treated meat, yet they present drawbacks due to being destructive and time-consuming (Campus et al., 2010). The stress relaxation test is one of the most important evaluation tools used for determining viscoelastic properties of materials and can be used for quality assurance of high-pressure-treated meat, being rapid, robust and non-destruction, and this method has been proven to be well consistent with structural protein changes and texture parameters determined by TPA testing, which can be used as markers to monitor the textural changes of muscle (Barroso et al., 2010; Herrero et al., 2010).

Although some studies have reported the effects of high-pressure treatment on the textural properties of seafood and meat products during storage (Campus et al., 2010; Chouhan et al., 2015; McArdle et al., 2013), few studies have monitored the rheological changes of post-rigor mutton during storage by stress relaxation test, and further established the correlations between pressurized meat quality characteristics and protein changes from the perspective of protein oxidation and changes in key myofibrillar skeleton proteins. Therefore, this study aimed to investigate the influences of different high-pressure treatments (200/500 MPa, 15 min, 18°C) on the quality characteristics (meat color, water retentiveness including Cel and WHC) of tan mutton during the chilled period (4°C, up to 7 days), and to characterize its textural properties more finely by stress relaxation test. Key myofibrillar protein changes are elucidated by SDS-PAGE and protein oxidation is determined, and their associations with the above quality characteristics are established.

2. Materials and methods

2.1. Experimental design and high-pressure treatment

Samples were collected from Xinhai pasture, Yanchi, Ningxia China. A total of 45 tan sheep with the same feeding conditions and management methods (about 8 months old, average carcass weight 36 ± 5 kg) were slaughtered. The longissimus dorsi muscles from the left and right carcasses of each sheep were removed separately and immediately transported back to the laboratory under the protection of an ice bag. Subsequently, visible fat and anodesma of the longissimus dorsi muscles were removed and uniformly trimmed to a size of approximately 10 cm × 4.0 cm × 4.0 cm chunks of meat, each sheep could obtain two chunks of meat from the left and right carcasses and assigned them to two different groups (M1 group and M2 group), one chunk of meat in each group, so a total of 90 chunks of meat were obtained from the carcasses of 45 sheep, and 45 chunks of meat from different carcasses were obtained from the two groups, respectively (M1 group = 45; M2 group = 45). Next, five chunks of meat were randomly selected from each group and packed in vacuum (DZ-400D, Shenzhen Packaging Machinery Co., Ltd. China) to ensure that each chunk of meat in the polyamide-polyethylene vacuum bag came from five different carcasses. A total of 18 vacuum packages were finally obtained from the two groups, and all vacuum packages were placed in a 4°C refrigerator for 48 h. Then, all meat chunks were taken out and randomly divided into three groups (control group, 200 MPa high-pressure treatment group, 500 MPa high-pressure treatment group). Each treatment group had six vacuum packages. After high-pressure treatment was completed, the meat chunks were again kept in a refrigerator at 4°C for 1 day, 3 days and 7 days. Two packages were taken from each treatment group at each storage time point for determination. Five chunks of meat in one package were used for the stress relaxation test. Five chunks of meat in another package were used for the determination of other indicators. High-pressure processing was performed with reference to Marcos and Mullen (2014) and McArdle et al. (2013)'s methods, sample chunks packed were placed in an ice bath (0–2°C) before high-pressure treatment to prevent adverse temperature effects. One polyamide-polyethylene vacuum bag containing five sample chunks was placed in a 5-L capacity high-pressure vessel (HPP-600, Baotou KeFa Co., Inner Mongolia, China) during each high-pressure treatment. The preset pressures of 200 MPa and 500 MPa were achieved by an ascending rate of 5 MPa/s and maintained for 15 min, the pressure release time was 10 s, the pressure-transmitting medium was distilled water, and the pressure temperature was set to 18°C.

2.2. Water retentiveness

The ability of muscle to retain water was characterized in terms of centrifugal loss (Cel) and water-holding capacity (WHC) according to Luca et al. (2010) and Campus et al. (2010), respectively.

2.3. Color assessment

Surface color was assessed at 25°C using a color difference meter (CR-400, Konica Minolta, INC., Japan) in the CIE L* a* b* scale in the reflectance mode. The lightness values (L*), redness values (a*), and yellowness values (b*) of tan mutton were measured according to the method of McArdle et al. (2013).

2.4. Protein oxidation analysis

Carbonyl content was evaluated by reaction with 2,4-dinitrophenylhydrazine (DNPH) to form protein hydrazones (Ali
et al., 2015). An extinction coefficient of 22,000 M$^{-1}$ cm$^{-1}$ was used to calculate the carbonyl content.

2.5. Stress relaxation test

Stress relaxation tests were performed with a TA-XT plus Texture analyzer (Stable Micro Systems Ltd., Surry, UK) equipped with Exponent 5.1.2.0 software using a 500 N load cell. Slices that were 20 ± 1 mm in thickness were cut out from different sample chunks of longissimus dorsi perpendicular to the muscle fiber orientation, and compressed by 30% with a 36 mm aluminum cylinder probe at a crosshead speed of 1 mm/s. Sample compressed surface was placed perpendicular to the direction of the muscle fiber, and the compression was kept constant for 60 s, allowing the stress to reach equilibrium. For each storage time (1, 3, 7 days), five sample chunks for each treatment condition were analysed (control, 200 MPa, 500 MPa), making a total of fifteen sample chunks per time of analysis. Texture Expert Exceed Software was used to acquire the data output. The force–time relationship in relaxation was fitted to a modified version of the Maxwell model represented by (Peleg, 2010), as follows:

$$\delta(t) = \sum_{i=1}^{n} C_i (e^{-t/t_i}) + \sigma_0$$

where $\delta$ is the stress (N) at a given time, $t$ (seconds), $C_i$ are stress relaxation constants (N), $\sigma_0$ is the equilibrium stress (N), $t_i$ (seconds) are the relaxation times of the Maxwell elements. Non-linear regression models with three exponential terms with $\delta_0$ were used as described by previous studies (Herrero et al., 2008, 2010).

2.6. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

The extraction of myofibrillar protein and SDS-PAGE were performed according to the procedures of Marino et al. (2013) and slightly modified. Briefly, SDS-PAGE was resolved in 12% polyacrylamide resolving gel with a 5% stacking gel. Protein concentration was adjusted approximately at 2 mg/mL. Twenty microgram of protein was loaded per lane. Low range molecular weight markers ranging from 150 kDa to 15 kDa were used for the estimation of protein’s molecular weight. Gels were run in a Mini-PROTEAN Tetra Cell system (Bio-Rad). Bio-safe Coomassie stain (Bio-Rad) was used to visualize bands. Gels were destained, and stained gel images were captured using a G-800 Densitometer (Bio-Rad). The density of the protein bands were quantified using Quantity One software (Version 4.6.20) and expressed as average intensity.

2.7. Microbiological analysis

Samples were analysed for Total Viable Counts (TVC) at 1d and after 7d. Microbiological analysis TVC was inspected based on standard plate count method by the method of Hussain et al. (2020). In brief, 5 g sample was taken aseptically from vacuumed bags and homogenized in 45 mL of sterile physiological saline (0.85% w/v) in stomacher bags for 2 min. For microbial enumeration, suitable serial dilutions were prepared using the same diluent, by following the protocol of the International Organization for Standardization’s (ISO, 2003). Then, the deriving suspension was serially diluted (1:10) in sterile physiological saline water and 1 mL samples of appropriate dilutions were poured into the petridishes, containing 15–20 mL of plates count agar (PCA). The number of bacterial colonies on the plates was enumerated after incubation for 72 h at 37°C and expressed as Log CFU (colony forming units)/g meat.

2.8. Statistical analysis

All analyses were repeated five times, and the results were expressed as the mean ± standard deviation. The relaxation curves were fitted with Origin Pro 2018 (Origin Lab Corporation, USA), using the Levemberg-Marquardt regression analysis method. The correlations between meat quality characteristics (Rheological properties, WHC, Cel, meat color, Carbyl content) and changes of myofibrillar proteins caused by HP treatments were analyzed by ANOVA with PLSR (APLSR) under Unscrambler X 10.4 Software (CAMO ASA, Trondheim, Norway). From an examination of the validated variance, two two Principal Components (PCs) models were established. On average, 75% and 13% of the validated total variation in Y indicators (meat quality characteristics and protein indicators) were revealed as relevant to X variables (different treatments, referred as control-1d (C-1d), control-3d (C-3d), control-7d (C-7d), 200 MPa-1d, 200 MPa-3d, 200 MPa-7d, 500 MPa-1d, 500 MPa-3d, 500 MPa-7d) in PC1 and 2, respectively. Besides, significant differences were compared using Fisher’s least significant difference (LSD) test at $P = 0.05$ through DPS v7.05 data processing system.

3. Results and discussion

3.1. Effect of high-pressure treatments on microbial numbers of post-rigor tan mutton

The total microbial count is one of the most important criteria for the quality evaluation of fresh and refrigerated seafood and meat products (Chouhan et al., 2015). Figure 1 represents the effect of different high-pressure treatments on the TVCs of tan mutton after 1 and 7 days of refrigerated storage. Microbial growth increased significantly $(P < 0.05)$ during storage and this increase was found to be higher in untreated meat. On the initial day, the application of 200 MPa pressure significantly reduced the TVC compared with untreated $(P < 0.05)$, and further pressurization to 500 MPa showed a stronger sterilization efficiency than other treatments $(P < 0.05)$. After 7 days of storage, the corresponding TVC in the control approached the unacceptable limit of 7 log cfu/g, while the significantly decreased TVCs were obtained in 200 MPa and 500 MPa pressure-treated samples $(4.67 ± 0.21$ and $1.40 ± 0.26$ log cfu/g for 200 MPa and 500 MPa, respectively). Cell membrane is considered to be a primary target for high-pressure inactivation of bacteria as it affects permeability and thereby disturbs transport mechanisms which leads to nutrient deficiency, pH modifications and eventual cell death (Chouhan et al., 2015). The effectiveness of HPP for reducing or inactivating microbial growth of seafood and meat products has been previously reported, including 250 and 350 MPa/10 min processed hilsa fillets (Chouhan et al., 2015), 200–400 MPa/10 min processed
Sea Bream (Campus et al., 2010), 400 MPa/5 min processed duck breast (Khan et al., 2019) and 200–600 MPa/20 min processed lamb (McArdle et al., 2013).

3.2. Effect of high-pressure treatments on water retentiveness of post-rigor tan mutton

Water retentiveness is a property of muscular tissue that withstands water loss (Roco et al., 2018). The water retentiveness of tan mutton under different treatments was together characterized in terms of WHC and CeL in this study, as shown in Figures 2 and 3. The effects of high pressure and storage time on the WHC of tan mutton were not obvious, but it could be noted that WHC decreased slightly with the increases of high pressure. The effect of high pressures on the water retentiveness of treated meat was more reflected in the CeL, as shown in Figure 3. The CeL of untreated samples and 200 MPa high-pressure-treated samples did not differ significantly in each storage day (P > 0.05), while a significant increase in CeL was observed when further pressurized to 500 MPa compared with other treatments (P < 0.05). The influence of

Figure 1. Changes in total aerobic count during storage in untreated samples and samples treated at 200 and 500 MPa for 15 min at 18°C.

Figura 1. Cambios en el recuento aeróbico total durante el almacenamiento en muestras no tratadas y en muestras tratadas a 200 y 500 MPa durante 15 minutos a 18°C.

Results are shown as the average value with the standard deviation (n = 3). The different lower case letters represent significant differences (P < 0.05).

Los resultados se indican como el valor medio ± la desviación estándar (n = 3). Las distintas letras minúsculas representan diferencias significativas (P < 0.05).

Figure 2. Changes in water-holding capacity during storage in untreated samples and samples treated at 200 and 500 MPa for 15 min at 18°C.

Figura 2. Cambios en la capacidad de retención de agua durante el almacenamiento en muestras no tratadas y en muestras tratadas a 200 y 500 MPa durante 15 minutos a 18°C.

Results are shown as the average value with the standard deviation (n = 5). The different lower case letters represent significant differences (P < 0.05).

Los resultados se indican como el valor medio ± la desviación estándar (n = 5). Las distintas letras minúsculas representan diferencias significativas (P < 0.05).
high pressure on the water retentiveness of meat depends on the pressure level, holding time and temperature (Sun & Holley, 2010). Kim et al. (2007) indicated the bovine semitendinosus muscles obtained higher CEL than the controls after 5 min of high-pressure treatment with 200–500 MPa in a hydrostatic fluid medium at 15 ± 3°C. Hong et al. (2005) discovered that the increase of pressure from 50 MPa to 200 MPa at an ambient temperature significantly decreased the WHC of pork longissimus dorsi (P < 0.05). The decrease in water retentiveness of meat treated with high pressure may be attributed to protein denaturation and changes in the intracellular architecture of fibrils induced by high pressure (Fabiano et al., 2017).

3.3. Effect of high-pressure treatments on meat color of post-rigor tan mutton

The color of meat depends on the optical properties of the meat surface as well as on the myoglobin content of the muscle (Marcos & Mullen, 2014). Different high-pressure treatments had significant effects on the color of tan mutton during storage, as shown in Figure 4. The redness (a*) of untreated and 200 MPa treated samples did not differ significantly from each storage day except the third day (Figure 4(a)). Continuing to increase the pressure to 500 MPa, it was found that the a* values during the whole storage period were significantly lower than those of untreated and 200 MPa treatments (P < 0.05). The negative effects of high pressure on meat color were also reflected in the yellowness (b*) of processed meat, as shown in Figure 4(b). 200 MPa high-pressure application only apparently advanced the b* values of the treated samples on the third day (P < 0.05), whereas no significant differences with the untreated samples at other storage times (P > 0.05). Further pressurization to 500 MPa significantly elevated the yellowness of treated meat, showing a significant increase in b* values than the control and 200 MPa treatment during the storage period (P < 0.05). In addition, the lightness (L*) of tan mutton also responded differently to various high-pressure levels (Figure 4(c)), 200 MPa pressure substantially increased the L* values of the treated samples on the first and third days (P < 0.05), while the application of 500 MPa pressure further promoted this parameter of the treated meat throughout the storage period by comparing the 200 MPa treated and untreated groups (P < 0.05). It is generally accepted fact that high pressure induces changes in the color of processed meat, which depends on the intensity of the HP process (pressure and time). Similar to our findings, significantly increased L* values were observed in abalone treated at 200–500 MPa for 3 min. Meanwhile, a decrease in the a* value of abalone was observed after 2–3 weeks depending on the pressure level (P < 0.05). The abalone treated at <200 MPa showed decreases in a* after 2 weeks of storage, whereas the a* value of those treated at >300 MPa decreased after 3 weeks (Jo et al., 2014). In addition, carp muscle fillets also obtained significantly increased L* values when treated with 140–200 MPa pressures for 15 or 20 min (P < 0.05), while significantly increased b* values could be found when treated with pressures higher than 100 MPa regardless of treatment time (15/20 min, P < 0.05) (Sequeira-Munoz et al., 2006). According to the authors, increased L* parameters in HP meat may be associated with protein coagulation, which would affect sample structure and surface properties, while increased b* and decreased a* parameters may be attributed to the pressure-induced oxidation of ferrous myoglobin to ferric metmyoglobin and denaturation of myoglobin.

3.4. Effect of high-pressure treatments on protein oxidation of post-rigor tan mutton

The data related to the carbonyl contents of tan mutton samples after different treatments are shown in Figure 5. The carbonyl contents of samples corresponding to each
treatment increased significantly with storage time ($P < 0.05$). 200 MPa high-pressure-treated samples obtained significantly increased carbonyl contents on the third and seventh days of storage compared with the control ($P < 0.05$), while the application of 500 MPa pressure further increased the carbonyl content at the corresponding each storage day compared with 200 MPa treated and untreated ($P < 0.05$). Higher carbonyl contents indicated more production of protein oxidation.
products and implied lower oxidation stability under these high-pressure treatments. The increase in protein oxidation under pressure may be associated with the potential formation of free radicals, pressure associated denaturation of myofibrillar proteins could cause the release of Fe^{2+}, which promoted the site-specific metal-catalyzed oxidation occurred in the side chains of lysine, arginine, proline, and threonine residues in the tan mutton samples, to which gave rise to the carbonyl derivatives (Jung et al., 2013; Khan et al., 2019).

3.5. Effect of high-pressure treatments on rheological characteristics of post-rigor tan mutton

The generalized Maxwell model was often used to describe of relaxation behavior of materials (Nobile et al., 2007). It was reported that the number of elements taking part in the establishment of the generalized Maxwell model for food materials was usually 2 to 3 (Peleg, 2010), and the three-term model with $\delta_0$ has been chosen for some foods such as pork, poultry meats, and seafoods, as best fitted to interpret the relaxation curves (Campus et al., 2010; Herrero et al., 2008, 2010; Myhan et al., 2015). Therefore, a three-term model with $\delta_0$ was established and used to attempt to fit the experimental data in this study. Curves obtained from relaxation experiments were fitted considering the relaxation part, taking not into account the first part of the curve, namely from the start of the test to the reaching of the maximum force (Zhao et al., 2017). Fitting results by a three-term model with $\delta_0$ to relaxation data are presented in Table 1, where the fitting capability of each model was evaluated based on the maximum relative difference (MRD) and the Percent Explained Variation ($R^2$). The results showed that the three-term model with $\delta_0$ produced an excellent representation of experimental data ($R^2 > 0.99$ and MRD < 5), and regression analysis results can be used for subsequent analysis.

As the decay forces of the Maxwell model, $C_i$ represent the elastic components and can be used as a measure of material elasticity (Campus et al., 2010). As shown in Table 1, it was observed that the $C_i$ values ($C_1$, $C_2$ and $C_3$) in the control after 7 days of storage were significantly lower than those on the first day ($P < 0.05$), while no significant differences in $C_i$ size between the seventh day and the first day for 500 MPa treated samples ($P > 0.05$). Conversely, the application of 200 MPa high pressure appreciably elevated the level of $C_i$ parameters as storage progressed, and a prominent enhancement in $C_i$ values appeared on the seventh day compared to its initial levels on the first day ($P < 0.05$). Comparison of the amplitude of $C_i$ parameters among different treatments revealed that 200 MPa high-pressure treatment significantly decreased the amplitude of these parameters when just being treated relative to the untreated ($P < 0.05$), while further increasing the pressure to 500 MPa promoted them significantly ($P < 0.05$). At the end of the whole storage period, the $C_i$ values in the 200 MPa and 500 MPa pressurized samples were obviously higher than these in the untreated ($P < 0.05$), and there were no significant differences in $C_i$ magnitude between the applied high-pressure levels ($P > 0.05$). Campus et al. (2010) pointed out that $\tau_i$ can also be used to measure muscle firmness and elasticity, but this parameter other than $\tau_1$ did not seem to be significantly affected by high-pressure treatment and refrigeration storage in this study ($P > 0.05$), as depicted in Table 1. Meanwhile, the magnitude of $\delta_0$ is often used to measure the “stiffness” of materials (Campus et al., 2010), and its change with storage time in each treatment group was basically consistent with the $C_i$ parameters.

According to the analysis results of stress relaxation parameters, the post-rigor tan mutton may suffer from the deterioration of texture after being refrigerated for 7 days, accompanied by becoming softening and less elastic, while the processed meat with 200 MPa and 500 MPa high pressures maintained higher stiffness and elasticity after storage. Similarly, Campus et al. (2010) also assessed the rheological changes of sea bream muscle after high-pressure treatment using a three-element Maxwell model with $\delta_0$ and found that the $C_i$ values of untreated and 200 MPa treated samples for 10 min decreased significantly ($P < 0.05$), while in samples treated at 300 MPa and 400 MPa the magnitude of this parameter, although decreasing, was kept significantly higher during 7 days of chilled storage ($P < 0.05$). Moreover, $\tau_i$ values decreased drastically in untreated sea bream muscles after 13 days of chilled storage, while the values of the parameter in high-pressure-treated samples were kept more constant, especially in samples treated at 300 MPa and 400 MPa. These results were consistent with our finding that tan mutton treated at 200 MPa and 500 MPa after 7 days of chilled storage had higher $C_i$ and $\tau_i$ values than untreated samples, which indicated that samples treated with high pressure retained more “elasticity” and “solidity” than the control during storage. The increased meat texture induced by high-pressure treatment could be associated with myofibrillar protein denaturation and aggregation (Christensen et al., 2017).

| Table 1 | \begin{tabular}{|c|c|c|c|c|c|c|c|c|c|c|} 
|  \hline | \textbf{0.1 MPa} | & \textbf{200 MPa} | & \textbf{500 MPa} | 
| \hline | 1d & 3d & 7d & 1d & 3d & 7d & 1d & 3d & 7d | 
| \hline | $\delta_0$ & 1.50 cd & 1.38d & 1.61bc & 1.28d & 1.78bc & 1.95ab & 1.78bc & 1.66bc & 2.37a | 
| $C_1$ & 1.77bc & 1.19d & 1.29d & 1.10d & 1.54c & 2.02a & 1.92ab & 1.15d & 1.91ab | 
| $\tau_1$ & 0.33a & 0.34a & 0.34a & 0.36a & 0.35a & 0.36a & 0.36a & 0.36a & 0.35a | 
| $C_2$ & 1.72b & 1.13d & 1.26 cd & 1.12d & 1.44 c & 2.01a & 1.91ab & 1.25 cd & 1.99a | 
| $\tau_2$ & 2.97a & 3.01a & 2.88a & 3.09a & 3.12a & 3.05a & 3.05a & 3.05a & 2.92a | 
| $C_3$ & 2.11abc & 1.40d & 1.37d & 1.38d & 1.92bc & 2.53a & 2.36a & 1.74 cd & 2.28ab | 
| $\tau_3$ & 24.86bc & 27.75ab & 24.65 c & 26.89abc & 28.97a & 26.55abc & 26.03bc & 27.04abc & 27.23abc | 
| $R^2$ & 0.99957 & 0.99939 & 0.9972 & 0.99947 & 0.99901 & 0.99932 & 0.99969 & 0.99945 & 0.99957 | 
| MRD(%) & 4.03 & 3.95 & 4.04 & 3.81 & 4.06 & 3.70 & 3.66 & 3.88 & 3.57 | 
\hline \end{tabular} | 

The mean values of 5 measurements for each storage time corresponding to different treatments were reported. The different lower case letters on the same line represent significant differences ($P < 0.05$).

Se constaron los valores medios de 5 mediciones para cada tiempo de almacenamiento correspondiente a diferentes tratamientos. Las distintas letras minúsculas en la misma línea representan diferencias significativas ($P < 0.05$).
3.6. Effect of high-pressure treatments on myofibrillar protein of post-rigor tan mutton

In order to better reveal the effect of high-pressure application on myofibrillar proteins, SDS-PAGE was performed and shown in Figure 6. Major myofibrillar protein bands were identified starting from the positive electrode, as myosin heavy chain (MHC), C-protein, α-actinin (α-act), troponin-T, myosin light chains (MLC1, MLC2 and MLC3) and a low molecular weight region (<24 kDa) in Figure 6, and their corresponding band densities are represented in Table 2. It can be seen from the table that 200 MPa high-pressure treatment significantly increased the band densities of troponin-T and tropomyosin on the first and seventh days (P < .01), while further increasing the pressure to 500 MPa made their band strength notably increased.

![Figure 6](image_url)

**Figure 6.** SDS-PAGE profile of myofibrillar proteins of untreated samples and 200 MPa and 500 MPa treated samples for 15 min at 18 °C with storage time.

MHC, myosin heavy chain; α-act, α-actinin; MLC, myosin light chain.

**Figura 6**. Perfil de SDS-PAGE de las proteínas miofibrilares de las muestras no tratadas y de las muestras tratadas a 200 y 500 MPa durante 15 minutos a 18°C con tiempo de almacenamiento.

MHC, cadena pesada de la miosina; α-act, α-actínica; MLC, cadena ligera de la miosina.

**Table 2.** Effect of different high-pressure treatments (200 MPa/500 MPa, 15 min, 18°C) and storage time on myofibrillar proteins of post-rigor tan mutton.

|        | 0.1 MPa |          |          |          | 200 MPa |          |          |          | 500 MPa |          |          |          |
|--------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
|        | 1d       | 3d       | 7d       | 1d       | 3d       | 7d       | 1d       | 3d       | 7d       | 1d       | 3d       | 7d       |
| MHC    |          |          |          | 160.14   | 143.24   | 158.63   | 86.40    | 94.22    | 109.83   | 46.06    | 77.40    | 62.72    |
| α-actinin | a,A   | b,B      | a,A      | 121.20   | 105.80   | 100.64   | 74.69    | 69.14    | 80.68    | 47.67    | 71.59    | 40.13    |
| Actin  | c,B      | b,A      | f,E      | 224.50   | 226.49   | 212.09   | 228.83   | 220.34   | 215.67   | 164.40   | 190.83   | 146.97   |
| Troponin-T | e,D  | b,A     | h,G      | 203.31   | 219.60   | 169.18   | 218.66   | 207.74   | 181.44   | 211.32   | 222.07   | 192.50   |
| TPM    | d,D      | e,E      | h,H      | 209.51   | 205.89   | 155.62   | 223.78   | 200.19   | 177.71   | 229.63   | 237.02   | 222.35   |
| MLC3   | g,F      | f,G      | h,G      | 73.13    | 74.05    | 66.57    | 83.57    | 75.05    | 96.28    | 127.87   | 112.49   | 121.91   |
| < 24 kDa | 72.05   | 74.36    | 72.53    |          |          |          | 93.75    | 94.15    | 114.28   |          |          |          |
| MLC2   | e,E      | e,E      | e,E      | 144.85   | 125.65   | 131.90   | 131.98   | 150.63   | 171.58   | 173.45   | 174.12   | 158.48   |
| MLC1   | d,C     | f,E      | e,D      | 172.68   | 157.93   | 128.16   | 145.94   | 145.35   | 150.17   | 174.98   | 149.24   | 156.10   |

All bands shown in table were expressed as the average band density values obtained from the quantity one software. Different lower case letters on the same line indicated a significant difference in P = 0.05 level, while different upper case letters indicated a significant difference in P = 0.01 level. MHC = myosin heavy chain; TPM = tropomyosin; MLC3 = myosin light chain 3; MLC2 = myosin light chain 2; MLC1 = myosin light chain 1.

Todas las bandas indicadas en la tabla representan los valores medios de la densidad de banda obtenidos con la aplicación del software Quantity One. Las distintas letras minúsculas en la misma línea indican una diferencia significativa en P = 0.05, mientras que las distintas letras mayúsculas indican una diferencia significativa en el nivel de P = 0.01. MHC = cadena pesada de miosina; TPM = tropomiosina; MLC3 = cadena ligera de miosina 3; MLC2 = cadena ligera de miosina 2; MLC1 = cadena ligera de miosina 1.
throughout the storage period ($P < .01$), compared to the non-pressurized control. Similar band reinforcement in troponin-T and tropomyosin were also observed in 200 MPa and above pressures treated pork myofibrillar protein, which may be due to an increased concentration of these proteins, indicating an increased fraction of solubilization (Grossi et al., 2016). Meanwhile, it was also noticed that MHC, actin and α-act bands became less intense after high-pressure treatment of 500 MPa during the storage period than the untreated ($P < .01$), and accompanied by a significant increase in band magnitude of MLC3, MLC2 and MW-24 kDa ($P < .01$). A similar loss and disappearance of the α-act band in the soluble proteins fraction from bovine muscle was observed after pressure treatment above 300 MPa (Chapleau & Lamballerie-Anton, 2003), which was considered to be associated with decreased protein solubility. Also, more intense low molecular weights protein bands (<24 kDa) also appeared in carp fillets treated with 200 MPa for 30 min (Sequeira-Munoz et al., 2006) and sea bass treated with 250 MPa for 30 min (Teixeira et al., 2014), which may be the result of the fragmentation of myofibrillar proteins, leading to an increase in solubilization of these proteins.

3.7. Relationships between protein changes and quality characteristics of post-rigor tan mutton after high-pressure treatments

As indicated in Figure 7, most of the Y indicators located between the inner 50% and outer 100% explained variance circles of the APLSR correlation loading plot, indicating that they were well explained by the 2 PC model. Different treatments involving three different pressure levels (0.1 MPa control), 200 MPa and 500 MPa) were clearly separated and distinguished on the APLSR plot, in which the 500 MPa treatment was located on the right side of the PC2 axis, the control was located on the left side of the PC2 axis, and the 200 MPa treatment was distributed along the PC2 axis. The control (C-1d, C-3d) and 500 MPa treatment (500 MPa-1d, 500 MPa-3d and 500 MPa-7d) were distributed along the PC1 axis and located near the small ellipse, suggesting that both more markedly affected Y indicators that were mostly reflected by PC1 than the other treatments. Most of the Y indicators were spread along both sides of PC1 axis and were mainly interpreted by PC1 (75%). The relationships between meat quality and protein changes were very clear and mainly influenced by 500 MPa treatment.

In terms of the relevance between myofibrillar proteins and stress relaxation constants, δ0, C1, and τ1 were markedly affected by 500 MPa treatment, especially on the first and seventh days. C1 and τ1 derived from the first of the three terms of Maxwell model are generally considered to be a major contribution to the total modulus (Campus et al., 2010; Zhao et al., 2017). δ0, C1 and τ1 strongly negatively correlated with MHC, actin and α-act, and positively correlated with MLC2, MLC3, TPM. The increased stress relaxation constants indicated the increase of elasticity and firmness of pressurized meat, which may be due to pressure-induced denaturation and aggregation of myofibrillar proteins (Chouhan et al., 2015). High-pressure-induced aggregation involved the dissociation of myosin heavy and light chains followed by aggregation of the heavy chains, which reduced MHC and increased MLCs (Speroni et al., 2014). The unfolding of actin and aggregation of myosin molecules contributed to the enhanced texture of treated meat (Ramirez-Suarez & Morrissey, 2006).

In terms of the relevance between myofibrillar proteins and water retentiveness, 500 MPa treatments (500 MPa-1d and 500 MPa-7d) were markedly positively correlated with

![Figure 7](image_url)

**Figure 7.** The APLSR correlation loading plot for the first two principal components (PCs). Nine 0/1 indicator variables (different treatments, referred as C-1d (control-1d), C-3d (control-3d), C-7d (control-7d), 200 MPa-1d, 200 MPa-3d, 200 MPa-7d, 500 MPa-1d, 500 MPa-3d, 500 MPa-7d) in the X-matrix, and changes of myofibrillar proteins and meat quality characteristics in the Y-matrix. Ellipses represent $r^2 = 0.5$ (50%) and 1.0 (100%).

**Figura 7.** Gráfico de carga de correlación del APLSR para los dos primeros componentes principales (PC). Nueve variables indicadoras 0/1 [diferentes tratamientos, denominadas C-1d (control-1d), C-3d (control-3d), C-7d (control-7d), 200 MPa-1d, 200 MPa-3d, 200 MPa-7d, 500 MPa-1d, 500 MPa-3d, 500 MPa-7d] en la matriz X, y cambios de las proteínas miofibrilares y las características de calidad de la carne en la matriz Y. Las elipses representan $r^2 = 0.5$ (50%) y 1.0 (100%).
CeL, and negatively correlated with WHC. Meanwhile, CeL intimately negatively correlated with MHC, actin and α-act, and positively correlated with MLC2, MLC3, TPM and MW < 24 kDa. Myosin and actin, and to some extent tropomyosin are known as the main water-binding components in muscle tissue (Marcos & Mullen, 2014). HP induced protein denaturation of structural proteins like myosin and actin, causing reduced WHC and increased CeL of the proteins (Christensen et al., 2017). Low molecular weight proteins (MW < 24 kDa) were dramatically negatively correlated with MHC and actin, and were very close to the position of CeL in the APLSR plot, indicating that increased depolymerization and fragmentation of myofibrillar proteins due to high pressure contributed markedly to the increase of muscle water loss (Teixeira et al., 2014).

In terms of the relevance between myofibrillar proteins and meat color, 500 MPa treatment, especially the first and seventh days, had a remarkable negative correlation with a* and positive correlation with b* and L*. Changing the color coordinates a* and b* were considered to be related to denaturation of myoglobin and oxidation reactions (Fabiano et al., 2017). The application of 500 MPa high pressure denatured myoglobin and promoted oxidation of ferrous myoglobin to ferric metmyoglobin, resulting in a yellower and less red muscle. L* substantially positively correlated with CeL, and negatively correlated with MHC, actin, and α-act. This can be explained by the fact that 500 MPa treatment induced this structural proteins denaturation and formed larger insoluble protein aggregates, thus affecting the water distribution on the surface of pressurized meat and increasing light reflectance and scattering (Olsen & Orlien, 2016).

In addition, the location of carbonyl contents with relaxation parameters δp, C, and τ in the same quadrant described its strong relationships with sample texture. The structural changes occurring in the muscle due to denaturation of myofibrillar proteins, mainly by oxidation of protein side chains, modified the muscle structures and brought about an increase in textural properties of high-pressure-treated samples (Khan et al., 2019). Meanwhile, the close positional relationships between carbonyl content and CeL and L* also emphasized the importance of the structural changes of myofibrils caused by oxidative damage to the loss of free water between myofibrils and increased light reflectance and scattering (Christensen et al., 2017).

4. Conclusions

Both moderate and high-pressure applications (200/500 MPa, 15 min) effectively reduced the microbial loads during the chilled storage of post-rigor tan mutton, while 500 MPa pressure caused significantly undesirable deterioration in meat color and water retentiveness. 200 MPa treatment could decrease the number of initial microorganisms in processed meat while maintaining no significant differences in WHC and meat color with untreated samples at the beginning of the application, and could further improve the texture characteristics of processed meat. However, meat processed under this pressure is not recommended for long-term refrigeration, as APLSR analysis results revealed that the pressure-induced oxidation and denaturation of proteins increased with storage time, which in turn led to the deterioration of water retentiveness and texture properties of processed samples during later storage.

Disclosure statement

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