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INFLUENCES OF STORAGE CONDITIONS ON BEEF TEXTURE

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This study aimed to explore the influences of different storage conditions on beef texture from both micro-perspective and macro-perspective. In this study, the postmortem beef was stored under three storage conditions, i.e. (1) freezing point temperature + vacuum packaging (Cv), (2) 0 ℃ + vacuum packaging (Zv), (3) 4 ℃ + vacuum packaging (Fv). During the storage, sulfhydryl group (-SH) content, myofiber diameter, sarcomere length (SL), myofibril fragmentation index (MFI) and shear force (SF) were determined regularly. The result showed that: under above three conditions, both -SH content and myofiber diameter gradually decreased while MFI increased. With the treatment of Cv, Zv and Fv, myofiber diameter decreased by 46.67 %, 52.27 % and 57.23 % respectively on the 24th day. The SL was minimized at the 1st day with Zv and Fv condition and at the 4th day with Cv condition. The SF of three types of samples behaved in the similar pattern as increasing firstly and then decrease, however, the change of samples with Cv condition was much slower than those with Zv and Fv condition. Therefore, we conclude that Cv condition can effectively delay the rigor mortis and rigor-off processes of beef, and thus, enable the beef maintain good quality for a long time, following by Zv condition and then Fv condition.

Keywords: controlled freezing point; sulfhydryl group; myofiber diameter; sarcomere length; MFI; shear force.

Introduction

Beef has a large customer market and enjoys an increasing demand for its high protein, low fat and delicious taste. The traditional cold storage technology can only maintain beef freshness for a short period, which result in the imbalance between demand and supply and so brings some economic and demand losses for merchants and customers. In addition, the freezing technology has some disadvantages in maintaining the quality of beef such as flavor deteriorating quickly and nutrients losing rapidly. The shelf life of fresh material can be prolonged by controlled freezing point storage at non-freezing temperature-zone between the freezing point of water and that of an individual material with good quality retention [1, 2].

In recent years, controlled freezing point (CFP) technique has been applied in the storage of blueberry, spinach, green beans, pears, Penaeus vannamei and chicken [3–8]. Researchers have showed that the shelf life of fresh pork can be prolonged appreciably by using CFP [2, 9]. However, the study about CFP on the storage of beef storage is rarely reported comparing to the wide application of CFP in fruit, vegetable and aquatic products. Therefore, in this study, we focused on the possibility of using CFP to promote the shelf life of beef.

During the storage of meat products, the degradation and oxidation of proteins were the main reasons for the disordered and damage muscle structure. The main purpose of this study was to used different texture indicators (-SH content, myofiber diameter, SL, MFI and SF) to evaluate the influences of Cv, Zv and Fv on the beef texture.

Materials and Methods

Animal management

Bulls of Huanghua breed (aged 24 months, provided by Wuqing Slaughterhouse, Tianjin.) at a live weight of 450 kg were slaughtered following the traditional way. The muscles (longissimus dorsi) between the 12^{th} and 13^{th} spine were excised after postmortem, about 200 g per block. The blocks were vacuum packaged (0.9 mpa, PE/nylon bag) and then stored at 4 ℃, 0 ℃ and –1.9 ℃ respectively (conventional mechanical cold store and BW-120-Controlled-Freezing-Point storehouse, provided by National Engineering and Technology Research Center of Agricultural Products Freshness Protection, Tianjin, China). The content of -SH, myofibril diameter, SL, MFI and SF were initially determined at 4h after postmortem. Each item was determined for three times but six times for SF and then the mean values were taken.

Freezing point determination

Digital thermometer was applied to measure the center temperature of the beef block (5^3 cm^3)

which was stored in the frozen refrigerator (–18 ℃). The display of digital thermometer was out of the refrigerator to show the changes of temperature of beef block. Recorded the time of each 0.1 ℃ decline from 0 ℃ and made the variation curve of temperatures with time. If the temperature slightly increased after declining to a value below 0 ℃, and remained at this point for a period of time and then continuously declined, such inflection point temperature was taken as the freezing point of beef [9, 10].

Extraction of total protein

Total protein was extracted from 1 g muscle using 20 ml of ice-cold 1.1M potassium iodide in 0.1M phosphate buffer (pH 7.2) [11]. The samples were minced, homogenized on ice with a Polytron on the lowest setting, and then left on a shaker at 4 ℃ overnight. Samples were centrifuged at 1500 g for 20 min and the supernatant was obtained for the determination of -SH content.

The sulfhydryl group (-SH) content determination

The -SH content was determined by modification of the procedure of Beveridge et al. [12]. 0.5ml of total protein was added to 2.5 ml of 8M urea in Tirs-Gly (10.4 g Tris, 6.9 g glycine and 1.2 g EDTA per liter, pH 8.0, denoted as Tris-Gly) and 0.02 ml of Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid in Tris-Gly, 4mg/ml). Absorbance was measured at 412 nm on spectrophotometer (SPECTRONIC GENESYS 5, Milton Roy Company, USA). The sample concentration was determined by weighing 1 ml total protein.

$$\mu M\ SH/g = \frac{73.53 \times A_{412} \times D}{C}.$$

Where A_{412}= the absorbance at 412 nm; C= the sample concentration in mg solid/ml; D= the dilution factor, 6.02 for total protein; and 73.53 is derived from $10^6/(1.36\times10^4)$; 1.36×10^4 is the molar absorptivity (Ellman, 1959) and 10^6 is for conversions from the molar basis to the μM/ml basis and from mg solids to g solids.

Myofibril diameter and sarcomere length (SL) determination

The samples were cut into $0.5\times0.5\times1.0$ cm^3 and conserved in small centrifuge tubes filled with FAA until determination. 10 μm thick slices were obtained by freezing microtome (Leica CM1850, Germany). Then put the slices into distilled water (three times), 70 % alcohol, 80 % alcohol and 90 % alcohol in sequence to fix them to slides for 5 min respectively. The fixed slices were dyed by Eosin solutions for 15–20 min and then dehydrated by 95 % alcohol, 95 % alcohol and 95 % alcohol respectively for 5 min per stage. After using dimethylbenzene to make slices transparent for 5 min twice, used resinene to sealing and dried in dark and ventilated place for 24 hours. Images were recorded by laser scanning confocal microscope (Zeiss LSM 510, Carl Zeiss co., LTD, Germany) and analysed by LSM 5 Image Examiner.

Myofibril fragmentation index (MFI) determination

MFI was determined according to Culler et al. [13]. Four grams of minced muscle were homogenized for 30 set in 10 vol (v/w) of a 2 °C isolating medium consisting of 100 mM KCl, 20 mM K phosphate, 1 mM EDTA, 1 mM $MgCl_2$, and 1 mM sodium azide. The homogenate was sedimented at 1000×G for 15 min and then the supernatant was decanted. The sediment was next resuspended in 40 vol (v/w) of isolating medium by using a stir rod, sedimented again at 1000 x G for 15 min and the supernatant decanted. The sediment was resuspended in 10 vol (v/w) of isolating medium and passed through a polyethylene strainer (150 mesh) to remove connective tissue and debris. An additional 10 volumes (v/w) were used to facilitate passage of myofibrils through the strainer. Protein concentration of the suspension of myofibrils was determined by the biuret method of Gornall et al. [14]. An aliquot of the myofibril suspension was diluted with isolating medium to a protein concentration of 0.5±0.05 mg/ml. Protein concentration was again determined by the biuret method of Gornall et al. [14]. The diluted myofibril suspension was stirred and poured into a cuvette; absorbance of this suspension was measured immediately at 540 nm with the SPECTRONIC GENESYS 5 spectrophotometer (Milton Roy Company, USA). Absorbance was multiplied by 200 to give a MFI for each sample.

Shear force (SF) determination

After the respective ageing time, meat samples were prepared for SF analysis according to Stadnik [15] and Culler et al. [13]. Blocks of $50\times30\times30$ (mm^3) were cooked individually in plastic bags immersed in a water bath (PolyScience, Taicang experimental equipment factory , Jiangsu) at 80 ℃ for 30 min. The cooked meat was cooled in running water for 30 min. Three 1.27 cm cores were removed parallel with the muscle fibers for W-B shear determinations.

Each core was sheared twice, and the 6 values were averaged and then converted to kg force per square cm.

Results and Discussion

Freezing point

During the freezing process, a slight fluctuation was obtained from –2 ℃ to –1.9 ℃ and then the temperature was stabilized at –1.9 ℃ until reaching to 1628s (Fig. 1). Thus, the freezing point of the samples was –1.9 ℃. The previous studies revealed that freezing point of beef fell within the range of –0.6 ℃–1.7 ℃ [16], which was different from the result in this study. It was possibly caused by the differences in breed and gender et al. of cattle. In preliminary experiment, samples had not been frozen in the refrigeratory of –1.9 ℃, so the result was reliable.

Content of -SH

Fig. 2 showed -SH content changes at different storage temperatures. Along with the extension of storage time, -SH contents at different temperatures all tended to gradually decrease (decrease speed: Fv>Zv>Cv). The significance analysis revealed that there were significant differences ($p<0.01$) in -SH content among three conditions. As shown in Fig. 2, -SH content would decrease appreciably with the increase of temperature, which was also evidenced by other researchers [17]. In case of temperature rise, the oxidation rate of proteins accelerated.

Conversion of sulfhydryl groups into disulfides and other oxidized species is one of the earliest observable events during the radical-mediated oxidation of proteins [18]. The loss of the sulfhydryl groups may be due to the formation of disulfide bonds either within polypeptides or between polypeptides and also due to degradation reactions [19]. During the process of low temperature storage, the conformation changes happened to myofibrillar protein. The changes of actomyosin structure, especially the fore area, caused the -SH inside the molecules to be exposed. The exposed -SH easily

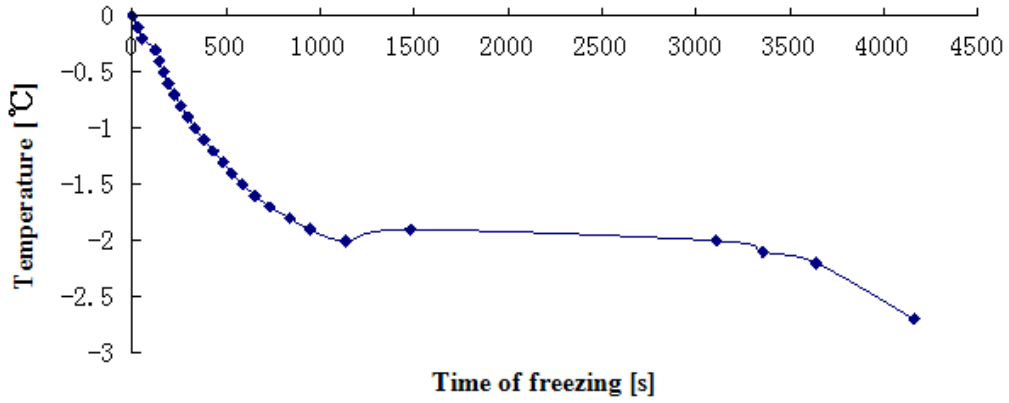

Fig. 1. Freezing curve of beef longissimus dorsi

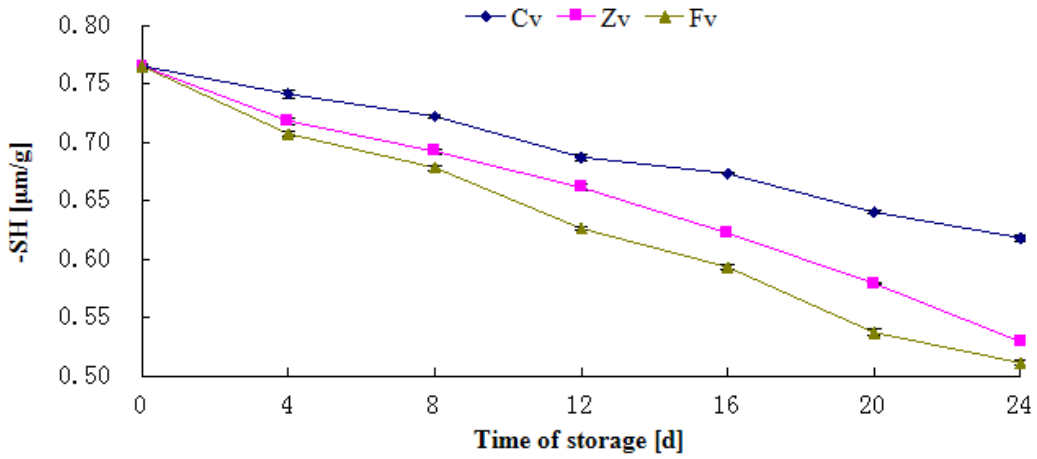

Fig. 2. Changes of -SH contents in different conditions

oxidated to form disulfide bonds, resulting in the decrease of -SH content and the increase of disulfide bond content [20]. Some researchers have supported that the main reason for the reduction of ATPase activity was that -SH was oxidated to form disulfide bonds and cause molecular aggregation [21]. Li combined the -SH content changes with Ca^{2+}-ATPase activity changes, and found that Ca^{2+}-ATPase activity constantly declined during the storage, thus evidenced the oxidation and content decrease of -SH. Cv condition significantly inhibited protein oxidation, and reduced the activity of enzymes [17].

Myofiber diameter

Myofiber diameter at three conditions all showed a decrease trend (Fig. 3). Under Cv, Zv and Fv condition, myofiber diameter decreased by 46.67 %, 52.27 % and 57.23 % respectively on the 24th day. There were significant differences ($p<0.01$) in myofiber diameter among three conditions (Cv, Zv and Fv). Low temperature delayed the rigor mortis and rigor-off processes of beef and slowed down the rate of combination of thick filaments of myosin and thin filaments of actin, which decreased the rate of water loss in cells and also reduced the protease activity.

Myofiber diameter is an important indicator to evaluate beef quality in terms of texture. In the process of storage, myofiber diameter gradually decreased, which was consistent to others' work [22]. During the rigor mortis process, combination occurred between thick filaments of myosin and thin filaments of actin, which caused muscle tissue contraction and shortened sarcomere length, accordingly resulting in the shrink of myocyte. Meanwhile, in the process of ageing, glycolysis generated lactic acid to decline the pH value. When it was close to the isoelectric point of myoprotein, the electrostatic repulsion force between the myofibrils elevated gradually which intensified the contraction of longitudinal myofiber and then the loss of water accelerated [23], leading to the shrink of myocyte. Along with the extension of storage time, endogenous enzymes and micro-organisms in muscles would cause the degradation of myofibrils and bring more myofibril fragmentations [24–27], resulting in the loss of tissue fluid and the continuous reduction of myofiber diameter. Most of the myofibrillar proteins could be hydrolyzed by calpain and lysosomal protease (cathepsin) [28–32]. Zhu et al. measured the myofiber diameter of Longissimus dorsi of Qinchuan cattle [33], and the data differed from those in this paper, which might be the results of different factors such as breed and gender.

Sarcomere length (SL)

There was a decrease in SL early post mortem and then an increased was observed with continuous storage (Fig. 4). SL of live mammalian is generally 2.5 μm. ATP

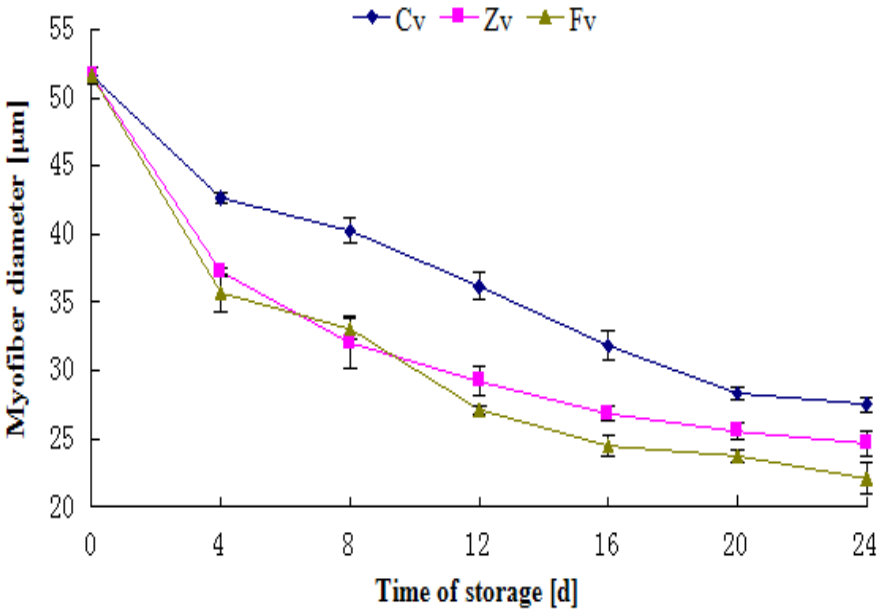

Fig. 3. Changes of myofiber diameter in different conditions

concentration in muscle reduces rapidly after slaughter and therefore Mg-ATP complex which inhabits myosin/actin interaction dissociated. So myosin combines with actin to form actomyosin, thus shortening SL [34, 35]. When the rigor mortis is maximized, SL reaches minimum. Further, the extended contraction force causes the destruction of Z-line and binding between myosin and actin, and finally SL begins to increase. In this study, SL was 2.09 μm initially. $SL_{(Zv)}$ and $SL_{(Fv)}$ reached the minimum (1.48 μm, 1.42 μm) on the 1st day and there was no difference between them ($p > 0.05$) while $SL_{(Cv)}$ reached the minimum (1.45 μm) on the 4th day. The studies have shown that beef reached maximum rigor mortis at 24h in case of refrigerated temperature or at nearly 48h in case of near freezing point temperature (–1 ℃~1 ℃) [36]. So it was predicted that it had approached or reached maximum rigor mortis under both Zv and Fv condition at about 24h and SL was about minimized, and samples with Cv condition reached maximum rigor mortis about on the 4th day. An increase was observed after minimum SL. And then the increasing rate of SL had decreased in each condition and there had been little difference between $SL_{(Zv)}$ and $SL_{(Fv)}$ since the 8th day ($p > 0.05$). $SL_{(Cv)}$ gradually increased after minimum SL, approximated to $SL_{(Zv)}$ and $SL_{(Fv)}$, and finally showed a relatively steady state after the 16th day. On the 24th day, $SL_{(Cv)}$, $SL_{(Zv)}$ and $SL_{(Fv)}$ were 1.73 μm, 1.75 μm, 1.72 μm respectively. The possible reason was that beef had long ageing time at low temperatures, and Cv condition delayed the ageing of beef comparing with Zv and Fv conditions. But under each condition, SL would remain relatively stable after recovering to a certain extent, which was consistent with the study of Wheeler et al. [34].

Many studies have shown that SL is closely related to beef tenderness and beef entering rigor-off state earlier would enter the tenderization process earlier [37, 38]. In this study, Cv condition couldn't make beef reach the ideal tenderness quickly for its low change rate, but it alleviated the degradation of muscle fibers, slowed down the changes in physicochemical properties of samples, and so had a good effect on extending the storage time of beef.

MFI

MFI reflects the integrity degree of myofibril and its cytoskeletal proteins. High MFI indicates that myofibrillar proteins are seriously decomposed and structural integrity is severely damaged [39]. Wheeler et al. reported that the breakdown of myofibrils after slaughter might due to the hydrolyzation of some key proteins by some enzymes, leading to the formation of fragmentation [34]. In addition, actomyosin, forming during the rigor mortis, would dissociate into actin and myosin in the ageing process and myofibrils would be degraded into fragments which were composed of sarcomeres [40].

In this study, MFI all increased along with the extension of storage time (Fig. 5). The change rate of $MFI_{(Cv)}$ was lower than those of $MFI_{(Zv)}$ and $MFI_{(Fv)}$. The increase speed of MFI complied with the principle of Fv>Zv>Cv. There were significant differences ($p<0.01$) in MFI among three groups. $MFI_{(Cv)}$ increased slightly in the

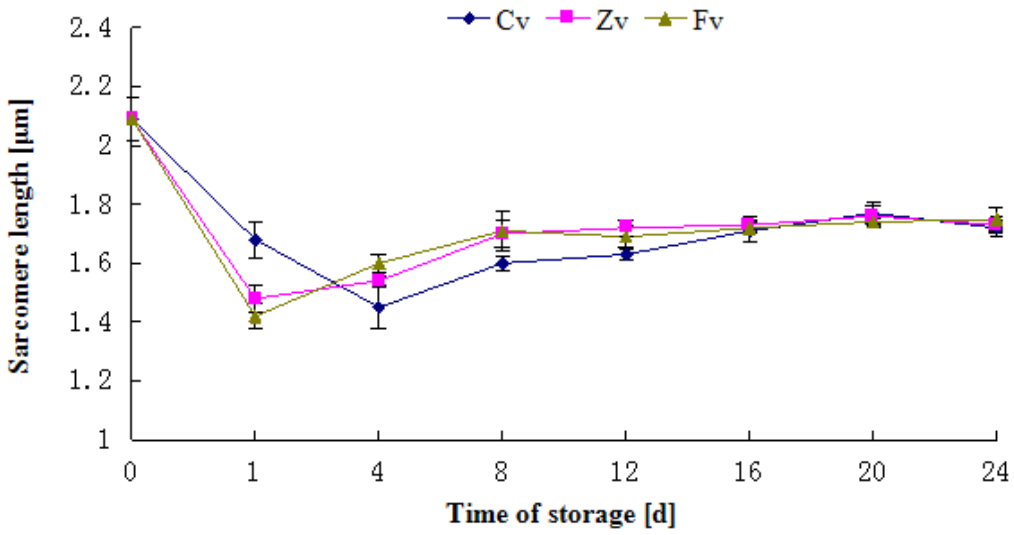

Fig. 4. Changes of sarcomere length in different conditions

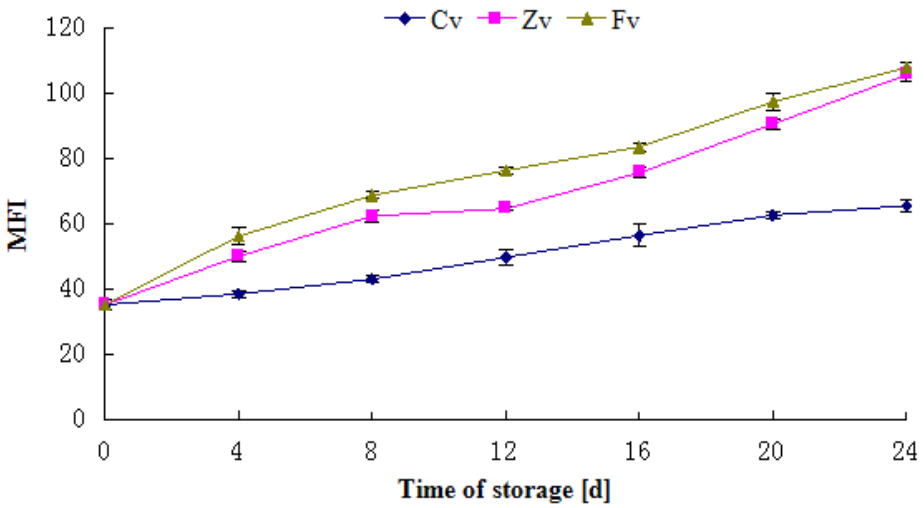

Fig. 5. Changes of MFI in different conditions

whole storage, which indicated that, Cv condition prolonged the rigor mortis and ageing process and decreased the speed of protein degradation and thus made the physicochemical properties of beef texture stable relatively and extended the shelf life. During the later stage of storage, the difference between MFI $_{(Zv)}$ and MFI$_{(Fv)}$ decreased possibly due to beef spoilage.

Along with the increase in MFI, beef tenderness got improved. Many studies have shown that there is a significant relevance between MFI and beef tenderness, so MFI can be used as an indicator for evaluating beef tenderness [41, 42]. Culler et al. graded tenderness of beef sirloin according to MFI, and found that beef would be very tender in case of MFI ≥ 60 [13]. As per this standard, this study showed that beef reached "very tender" grade on the 4th day under both Zv and Fv condition while on the 12th day under Cv condition but it stayed in this grade for a long time under Cv condition. So Cv condition can be associated to good beef quality within a long period of storage time.

Shear Force (SF)

SF$_{(Cv)}$ and SF$_{(Zv)}$ showed a trend of increasing firstly and then decreasing, which confirmed the finding of previous study [15], but SF$_{(Fv)}$ continuously decreased (Fig. 6). The post-mortem muscles entered the stage of rigor mortis, the interaction between myosin and actin caused muscle contraction [34,35], and SF of muscle tissue reached the maximum value when the rigor mortis was maximized. Along with the ending of rigor mortis and the starting of ageing process, the interaction between proteins became weak. In addition, protein degradation for the reasons of endogenous enzymes and micro-organisms caused SF to decrease [24–27]. But mass propagation of micro-organisms during the later stage resulted in water loss [43], and vacuum packaging also increased the loss of tissue fluids. These might be the main reasons for preventing SF from continuing to decrease.

With three treatments, change rates of SF were low during the later stage of storage. SF$_{(Cv)}$ and SF$_{(Zv)}$ reached the maximum value on the 4th day but maximum SF$_{(Zv)}$ was much lower than maximum SF$_{(Cv)}$. Deligeersang et al. found that beef reached maximum rigor mortis at 24h in case of refrigerated storage or at nearly 48h in case of near freezing point temperature (–1 ℃~1 ℃) [36]. So it was predicted that SF$_{(Cv)}$ had reached the maximum value on the 4th day or so, but maximum SF$_{(Zv)}$ and SF$_{(Fv)}$ had happened before the 4th day and had already entered a continuous decline phase since maximum SF. The change rate of SF complied with the principle of Fv>Zv>Cv. There were significant differences ($p<0.01$) in SF among three conditions. Low temperature delayed the rigor mortis and rigor-off processes, and the arrival time of maximum SF also complied with the principle of Fv>Zv>Cv. SF is used to evaluate the beef tenderness. Generally beef tenderness would increase along with the decrease in SF within a suitable range, beef spoilage was unaccepted. Cv condition was the most effective way to maintain the acceptable beef tenderness for a long time following by Zv condition and then Fv condition.

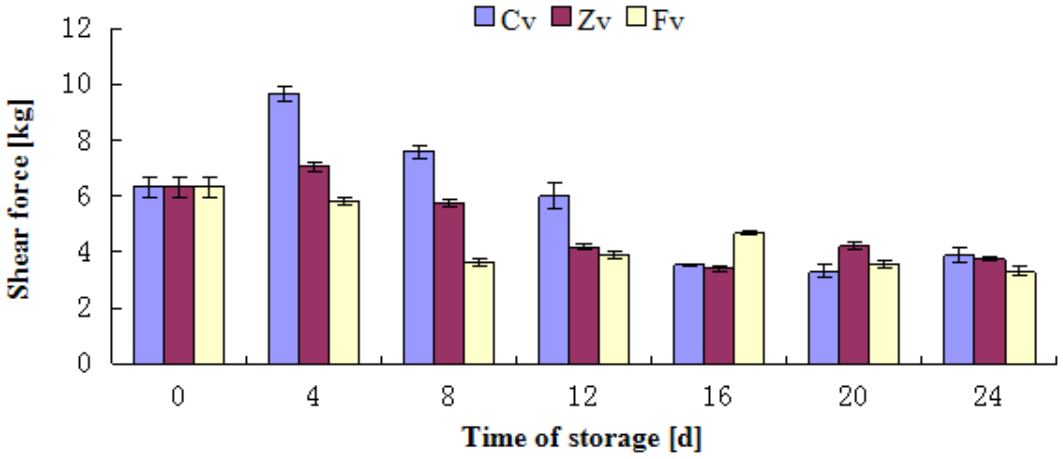

Fig. 6. Changes of shear force in different conditions

Conclusion

This study analyzed the texture of Longissimus dorsi of beef. The oxidation of proteins caused the continuous decrease in -SH content; during the rigor mortis and rigor-off processes, SL initially decreased and then increased as the decrease in myofiber diameter; MFI constantly increased with the function of endogenous enzymes and micro-organisms; SF of meat decreased initially and then increased, and meat tenderness changed accordingly. In summary, the lower temperature brought the better quality at a time point. Cv condition significantly delayed the process of qualitative change in meat products, and played a positive role in assuring meat quality, comparing with Zv and Fv condition.

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ВЛИЯНИЕ УСЛОВИЙ ХРАНЕНИЯ НА ТЕКСТУРУ ГОВЯДИНЫ

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Это исследование было направлено на изучение влияния различных условий холодильного хранения на структуру парного мясного сырья и характер протекания процессов автолиза. Говядина хранилась при следующих параметрах среды: 1) при температуре –1,9 °С (точка замерзания), 2) 0 °С, 3) 4 °С. Все образцы были упакованы под вакуумом и им были присвоены уловные обозначения Cv, Zv и Fv, соответственно. Хранение осуществлялось в течение 24 дней. Во время хранения регулярно определяли содержание сульфгидрильных групп (-SH), диаметр миофибрилл, длину саркомера (SL), индекс фрагментации миофибрилл (MFI) и силу сдвига (SF). В результате исследования было установлено, что при вышеуказанных условиях содержание сульфгидрильных групп и диаметр миофибрилл постепенно уменьшались, в то время как индекс фрагментации миофибрилл увеличивался. Для всех трех трупп образцов (Cv, Zv и Fv) диаметр миофибрилл уменьшился на 46, 7, 52,3 и 57,2 % соответственно. Изменение силы сдвига для всех групп образцов имело аналогичную динамику: в первые 12 дней хранения значения показателя имели тенденцию роста, а затем снижались, однако изменение параметра для образцов с условием Cv было менее выражено, по отношению к образцам с условием Zv и Fv. Таким образом, можно отметить, что хранение парного мясного сырья при температуре –1,9 °С может эффективно задерживать процессы окоченения и позволяет говядине сохранять хорошее качество в течение длительного времени.

Ключевые слова: автолиз, миофибриллы, саркомер, холодильное хранение, говядина, ясное сырье.

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