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Changes in Meat Quality Characteristics and Calpains Activities in Gannan Yak (*Bos grunniens*) Meat during Post Mortem Ageing

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Abstract: An experiment was conducted to investigate the effects of post mortem ageing on the changes of meat quality characteristics and calpains activities of Gannan yak (*Bos grunniens*) meat. Twelve, 3 years old yaks (body weight, 230±10 kg) were used in this study and Longissimus dorsi muscle was removed from the carcasses immediately after slaughter and cut into chops with an average weight of 50 g, vacuum-packed into pouches, taken to the laboratory under refrigerated conditions. The samples were aged at 4°C for 0, 12, 24, 48, 72, 120, 168 and 192 h, respectively. The results indicated that the pH value of meat fell gradually to the minimum of 5.56 within the first 72 h ($p<0.05$) and the value did not change in the next 120 h. Water Holding Capacity (WHC) had a decreased trend in the first 72 h ($p<0.05$) but was s since. Tenderness of yak meat was improved in ageing days evaluated by Shear Force (SF) and Myofibril Fragmentation Index (MFI) ($p<0.05$). Hardness, springiness and chewiness were significantly decreased by analyzing the texture profile which reflected the progressive softness of the meat ($p<0.05$). Activity of μ-calpain was decreased continuously in first 72 h and activity of m-calpain was declined slightly only in first 12 h. However, activity of calpastatin was stable in first 72 h and then decreased gradually. The results demonstrated that the meat quality properties of yak meat were improved during ageing after postmortem and μ-calpain might be the main contributor of tenderization.

Key words: Yak meat, ageing time, meat quality, tenderization, calpains activity

INTRODUCTION

Chinese yaks (*Bos grunniens*) live in the steppes of the Himalayan highlands and adapt to the cold and the low oxygen environment. The population of Chinese yak is about 14 million, accounting for 92% of the global population (Guo *et al*., 2008). The Chinese yak meat is famous for its low fat, high protein, fine texture and rich amino acids compared with that of cattle (Xiaoling and Tinghua, 2004). However, the quality of the yak meat is badly influenced by incorrect processing ways.

Meat tenderness is one of the most important eating quality in all the meat traits (Miller *et al*., 2001) and which will determine whether the purchase is repeated in the future (Morgan *et al*., 1991). Ageing is one effective way to enhance tenderness. The process is normally finished within 8-11 days at refrigeration temperatures (Smith *et al*., 1978) accompanied by a series of biochemical changes involving pH and ionic strength, MFI and so on (Ouali, 1991; Taylor *et al*., 1995). The final tenderness of meat relies on the degree of changes and weakening of myofibrillar structures caused by endogenous proteolytic enzymes (Kemp *et al*., 2010) these endogenous proteolytic enzymes includes calpains, cathepsins, proteasomes and so on. It is widely accepted that proteolytic calpain activity does contribute to meat tenderization (Koohmaraie and Geesink, 2006). In skeletal muscle, the calpain system includes three proteases, ubiquitously expressed isoforms μ-calpain, m-calpain, p94 and calpastatin which is the inhibitor of μ and m-calpain (Wendt *et al*., 2004).

Although, earlier reports showed that the role of calpains in postmortem proteolysis and tenderization (Delgado *et al*., 2001; Chereta *et al*., 2007; Veiseth *et al*., 2004), few studies had been performed to evaluate the effects of postmortem ageing days on Chinese yak meat quality, in particular, calpains and calpastatin of yak meat. Therefore, the objectives of the research were to demonstrate the changes of physiochemical traits and the activity of calpains and calpastatin during yak meat ageing and to discover some possible relationship among them.

MATERIALS AND METHODS

Animal, sampling and reagent: Twelve, 3 years old yaks (body weight, 230±10 kg) were used from Xiahe county, Gansu Province, China. Longissimus Dorsi (LD) muscle

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was removed from the carcasses immediately after slaughter and cut into chops with an average weight of 50 g, vacuum packed into pouches, taken to the laboratory under refrigerated conditions. The samples were aged at 4°C for 0, 12, 24, 48, 72, 120, 168 and 192 h, respectively. WHC, pH, SF and TPA parameters analysis were made in fresh meat samples. Other samples were stored at -80°C until tested for MFI and calpain activity.

Casein, Tris; EDTA, 2-Mercaptoethanol (MCE), Leupeptin, NaN_3, Phenylmethy-lsulfony l Fluoride (PMSF); ovomucoid was obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals used in this research were also from commercial sources and of analytical grade.

Analytical method

pH value determination: pH value of yak meat (LD muscle) were measured using a pH meter (PHS2-25, Shanghai, China) with a piercing pH electrode. Measurements were taken by inserting the pH probes in the LD muscle.

Water Holding Capacity (WHC): WHC was evaluated in two ways: Cooking Loss (CL) and Pressing Loss (PL) (Franco *et al*., 2009). For CL test, the samples were cooked in bags submerged in boiling water until the internal temperature reached to 75°C, a thermocouple was inserted into the center of each sample to measure the temperature during cooking. After cooking, the samples were cooled to room temperature (20°C) dried with blotting paper and re-weighed immediately. CL was calculated by measuring the difference in weight between the cooked and raw samples according to the following equation:

$$CL\ (\%) = \frac{(\text{Raw weight-Cooked weight})}{\text{Raw weight}} \times 100$$

PL was measured according to the procedure outlined by Wan *et al*. (2011).

Tenderness: The tenderness was manifested by the measurement of Shear Force (SF) and Myofibril Fragmentation Index (MFI).

Shear force: For shear force measurements, muscles were cooked as the described for CL. Three to five cores (φ1.27) were cut from the steaks parallel to the muscle fiber orientation. Each sample was sheared with the long axis of the fibers running perpendicular to the blade using a tenderometer (C-LM4, College of Engineering, Northeast Agricultural University, Harbin, China) (Wiklund *et al*., 2010). The results were expressed in Newtons.

Myofibril Fragmentation Index (MFI): MFI was determined according to the procedures of Kriese *et al*. (2007).

Texture measurement: Muscle texture was measured according to the Texture Profile Analysis (TPA) procedures described by Boume (1982) with some modifications with a Texture Analyser (Food Technology Corporation, Sterling, Virginia, USA). The test involved driving a 0.50 cm diameter shaft twice, in parallel to muscle fibre into a sample down to 50% of their original height using a crosshead speed of 50 mm min^{-1} and a load cell of 50 N. The force deformation curve obtained during the TPA test served to calculate meat hardness, cohesiveness, cohesiveness and springiness. The TPA test was repeated 10 times on each sample of muscle.

Calpains and calpastatin activities: The procedure to extract, separate and assay calpains and calpastatin was a modification of the method described by Delgado *et al*. (2001) and Morton *et al*. (1999). Briefly, 50 g samples from each of the twelve yaks were removed immediately after death or after 12, 24, 48, 72, 120, 168 and 192 h of postmortem storage. The samples were trimmed free from visible fat and connective tissue and were homogenized at 4°C in 3 volumes (vol/wt) of 100 mM Tris-HCl, pH 8.3; 10 mM EDTA; 10 mM 2-Mercaptoethanol (MCE) and a cocktail of protease inhibitors (5 mg L^{-1} leupeptin, 100 mg L^{-1} ovomucoid and 2 mM phenylmethy-lsulfonyl fluoride) by using a waring blender (Dynamics Co., of America; New Hartford, CT) at high speed five times for 3 min each time with 3 min cooling periods between each time. The homogenate was centrifuged for 120 min at 18,000×g, the volume of the supernatant was recorded and the supernatant was salted out between 0 and 45% ammonium sulfate saturation. The sediment protein was dissolved in approximately 25 mL of homogenizing buffer, placed in a dialysis bag and dialyzed (12-14,000 MW cutoff) overnight against 25 volumes of 40 mM Tris-HCl, pH 7.35; 5 mM EDTA; 10 mM MCE. After dialysis, The dialyzed extract was clarified by centrifugation at 18,000×g for 1 h and filtered through Miracloth (Calbiochem) and was loaded onto a 1.6×20 cm DEAE-Sephacel column. The DEAE columns were eluted with 50 mM Tris-HCl, pH 7.5; 0.5 mM EDTA; 10 mM MCE (3 column volume) until the A278 reached the baseline, the bound proteins were eluted with a linear 0→400 mM NaCl gradient (500 mL of each) in 50 mM Tris-HCl, pH 7.5; 0.5 mM EDTA; 10 mM MCE. Flow rate was 0.5 mL min^{-1}; 3.0 mL fractions were collected. Calpastatin eluted between 20 and 110 mM NaCl, μ-calpain between 110 and 165 mM NaCl and m-calpain between 220 and 330 mM NaCl from this column.

Calpain activity was determined using casein as a substrate. Aliquots (1 mL) of the column fractions were incubated at 25°C with 1 mL of 0.7% casein in 100 mM Tris-HCl, 10 mM mercaptoethanol, 1 mM NaN_3, pH 7.5 containing 100 mL of either 0.1 M $CaCl_2$ or 0.2 M EDTA. The reaction was stopped after 30 min with 2 mL of 5% TCA and the mixture centrifuged at 3500 g for 15 min. The absorbance of the supernatant was read at 278 nm with a HITACHIU-2000 spectrophotometer. One unit of calpain was determined as the amount which gave a Ca-dependent increase of 1.0 unit of absorption in 1 h. Calpastatin was determined and expressed as inhibitory equivalents of m-calpain.

Statistical analysis: Statistical analysis was performed with the Analysis of Variance (ANOVA) using SPSS (SPSS Version 16.0 for windows; SPSS, Chicago, IL, USA). All the data were subjected to analysis of variance where statistical significance was observed and the differences between means were carried out using Duncan's multiple range test.

RESULTS AND DISCUSSION

Changes of physiochemical traits during ageing pH: As was seen from Table 1, a significant decrease in pH values of LD muscle was found during the first 72 h post mortem ($p<0.05$). However, the pH values remained s after 72 h. A final value of pH was attained in 72 h. The result was compared with earlier research. Wan *et al.* (2011) has reported an effect on pH was found during yak meat ageing but the most declining phase occurred at 24 h. Other researchers also claimed that pH value of bovine meat decreased after slaughter and reached the minimum at 24 h (Silva *et al.*, 1999; Zamora *et al.*,1996; Marsh *et al.*, 1988). It is obviously descent rate of pH in this study is inconsistent with earlier study. Descent rate of pH can be influenced by several factors such as electrical stimulation, Stunning Method and chilling treatments (Barton-Gade, 1993; Kastner *et al.*, 1993; Rhee *et al.*, 2006). The possible reason of the result of this study might be attributed to the chilling treatments.

WHC: Significant increasing ($p<0.05$) in CL were found during 0-72 h and no differences during the next 120 h. PL had similar changes (Table 1). WHC of the meat during ageing was reflected by the results of CL and PL.

WHC is defined as the ability of meat to retain its water during application of external forces such as cutting, heating, grinding or pressing (Zhang *et al.*, 2005). WHC of meat is mainly affected by pH (Offer and Knight, 1988). The pI of meat is in the pH range of 5.0-5.5 which is also the pH of meat after it has gone through rigor mortis increasing or decreasing the pH away from the pI will result in increased WHC by creating a charge imbalance (Zhang *et al.*, 2005). Purchas (1990) reported that the greater the pH, the greater the WHC. Zhang *et al.* (2005) also claimed that higher WHC in high pH meat than in normal pH meat. The results of this research are consistent with Purchas's conclusions.

Tenderness: The changes of SF and MFI value for yak meat during postmortem aging are shown in Table 1. Significant increase and decrease in SF was found in the first 72 and 72-120 h after ageing, respectively. The initial gradual increase in meat shear force is a typical of muscle going into rigor mortis, the decrease thereafter is what could be expected as meat is aged (Thomas *et al.*, 2004).

MFI shows a significant increase during 72 h and then remains constant. MFI is a useful indicator of the extent of myofibrillar protein degradation (Olson *et al.*, 1976). Some researchers reported MFI increased with ageing (Olson *et al.*, 1976; Watanabe and Devine, 1996). Results of this study are consistent with the earlier findings.

Changes of TPA in yak meat during aging: Table 2 reflected a progressive softening in the meat from texture parameters. A significant increase in hardness was found in 72 h post mortem ($p<0.05$) and a slight decline was found from 72-120 h ($p<0.05$) then the value remained constant. Similar situation was also found in cohesiveness and chewiness; however, there are no significant differences in cohesiveness. The springiness of the meat showed significant decrease in first 72 h ($p<0.05$), afterwards, the values remained stable. Hardness is one of the major factors to decide the commercial value

Table 1: Physicochemical changes of Gannan yak meat during ageing

	Ageing time (h)							
Items	0	12	24	48	72	120	168	192
pH	6.79±0.05^a	6.34±0.04^b	6.29±0.07^b	5.93±0.06^c	5.56±0.05^d	5.58±0.07^d	5.60±0.06^d	5.65±0.09^d
CL (%)	27.98±1.54^d	31.2±1.380^c	35.98±1.29^b	36.27±1.56^b	40.21±1.43^a	32.97±2.16^c	32.54±1.89^c	31.95±2.45^c
PL (%)	5.60±0.42^d	11.56±1.95^c	15.79±1.78^b	16.12±1.56^b	23.98±1.02^a	12.05±1.33^c	11.89±1.13^c	11.09±2.13^c
SF (N)	49.92±3.29^d	50.1±2.650^d	63.79±4.98^c	70.25±3.16^b	88.94±3.64^a	52.14±2.16^d	41.89±1.88^e	40.78±2.07^e
MFI	35.5±5.460^e	40.6±4.200^e	50.8±3.590^d	70.29±8.21^c	130.2±8.490^b	155.7±7.130^a	160.2±8.350^a	162.36±9.16^a

$^{a-e}$Means with different letters in the same row differ ($p<0.05$)

Table 2: Changes of TPA of Gannan yak meat during aging

	Ageing time (h)							
Items	0	12	24	48	72	120	168	196
Hardness (N)	41.5±4.1200^{c}	43.27±4.270^{c}	44.89±3.430^{c}	50.90±5.130^{b}	70.3±4.5900^{a}	49.02±6.21^{b}	36.80±3.320	35.60±3.980^{d}
Cohesiveness	0.60±0.020	0.62±0.040	0.65±0.070	0.70±0.050	0.71±0.070	0.64±0.08	0.61±0.050	0.60±0.060
Springiness (mm)	4.35±0.120^{a}	4.30±0.170^{a}	3.90±0.150^{b}	4.08±0.180^{b}	3.77±0.200^{c}	3.70±0.22^{c}	3.62±0.240^{c}	3.60±0.200^{c}
Chewiness (J)	108.31±13.11^{c}	115.36±15.69^{c}	114.10±12.13^{c}	145.35±23.15^{b}	188.17±13.25	116.08±15.1^{c}	81.26±17.89^{d}	76.89±10.58^{d}

$^{a-e}$Means with different letters in the same row differ ($p<0.05$)

Table 3: Activitiy changes of calpains and calpastatin during ageing

Ageing time (h)	μ-Calpain (U)	m-Calpain (U)	Calpastatin (U)
0	2.16±0.12^{a}	2.06±0.12^{a}	3.25±0.15^{a}
12	1.20±0.04^{b}	1.98±0.09^{a}	3.20±0.14^{a}
24	0.80±0.06^{c}	1.78±0.10^{b}	3.16±0.17^{a}
48	0.75±0.05^{c}	1.75±0.15^{b}	3.18±0.13^{a}
72	0.72±0.08^{c}	1.67±0.16^{b}	3.10±0.18^{a}
120	0.32±0.05^{d}	1.63±0.18^{b}	1.88±0.20^{b}
168	0.28±0.04^{d}	1.60±0.20^{b}	1.08±0.16^{c}
192	0.26±0.09^{d}	1.62±0.21^{b}	1.02±0.10^{c}

Standard casein assay (activity given as units/g of muscle). $^{a-e}$Means with different letters in the same row differ ($p<0.05$)

of the meat (Chambers and Bowers, 1993). It is affected by the contents of connective tissue (Franco *et al*., 2009). Values of hardness, springiness and chewiness assessed by means of TPA decreased as ageing time (De Huidobro *et al*., 2003). The results are almost consistent with Ruiz's conclusions.

Changes of calpains and calpastatin in yak meat during aging: Table 3 shows the activity changes of μ-Calpain, m-Calpain and Calpastatin. The activities of μ-Calpain displayed a significant decline during 72 h ($p<0.05$) and then remained unchanged. The activities of m-Calpain had a slight decrease from 0-12 h postmortem, afterwards the values were s. The activity of calpastatin had a decline tendency as ageing time ($p<0.05$).

The μ-Calpain and m-Calpain requires Ca^{2+} to be proteolytically active and the presence of sufficient Ca^{2+} will undergo autolysis. Autolysis of μ-calpain causes the enzyme to be less s and ultimately leads to loss of activity (Goll *et al*., 2003). The loss of calpastatin activity during postmortem storage was caused by degradation of the calpastatin polypeptides (Koohmaraie *et al*., 1987). Several studies on the changes in activities of calpain and calpastatin during postmortem storage have been reported (Boehm *et al*., 1998; Delgado *et al*., 2001; Zamora *et al*., 1996). These results suggested that extracable μ-calpain and calpastatin activities were decreased during the postmortem storage whereas less obvious changes were found on m-calpain activity during the postmortem storage. However, in a recent study using five different bovine muscles showed a significant decrease in the m-calpain activity of around 80% was observed during the first 144 h of post-mortem storage (Camou *et al*., 2007). The results of this study are almost consistent with studies reported earlier (Boehm *et al*., 1998; Delgado *et al*., 2001).

In post-mortem of skeletal muscles from meat animals, the proteolytic activity of the calpain system on the myofibrillar proteins has the primary influence on meat tenderness (Veiseth *et al*., 2004). In the study, researchers observed MFI had a significant increase during the first 72 h, its increases showed the extent of myofibrillar protein degradation.

At the same time, researchers also observed a significant decline of activities of μ-calpain during 72 h but the activities of m-calpain and calpastatin had only slight changes in first 72 h. Based on the available evidence, researchers could conclude that the changes of calpains proteolytic activity possibly caused the increase in MFI, weakening of the myofibrils and tenderization, μ-calpain might be the main contributor of tenderization.

CONCLUSION

In the study, physiochemical traits and μ-calpain, m-calpain and their inhibitor calpastatin had been studied during yak meat postmortem ageing. Ageing improved the tenderness and declined the activities of u-calpain, m-calpain and calpastatin. Researchers discussed the roles of μ-calpain, m-calpain and calpastatin in the tenderisation process of yak meat and μ-calpain may be the main contributor of tenderization. However, meat tenderization is a very complicated process because there are other enzymes are related to tenderization such as cathepsins, caspases and proteasomes and so on. Therefore, further in depth studies are needed to discover the tenderization of yak meat during ageing.

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