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Research Article

Comparison of Tenderness and Calpains Activity of Yak Meat in Different Ages During Postmortem Aging

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Abstract

Yak is a rare breed of resources in Tibetan Plateau, with high protein and low-fat content, and has other important application value. Yaks are managed by herders and slaughtered in various ages, changing from three years to twelve years of age, and there has a significant difference on meat tenderness in different ages. This study was conducted to investigate the effects of age at harvest, ageing time and the correlation analysis to meat tenderness calpains activity. The work may be used to establish a classification system and grading standards for yak meat. Four groups were established as fewer than 3 years-old, 3-5 years-old, 5-7 years-old and more than 7-years old. The results demonstrated that the content of tenderness has significantly different ($P<0.05$) in various ages and it increased and then decreased during postmortem aging. The muscle fiber diameter has decreased with the increase of slaughter age, while the MFI showed the contrary. The MFI increased with aging. The myofibrils' ultrastructure was completely disrupted. Calpains activity was significantly decreased ($P<0.05$) in the first 3 d and then decreased with aging. The calpains activity of yak meat in different ages has increased and then decreased with the different ages. The nitration of μ-calpain promoted its ability in degrading a part of myofibrillar proteins and the degrading of myofibrillar proteins related to the tenderness of meat.

Keywords: *Yak meat, Ages, Postmortem aging, Meat tenderness, Calpains*

Farklı Yaşlardaki Yaklara Ait Etlerin Postmortem Olgunlaştırma Sürecinde Yumuşaklık ve Kalpain Aktivitelerinin Karşılaştırılması

Öz

Yak, Tibet Platosu'nda yaşayan, yüksek protein ve düşük yağ içerikli etinin yanı sıra uygulamada önemli değere sahip diğer nitelikleri ile nadir bir cinstir. Çobanlar tarafından idare edilen Yakların, 3-20 arasında değişen yaşlarda kesimleri gerçekleştirilir ve bu yaş farklılıkları etin yumuşaklığı üzerinde belirgin bir fark oluşturur. Bu çalışma, kesim yaşı, olgunlaştırma zamanı ve korelasyon analizinin etin yumuşaklık kalpain aktivitesine etkisini araştırmak üzere yürütülmüştür. Çalışma sonuçları, Yak etinin sınıflandırma sistemi ve derecelendirme standardının oluşturulmasında yararlı olabilir. Araştırmada 3 yaşından küçük, 3-5 yaş arası, 5-7 yaş arası ve 7 yaşından büyük olmak üzere 4 grup oluşturulmuştur. Bulgular, yumuşaklık göstergesi olan parametrelerin yaş gruplarına göre belirgin bir şekilde değiştiğini ($P<0.05$), postmortem olgunlaştırma sürecinde önce artıp sonra azaldığını göstermiştir. Kesim yaşı arttıkça kas lifi çapının azaldığı, ancak MFI için tam tersi bir durumun sözkonusu olduğu belirlenmiştir. MFI, olgunlaştırma ile birlikte artış göstermiştir. Kas liflerinin ultrayapısı tamamıyle bozulmuştur. Kalpainlerin aktivitesi ilk 3 günde belirgin biçimde azalmış ($P<0.05$) ve ardından olgunlaştırma süresince azalmıştır. Farklı yaşlardaki Yaklara ait etlerdeki kalpainlerin aktiviteleri artmış ve sonrasında yaş farklılıklarına bağlı olarak azalmıştır. μ-calpain nitrasyonu miyofibriler proteinlerin parçalanmasını hızlandırmış, böylece et yumuşaklığı üzerine etki göstermiştir.

Anahtar sözcükler: *Yak eti, Yaş, Postmortem olgunlaştırma, Et yumuşaklığı, Kalpainler*

INTRODUCTION

The yak meat is famous for its low fat, high protein, fine texture and rich amino acids compared to beef [1]. Consequently, more and more people choose the green and non-pollution yak meat [2]. Nowadays, on the source of Yak meat, ongoing research is underway on the yak meat composition [3,4]. Male Yak meat is rich in phosphorus and

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calcium, compared to female meat as well as cattle meat [5]. Wild Yak meat is rich in iron and various essential minerals [5]. Ageing is one effective way to enhance tenderness and meat tenderness is one of the most important factors in terms of consumer purchase [6,7]. Many researchers study Yak meat quality, and they are focused on the effect of the breed of beef, feeding time, gender, trophic levels, and feed additives on tenderness [8,9]. Postmortem tender process, color stability and water holding capacity of yak meat were also studied [9,10]. Meat processing and meat quality of yak in different ages during postmortem aging were also investigated [9,10]. However reports about the difference of yak meat tenderness and the mechanism are very few. Therefore, the aims of this research were to demonstrate the difference of meat tenderness and calpains activity of yak in different ages during postmortem aging.

MATERIAL and METHODS

Materials and Reagents

Sixty healthy yaks, selected after examination by a veterinarian, were used from Maqu country in Gannan. The fasting 24 h, 12 h water deprivation was compiled before yaks slaughtered. *Longissimus dorsi* muscle was removed from the carcasses immediately after slaughter and cut into chops with an average weight of 200 g, vacuum packed into pouches, taken to the laboratory under refrigerated condition. The samples were refrigerated at 4°C for 0, 1, 2, 3, 4, 5, 6 and 7 days, respectively. The shear force analysis was made in fresh meat samples. The samples tested for muscle fiber diameter are fixed in 10% formaldehyde solution, and those tested for myofibrils ultrastructure are fixed in 3% glutaraldehyde solution. Other samples were stored at -80°C until tested for muscle fiber diameter, myofibrillar fragmentation index, myofibrils ultrastructure and calpain activity. There were four experimental groups: ≤3 years, 3-5 years, 5-7 years, >7 years 4 groups.

Tenderness Measurement

Shear Force: Samples were packed in plastic bags and submitted for cooking in a water bath until the internal temperature reached the value of 75°C, maintain 15 min and then cool down to room temperature. Samples were cut into 1 cm^3, and analyzed on a texturometer C-LM4 [11].

Muscle Fiber Diameter: Formaldehyde fixed samples were taken out, stripped of muscle fiber, and then measured by micrometer at 400 times magnification. In each sample 100 muscle fibers were measured, and the averages were calculated.

Myofibrillar Fragmentation Index (MFI): MFI was determined according to the procedures described by Kriese et al.[12]. One gram muscle sample, free of external fat and visible connective tissue, were homogenized for 30 s in 20 mL of MFI buffer (100 mM KCl, 20 mM potassium phosphate, 1 mM EDTA, 1 mM $MgCl_2$ and 1 mM NaN_3 at pH 7.0). 1 mM EDTA, 1 mM $MgCl_2$ and 1 mM NaN_3 at pH 7.0). The homogenate was centrifuged at 10×10^3 rpm for 15 min at 4°C, the supernatant discarded and pellet resuspended in 20 mL of the MFI buffer and centrifuged at 10×10^3 rpm for 15 min at 4°C. The supernatant was discarded and the pellet suspended in 10 mL of the same MFI buffer. The myofibril suspension was poured through a strainer to remove connective tissue, and then assayed for protein concentration using the biuret method. Aliquots of the suspensions were diluted in the MFI buffer to a final protein concentration of 0.5 mg/mL. The diluted protein suspension was poured into a cuvette and the absorbance at 540 nm was immediately measured with a spectrophotometer. The MFI was expressed as A540 nm×200.

Myofibrils Ultrastructure: Myofibrils ultrastructure was determined according to the procedures of Xu et al.[13]. The samples were cut into 0.5×0.5×0.5 cm small squares, and put in 3% glutaraldehyde solution (0.1 mol/L, pH 7.3), fixed and stored 3 days or longer at 0~4°C. Then, the samples were rinsed with 0.1 mol/L phosphate buffer solution 10 min and then fixed 1 h in 1% osmium tetroxide (0~4°C). Samples were rinsed with 0.1 mol/L phosphate buffer 10 min. With a concentration of respectively 30%, 50%, 70%, 90% and 100% ethanol gradually elute and with 100% ethanol dehydration once again. Samples were processed with Epon 812 epoxy resin and cut into 50 mm flake and then dyed with uranyl acetate and lead citrate. the samples were observed under a transmission electron microscope, and photographed.

Calpain Activity

• *Crude Calpain Activity Determination*

Crude Enzyme Extraction: Extraction, separation and assay of calpains were done according to the method described by Delgado et al.[14]. Briefly, the samples from each of the yaks were removed after death or after 1, 2, 3, 4, 5, 6 and 7 days of postmortem storage. The samples were trimmed free from visible fat and connective tissue and were homogenized at 4°C and 1 L extraction liquid was added (pH 8.3, 100 mmol/L Tris-HCl, 10 mmol/L EDTA, 0.05% MCE, 100 mg/L, Ovomucoid inhibitor, 2 mmol/L PMSF, 6 mg/L Leupeptin) then centrifuged (4°C, 10000 r/min 1.5 h), the precipitate was discarded, and the volume of the supernatant was recorded and the supernatant was salted out between 0 and 45% ammonium sulfate saturation. Then the sediment protein was dialyzed (pH 7.5, 20 mmol/L Tris-HCl, 1 mmol/L EDTA, 0.1% MCE). After dialysis, the dialyzed extract was clarified by centrifugation at 4°C, 10.000 r/min for 1.5 h and filtered with 0.45 μm needle filter.

Crude Activity Measurement: The procedure to determin crude activity was a modification of the method described by Koohmarie [15] and Huang Ming [16]. The samples were taken 1 mL and the reaction mixture (100 mmol/L Tris,

10 mmol/L MCE, 5 mg/mL Casein-Hammerstein, the pH 7.5 with acetic acid) was added. The reaction was started adding 100 µL 0.1 mol/L $CaCl_2$, and it was stopped after 30 min with 2 mL of 5% TCA and the mixture was centrifuged at 6.000 g for 30 min. The absorbance of the supernatant was read at 278 nm.

• µ-Calpain in vitro Nitration of Myofibrillar Protein Degradation

The Separation and Purification of µ-Calpain: µ-Calpain was purified using DEAE-Sepharose-FF, selection the highest activity of crude enzyme calpain, and use of equilibrated solution TEMA (4ºC, pH7.5, 20 mmol/L Tris-HCl, 1 mmol/L EDTA, 0.1% MCE, 1 mmol/L NaN_3), then TEMA solution was used to elute with a concentration of 0~500 mmol/L NaCl, until the absorbance value of sample liquid less than 0.1 at A280 nm.

Myofibrillar Protein Extraction: The procedure to extract myofibrillar rotein was a modification of the method described by Liu [17]. The longissimus sample trimmed, cut into pieces, 10 volumes salt solution was added (20 mmol/L potassium phosphate buffer, 0.1 mol/L KCl, 2 mmol/L $MgCl_2$, 2 mmol/L EGTA, pH 6.8), and homogenated 10 s (13.000 r/min) then centrifuged 10 min (1000 r/min) at 4ºC, and the supernatant was discarded. Using 8 volumes salt solution rinse the precipitation, and using the 8 volumes salt solution (20 mmol/L potassium phosphate buffer, 0.1 mol/L KCl, 2 mmol/L $MgCl_2$, 2 mmol/LEGTA, 1% Triton-100, PH 6.8) what contain 1% Triton-100, then rinsing with 8 volumes 100 mmol/L KCl. After rinsing, the precipitate was dissolved in the incubation buffer without DTT (5 mmol/L Hepes, 100 mmol/L NaCl, 0.1% Chaps, 5 mmol/L NaN_3, pH 6.5) in.

Protein Concentrations Determination: Coomassie Brilliant Blue

ONOO- Preparation: The procedure to prepare ONOO- was a modification of the method described by Beckman et al.[18]. Mixe the dilution 100 mL containing 12.5 mL 0.6 mol/L HCl and 15 mL 0.7 mol/L H_2O_2, and prepared 100 mL 0.6 mol/L $NaNO_2$, then filtered to brown reagent bottle at -20ºC cryo- preservation. Before each test, with 1 mol/L NaOH diluted ONOO-, according to 302 nm absorbance values to determine the concentration of ONOO- (ε302 nm = 1670 L/mol • cm).

µ- Calpain In Vitro Nitration: Active enzyme was randomly divided into seven groups, with six different concentrations of peroxynitrite solution, to produce different levels of nitration of ONOO-, every groups were processed 30 min at 37ºC.

Nitration µ-Calpain Incubated Myofibrils: After the nitration µ-calpain oxide ended in different conditions, purified myofibrils and nitration µ-calpain were mixed immediately.

Incubation Conditions: 600 µL of myofibrillar protein (10 mg/mL), 15 µL µ-calpain, 37ºC water bath for 30 min. After the incubation, the samples were placed in ice immediately for stopping the reaction and an appropriate amount of sample reagent solution (0.6 mL 1 mol/LTris-HCl pH6.8, 5 mL 50% glycerin, 2 mL 10% SDS, 0.5 mL MCE, 1 mL BPB, 0.9 mL distilled water)was added in water bath at 100ºC. Then the samples were stored at -80ºC.

SDS-PAGE Electrophoresis Analysis: Electrophoresis conditions: separation gel concentrations of 12%, spacer gel concentrations of 5%, voltage 80v. With Coomassie Brilliant Blue R-250 staining 1 h after electrophoresis, and decolorization processing placed a night.

Data Analysis

Processing data using Microsoft Excel 2007; Using SPSS 19.0 for data analysis and significant pearson correlation analysis.

RESULTS

Shear Force

Shear force is the most important quality index of meat, which reflect the tenderness of meat. Lower the value of the meat is, tenderer it is. The results of the shear force in the mature process of yak in different ages during postmortem aging are shown in *Table 1*.

The changes of shear force for yak meat during postmortem aging are shown in *Table 1*. Significant increase and than decrease in shear force was found in the first 3 day and 3-7 days after aging, respectively. Among the different ages, yak meat tenderness significantly differed ($P<0.05$). The initial gradual increase in meat shear force is a typical of muscle going into rigor mortis, the decrease there after is what could be expected as meat is aged.

Table 1. *Difference in Share force values (kgf) during postmortem aging of yak meat in different ages*

Index	Mature Time (d)					
	0	1	2	3	5	7
≤3 years	$3.45\pm0.29^{a,B}$	$4.60\pm0.28^{a,C}$	$5.22\pm0.13^{a,D}$	$6.92\pm0.46^{a,DE}$	$5.31\pm0.35^{a,D}$	$2.87\pm0.23^{a,A}$
3~5 years	$4.11\pm0.66^{b,A}$	$5.02\pm0.65^{a,B}$	$7.21\pm0.18^{b,C}$	$9.38\pm0.03^{b,D}$	$6.60\pm0.35^{b,E}$	$3.82\pm0.27^{b,A}$
5~7 years	$5.31\pm0.65^{c,B}$	$7.60\pm0.73^{b,C}$	$9.40\pm0.54^{c,D}$	$10.40\pm0.70^{c,E}$	$7.98\pm0.02^{c,C}$	$4.04\pm0.26^{b,A}$
>7 years	$6.49\pm0.33^{d,B}$	$7.55\pm0.74^{b,C}$	$9.19\pm0.49^{c,D}$	$11.30\pm1.25^{d,E}$	$8.32\pm0.66^{c,C}$	$5.26\pm0.98^{c,A}$

Table 2. *Difference in muscle fiber diameter(μm) during postmortem aging of yak meat in different ages*

Index	Mature Time (d)					
	0	1	2	3	5	7
≤3 years	49.71±0.63^{a,A}	48.45±0.67^{a,B}	47.87±0.52^{a,C}	45.39±0.50^{a,D}	41.97±0.49^{a,E}	40.62±0.68^{a,F}
3~5 years	55.38±0.46^{b,A}	54.98±0.55^{b,A}	54.03±0.82^{b,B}	51.88±0.48^{b,C}	48.38±0.52^{b,D}	46.78±0.43^{b,E}
5~7 years	59.88±0.49^{c,A}	58.54±0.14^{c,B}	57.43±0.80^{c,C}	53.78±0.45^{c,D}	50.01±0.90^{c,E}	48.26±0.98^{c,F}
>7 years	63.33±0.98^{d,A}	61.32±0.66^{d,B}	60.65±0.53^{d,C}	56.89±0.57^{d,D}	53.21±0.44^{d,E}	51.08±0.43^{d,F}

Table 3. *Difference in MFI during postmortem aging of yak meat in different ages*

Index	Mature Time (d)					
	0	1	2	3	5	7
≤3 years	35.03±0.64^{a,A}	61.75±0.76^{a,B}	100.02±0.66^{a,C}	122.47±0.42^{a,D}	132.78±0.02^{a,D}	137.25±0.41^{a,D}
3~5 years	29.54±0.31^{b,A}	42.95±0.31^{b,B}	79.13±0.23^{a,C}	108.68±0.42^{b,D}	111.21±0.64^{b,DE}	115.07±0.62^{b,E}
5~7 years	21.03±0.21^{c,A}	30.11±0.32^{c,B}	64.24±0.07^{b,C}	97.32±0.03^{c,D}	102.45±0.42^{c,DE}	105.24±0.57^{c,E}
>7 years	13.54±0.41^{d,A}	22.28±0.42^{d,B}	56.44±0.25^{c,C}	77.24±0.67^{d,D}	83.38±0.75^{d,DE}	86.34±0.24^{d,E}

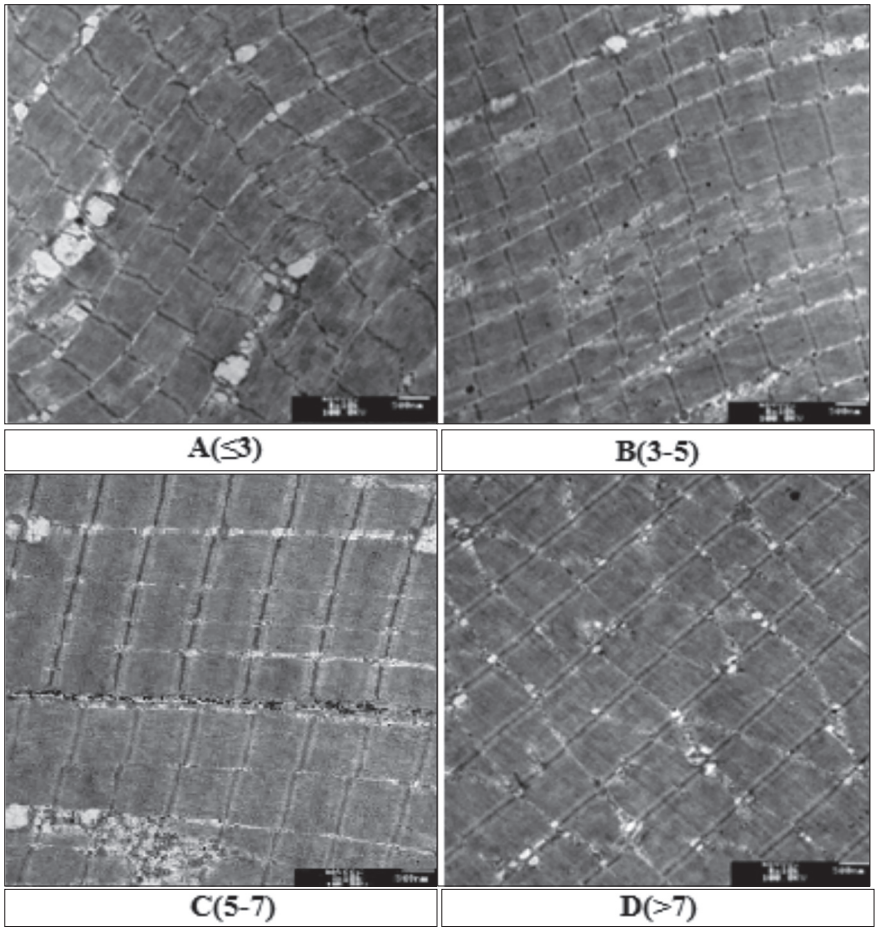

Fig 1. Myofibrils ultrastructure of yak meat in different ages (×10000)

Muscle Fiber Diameter

Muscle fiber diameter as an important index for muscle tenderness, and have a great impact on muscle tenderness. The measurement results of different ages during postmortem aging of muscle fiber diameters as shown in *Table 2*.

The changes of shear force for yak meat during postmortem aging are shown in *Table 2*. Significant decrease in muscle fiber diameter was found 0-7 days after ageing ($P<0.05$), and also significant differences between different ages were seen ($P<0.05$).

Muscle Fiber Fragmentation Index

The changes of MFI for yak meat during postmortem aging are shown in *Table 3*. It shows a significant increase during 0-7 days and then remains constant, and there iare also significant differences between the different ages ($P<0.01$). The higher the age, the lower the MFI of the samples.

Myofibrils Ultrastructure

Fig. 1 shows myofibrils ultrastructure of samples at different ages by transmission electron microscopy. Myofibrils ultrastructure are consistent on the form in different ages, and Z- line clearly. Between two Z lines of a sarcomere, the longer the sarcomere, the better the tenderness. As we can see from the *Fig. 1*, 3 years old yak meat myofibrillar ultrastructure sarcomere are relatively long, and 3 to 5 years old we can see the sarcomere are clearly shorter; 5 to 7 years and beyond 7 years old the sarcomere length compare to the 3 to 5 years old is even shorter.

Table 4. *Correlations analysis between age and myofibrils ultrastructure indicators*

Index	Age	Muscle Fiber Diameter	MFI
Age	1	0.737**	-0.387
Muscle fiber diameter		1	0.714*
MFI			1

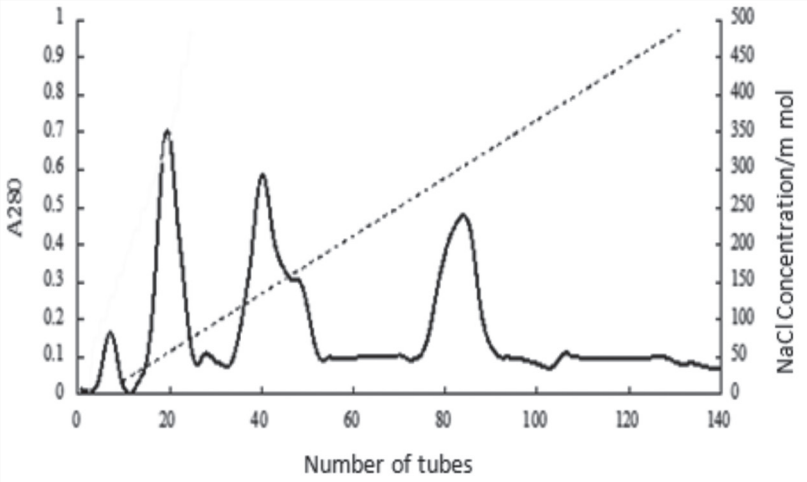

Fig 2. Elution profile of calpain system loaded onto DEAE-Sepharose-FF column

in calpain activity was found 0-2 days after ageing (P<0.05), and the rate of decline slowed down after 3 days. The crude calpain activity was initially increased, and it subsequently decreased in 0 day after aging, it is related to the body metabolism of different ages. Calpains activity of yak meat in different age decreased in all the tested postmortem aging groups. On one hand, it is due to lack of the calcium activated enzyme (consumed), chaplains can degrades the other proteins, and also can degrade itself.

Correlation Analysis Between Calpain Activity and Tenderness

As it can be seen from *Table 6*, shear force and muscle fiber diameter had a significant positive correlation (P<0.05) during postmortem aging. Shear, MFI and muscle fiber diameter and calpains activity correlation significantly (P<0.01). The shear force can be responsiable from the tenderness of the yak meat directly, and

Table 5. *Difference in calpains during postmortem aging of yak meat in different ages*

Index	Mature Time (d)					
	0	1	2	3	5	7
≤3years	0.738±0.019^{a,A}	0.549±0.023^{a,B}	0.249±0.064^{a,C}	0.191±0.045^{a,CD}	0.161±0.063^{a,D}	0.145±0.029^{a,D}
3-5years	0.830±0.034^{b,A}	0.628±0.055^{b,B}	0.380±0.053^{a,C}	0.273±0.045^{a,D}	0.247±0.074^{a,D}	0.231±0.051^{a,D}
5-7years	0.823±0.045^{c,A}	0.615±0.021^{c,B}	0.312±0.060^{a,Cb}	0.261±0.037^{b,C}	0.238±0.092^{b,C}	0.224±0.064^{b,C}
>7years	0.658±0.066^{c,A}	0.506±0.014^{c,B}	0.256±0.034^{b,C}	0.213±0.036^{b,C}	0.196±0.023^{b,C}	0.179±0.097^{b,C}

Table 6. *Correlation analysis between tenderness indexes of yak meat*

Index	Shear Force	MFI	Muscle Fiber Diameter	The Crude Enzyme Activity
Shear force	1	-0.012	0.489*	-0.291
MFI		1	-0.822**	-0.881**
Muscle fiber diameter			1	0.533**
The crude enzyme activity				1

Correlation Between Age, Muscle Fiber Diameter and the MFI

The correlations between the age, and the muscle fiber diameter and the MFI are shown in *Table 4*. Age and muscle fiber diameter were significantly positivly correlated (P<0.01), and MFI was negatively correlated. This shows that age has a greater impact on muscle tenderness. The bigger the yak's age, the smaller fiber diameter of the muscle, the smaller the MFI value, and the less tenderness of samples.

Changes in calpain activity for yak meat during post-mortem aging are shown in *Table 5*. A significant decrease

four indicators (shear force, MFI muscle fiber diameter, and the crude enzyme activity) can be used as indicators of the evaluation of yak meat tenderness.

In vitro Nitro μ-Calpain of Yak Meat Myofibrillar Protein Degradation

DEAE-Sepharose-FF ion exchange column was used to separate and purify the calpains, and Calpastatin, μ-calpain, m-calpain are separated completely *(Fig. 2)*. Calpastatin in the range of 30~110 mmol/L NaCl concentration was eluted, μ-calpain in the range of 130~180 mmol/L NaCl concentration was eluted and m-calpain in the range of

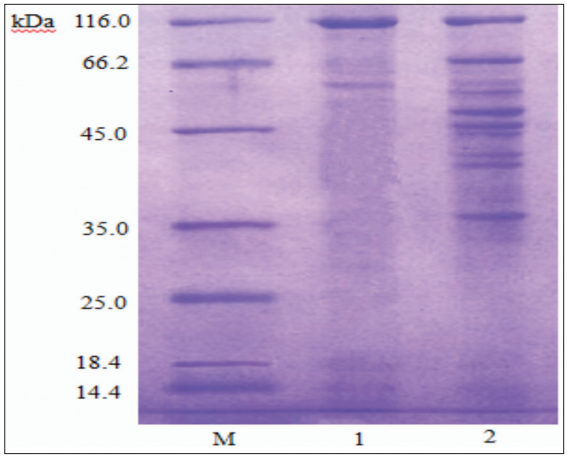

Fig 3. SDS-PAGE analysis of degradation of myofibrillar proteins incubated with μ-calpain nitrationed at different levels. M: Marker; 1: Control; 2: 0.2 mmol/LONOO-; 3: 0.4 mmol/LONOO-; 4: 0.6 mmol/LONOO-; 5: 0.8 mmol/L ONOO-; 6: 1.0 mmol/L ONOO-; 7: 1.5 mmol/L ONOO-

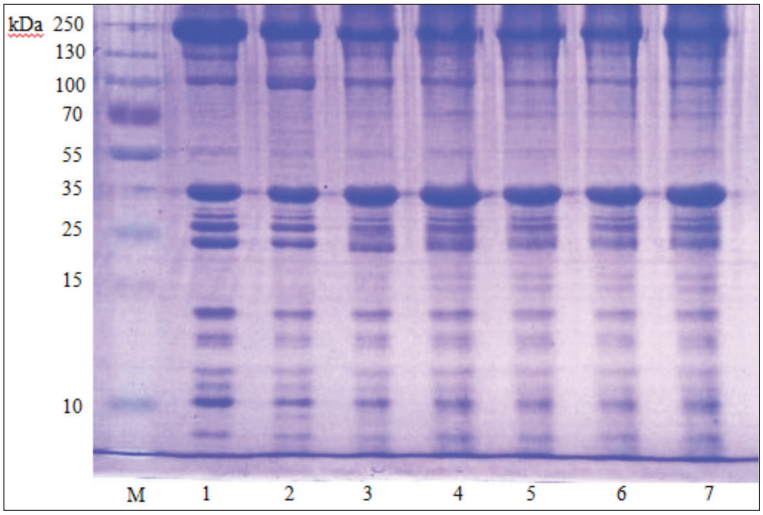

Fig 4. SDS-PAGE of purified μ-calpain. M: Marker; 1: 0~500 mmol/LNaCl; 2: 0~330 mmol/LnaCl

Table 7. *Changes in μ -calpain of different degradation*

Treatments	1	2	3	4	5	6	7
μ-calpains Activity	0.133±0.002[a]	0.163±0.006[b]	0.182±0.002[c]	0.208±0.002[d]	0.235±0.007[e]	0.268±0.004[f]	0.281±0.008[g]

260~330 mmol/L NaCl concentration eluted,the collected part what has μ-calpains activity was concentrated.

As can be seen from *Fig. 3,* the degradation ability of μ-calpain to myofibrillar protein was enhanced by its nitration. Compared to the control group, after the incubation of 1.0 mmol/L $ONOO^-$ and 1.5 mmol/L ONOO- treated μ-calpain and myofibrillar protein, two protein bands appeared at relative molecular mass of 15 kDa and 17 kDa. After the concentration of the nitrating reagent is reduced, the above-mentioned two bands disappeared. The protein bands at relative molecular mass of 250 kDa, 100 kDa, and 10 kDa gradually became lighter in pace with increasing the concentration of the nitrating reagent *(Fig. 4)*, but the protein bands of 35 kDa gradually strengthened as the concentration of the nitrating reagent increased. These changes are identical to the changes in μ-calpain activity after nitration *(Table 7)*.

DISCUSSION

From this study, it can be seen that both age and slaughter handling of yak meat muscle fiber diameter have a greater impact. If we grade the meat according to their age which is postmortem aging, and the use of appropriate ripening conditions will improve muscle tenderness.

The results showed that the active ingredient collected on SDS-PAGE showing a single band of molecular weight of about 100kDa after DEAE-Sepharose-FF ion exchange column purification *(Fig. 4),* it is the same to Hu Peng et al.[19] measured μ-calpain relative molecular weight of about 100kDa.

The value of shear force for yak meat during postmortem aging in the 0 day were 3.45 kgf, 4.11 kgf, 5.31 kgf, 6.49 kgf, these data showed that as the age increases the tenderness decreases. But after 7 days aging, the shear force value significantly decreased to 16.81%~22.01%. In different ages, the results were as similar to the findings of Hu Peng et al.[19]. Shear force was first increased and then decreased during postmortem aging, since sarcomere shortening leads to the rigor stage, and then muscle structure was destroyed under some endogenous enzymes, and the tenderness was increased [20].

Muscle fiber diameter as an important index for muscle tenderness, have a great impact on muscle tenderness. Light et al.[21] studied the different parts of the beef connective tissue content and features, and found that the more tender of the sample meat with a smaller diameter muscle fibers. Endomysium and muscle fibre are separated, and it lost with droplets during postmortem aging, this lead to reduces in fiber diameter value in yak meat in different age, after 7 days ($P>0.05$). Hence, postmortem aging can improve the tenderness of the yak meat.

Myofibrillar fragmentation index reflects the degree of myofibrillar protein degradation and muscle fiber structure is destroyed. The larger MFI, the better muscle tenderness, and myofibril structure damaged severely. In this study, MFI significantly increased in 0-3 day after slaughter ($P<0.05$), and its rise velocity and amplitude decreased and stabilized after 3 days, which was due to consumption of *in vivo* protease, and hence decreased activity, and the muscle fibrillin was no longer degraded [22].

The protein constituting the myofibrillar skeleton was destroyed by endogenous enzymes, myofibrillar was broken and degraded at Z-line and this change as an important indicator of muscle tenderness during post-mortem aging [23,24]. In this study, the myofibrillar ultra-structure was not significantly different in morphological point of view and Z-line clear. This test measured that with the age increases, the sarcomere length are shortened, which leads to aging of yak meat tenderness, and palatability greatly worsen. As it can be seen from the analysis of the correlation between the microstructure index, age and muscle fiber diameter, MFI values showed a highly significant correlation ($P<0.01$), it can be said that, when the age of yak is older, muscle fiber diameter is thicker and the MFI value is smaller, so the tenderness will decreased.

Tenderness is the most important indicator for meat quality evaluation, and the degradation of skeleton protein is a major factor to improved tenderness during postmortem aging. The present study has demonstrated that the calpain system plays a major role in postmortem muscle tenderness [25,26]. Calpain as a proteolytic enzyme, which is capable of hydrolyzing protein composition Z-line, it affects tropomyosin, M line protein, troponin T and so on [27]. In muscle tissue, calpains are divided into μ-calpain and m-Calpain, and μ-calpain is considered to be closely related to the meat tenderness during postmortem. The μ-Calpain activity decrease is a sign of hydrolysis [28]. During postmortem aging, muscle sarcoplasmic reticulum and mitochondria rupture, and Ca^{2+} is released, then calpains are activated and cause degradation of myofibrillar cytoskeletal proteins. Therefore, this process promotes increased tenderness [29].

The study found that the crude calpain activity increase and decrease in different age yak meat after aging. This may be because, the activity of calpains was lower before physiological maturity and when the aging begins, slow metabolic rate slowes, calpains activity is also reduced [30].

As it can be seen from the correlation analysis between the calpains activity and tenderness, shear forces, MFI and muscle fiber diameter and calpains activity correlated significantly ($P<0.01$). During postmortem aging, muscle sarcoplasmic reticulum and mitochondria rupture, and Ca^{2+} is released, then activation of calpains act on muscle fiber skeleton protein and make the muscle fibers weaken, thus it is improving the yak meat tenderness and playing a significant role in maturity and tenderness [15], Pringle [31] studied the different breeds of cattle, and found that the activity of calpains associated with tenderness. Cheng et al.[32] found that MFI and calpains activity can be used as important indicators on pork tenderness.

Oxidation phenomenon is widespread during postmortem aging and meat storage process. Some scholars think oxidative stress leads to protein oxidation [33] and oxidative stress, including reactive oxygen species; reactive oxygen species (ROS) and reactive nitrogen radicals (RNS). Some studies demonstrated that ROS on oxidative protein results in protein polymerization, crosslinking and forming groups and amino acid derivatives, these structural changes can increase susceptibility to proteolytic enzyme protein, thereby accelerating protein degradation, and reactive nitrogen radicals (RNS) allows protein tyrosine nitration reaction and produce 3-nitro-tyrosine (nitrotyrosine, NT) [33,34]. Studies have found that nitration of tyrosine residues can accelerate the proteolytic enzyme degradation of certain proteins, and the degradation rate associated with their degree of nitrification [34]. Thereby, nitration may changes the center of calpain active group and also change the degradation of myofibrillar proteins. Consequently, the study on nitration effects calpains is necessary.

This study demonstrated that nitration increases the activity of μ-calpain and promotes the degradation of myofibrillar proteins. There have been studies showning that μ-calpain activity in beef skeletal muscles increase with the degree of degradation [35]. Nitration is increasing the activity of μ-calpain, it probably because nitration μ-calpain inhibit their degradation. Myofibrillar protein degradation is believed to have a close relationship with the tenderness, it can be said that nitration to μ-calpain may improve muscle tenderness to some extent. However, this test mainly used isolated purified myofibrillar protein as a substrate, and is distinct with internal environment of meat. Therefore, the research of the mechanism about nitration of the postmortem meat tenderness are further needed.

Age and postmortem aging time had a significant effect on the tenderness of yak meat. After aging 7 days, shear force value is significantly decreased as compared to slaughter the first (0) day. The older of yak, the muscle fiber diameter thicker, and the sarcomere length shorter, the degree of fragmentation lower. After aging 7 days, muscle fiber diameter is thinner and the tenderness increases. Calpains activity was increased and then decreased during postmortem aging. Calpains activity was significantly decreased after postmortem before 2 days, and then decreased slowly. In vitro nitro μ-Calpain can improve this enzyme degradation of myofibrillar proteins, and μ-Calpain after nitration may improve muscle tenderness.

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