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Effect of refrigeration on the collagen and texture characteristics of yak meat

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1 **Effect of refrigeration on the collagen and texture characteristics of yak meat**

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20 Abstract

21 To investigate the effect of refrigeration on the collagen and texture characteristics of yak meat,
22 the changes in collagen content, solubility, shear force, hardness, cohesiveness, springiness,
23 gumminess, and chewiness of yak meat at $4\pm 1^{\circ}\text{C}$ were measured. The results showed that the
24 contents and solubility of collagen in yak meat significantly decreased ($P<0.05$). The myofibrillar
25 fragmentation index of yak meat significantly increased ($P<0.05$). The shear force, hardness,
26 cohesiveness, springiness, gumminess and chewiness of yak meat significantly decreased
27 ($P<0.05$). Correlation analysis and histological structure analysis confirmed that myofibrillar
28 fragmentation and collagen characteristics significantly affected the formation of yak meat texture
29 during refrigeration. After 5 days of refrigerated storage, yak meat has a higher quality. The
30 results provide a theoretical basis and data to support the deep processing and quality control of
31 yak meat.

32 **Key words:** yak, refrigerated, collagen, texture, histology

33 Introduction

34 The national beef and cattle yak industry reported that approximately 3.6 million yaks were
35 slaughtered in China, meat production was 370,000 tons, and the yak meat output value was
36 approximately 27 billion yuan in 2019 (Cao et al., 2010). In China, yak (*Bos grunniens*) is mainly
37 distributed on the Qinghai-Tibet Plateau and can survive 3,000-5,000 meters above sea level (Li et
38 al., 2019). For a long time, yak meat has been favored by consumers because it has "high protein

39 and low fat" contents. However, refrigerated yak meat and high value-added yak beef products are
40 limited due to the problems of "thick muscle fiber and poor tenderness" (Li et al., 2018; Yang et
41 al., 2020; Zuo et al., 2016).

42 Texture is the main quality of meat products, and it is also the main factor affecting the deep
43 processing of products and consumer purchases (Bayraktaroglu & Kahraman, 2011). Texture
44 characteristics can be measured by the shear force, hardness, cohesiveness, springiness,
45 gumminess and chewiness of meat products (Lana & Zolla, 2016). At present, the mechanism that
46 forms meat texture demonstrates that texture is mainly composed of background toughness and
47 rigor-induced toughness (Fang et al., 1999). The rigor-induced toughness caused by carcass
48 contraction after livestock and poultry are slaughtered can be improved by endogenous enzymes
49 during the aging process (Vitale et al., 2014; Kemp & Parr, 2012; Huang et al., 2016). Muscle
50 fibers can be degraded by calpain, cathepsin and apoptotic enzymes, which increase the
51 fragmentation of myofibrils and improve the texture of meat. Bhat et al. (2018) reported that μ -
52 calpain and m-calpain played an important role in the formation of meat tenderness. Cruzen et al.
53 (2014) indicated that endogenous enzymes can lead to the degradation of muscle fibers during
54 aging, which promotes the improvement of muscle tenderness. Wang et al. (2019) reported that
55 activated caspase-3 can promote the hydrolysis of myofibrin, increase the degree of
56 myofibrinolysis, and improve muscle tenderness. The background toughness is mainly affected by
57 connective tissue, and collagen content and solubility can affect the strength of connective tissue

(Cross et al., 1973; Powell et al., 2000; Lepeti 2007). Many studies have indicated that changes in collagen content and solubility of beef during aging improve the tenderness of beef (Judge et al., 2004; Nishimura et al., 1998; Stanton et al., 2005). Therefore, the texture of muscle is significantly affected by the characteristics of collagen and the degree of muscle fiber fragmentation. However, for yak meat, most studies have focused on the effect of endogenous enzymes during aging (Li et al., 2019; Wang et al., 2019; Yan et al., 2020) and ignored the effect of collagen on improving the tenderness of meat.

Therefore, the contents of soluble and insoluble collagen, fragmentation of myofibrils, shear force, hardness, cohesiveness, springiness, gumminess and chewiness of yak meat were studied under refrigerated storage to provide a theoretical basis and data to support deep processing and the quality control of yak meat.

Materials and methods

Raw material preparation

Animal welfare and experimental conditions were executed according to the guidelines laid down by the Canadian Council on Animal Care (1993) and approved by the Animal Ethic Committee of the Qinghai province.

Yak meat was obtained from a commercial abattoir (Qinghai Xia Hua Food Ltd). Thirty-two yak bulls (265 ± 19.36 kg) aged 42 ± 3 months with the same feeding background were selected and their *longissimus dorsi* were taken after slaughter, vacuum packed, and transported to the

laboratory at $4\pm 1^{\circ}\text{C}$. The yak meat was divided into 180 ± 10 g pieces and refrigerated at $4\pm 1^{\circ}\text{C}$. The textures and collagen characteristics were measured on days 0, 1, 3, 5 and 7. Approximately 10 g meat samples were cut and fixed in 4% paraformaldehyde to observe the microstructure changes of yak meat during refrigerated storage.

Collagen content and solubility

The contents of total collagen (TCC), soluble collagen (SCC) and insoluble collagen (ICC) in yak meat during refrigerated storage were measured based on the method of Chang Haijun (2010).

The standard curve for the determination of hydroxyproline content is $Y=0.102X+0.004$, $R^2=0.998$ (Y is the content of hydroxyproline; X is the absorbance).

Collagen solubility (CS%) = soluble collagen content/total collagen content $\times 100$

Myofibril fragmentation index (MFI)

According to the method of Delgado et al. (2001), 2.00 g meat were added to the mortar then 8 mL MFI buffer solution (100 mM potassium chloride, 20 mM potassium phosphate, 1 mM ethylene diamine tetraacetic acid, 1 mM magnesium chloride, 1 mM sodium azide, pH 7.1) was added for grinding, and the mixture was centrifuged at 1000 g for 15 min, the supernatant was discarded, precipitated and mixed with 8 mL MFI buffer solution evenly, and centrifuged at 1 000 $\times$ g for 15 min, and the supernatant was discarded. The 10 mM MFI buffer solution was added to the precipitate and mixed evenly. After passing through a 200 mesh sieve, the filtrate protein concentration was determined by the biuret method, and the controlled protein concentration was

0.5 mg/mL. The absorbance value was measured at 540 nm, and the MFI value was multiplied by 200.

Warner-Bratzler shear force (WBSF)

Each sample (length $\times$ width $\times$ height of 60 mm $\times$ 30 mm $\times$ 30 mm) was placed into a plastic bag and cooked in a water bath at 80°C until the central temperature reached 70°C (Li et al., 2019; Yan et al., 2020). Subsequently, all the samples were cooled in running water until their core temperatures were below 30°C. Then, each sample was cut into slices with a length $\times$ width $\times$ height of 50 mm $\times$ 10 mm $\times$ 10 mm (the fiber axis was in the 50 mm direction). Afterward, each sample was placed in a texture analyzer (CT-3, TexturePro CT, Brookfield Engineering Laboratories, Inc., USA) and tested by using a Warner-Bratzler V-shaped shearing device with a cross head speed of 1.5 mm/s. The shear force recorded in newtons (N).

Texture

The hardness, cohesiveness, springiness, gumminess and chewiness of yak meat were measured by a CT-3 texture analyzer from Brookfield Engineering Company. The meat sample were cooked in a water bath at 80°C until the central temperature reached 70°C (Li et al., 2019; Yan et al., 2020), then, sample were cut into a square of 10 mm $\times$ 10 mm $\times$ 10 mm. The TPA (texture profile analysis) model was used to simulate the chewing movement of the mouth, and the meat sample underwent two rounds of compression. The test parameters were as follows: the pretest rate was 2 mm/s, the test rate was 1 mm/s, the posttest rate was 1 mm/s, the trigger force was 5 g, the sample

height was 10 mm, and the compression ratio was 50%. The TPA results were analyzed by TPA-macro analysis. Each sample had 10 parallel measurements that were averaged.

Microstructure of the yak meat

According to the method of Ramos-Vara & Miller (2014), the main processes were fixed overnight in a 4% paraformaldehyde solution, the gradient was eluted with 30% ~ 100% ethanol, the xylene elution underwent reoccupation 2 times, the samples were biopsied by HE (hematoxylin-eosin) made of biopsy specimens after dyeing processing, optical microscopy was performed with a 40 times magnification under the photo-taking conditions, and the microstructure of yak meat changes during refrigerated storage were observed.

Statistical analysis

Statistical analysis of the difference was performed using one-way ANOVA by SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Significance among the groups was determined with Duncan's multiple range tests. The significance level was set at $P < 0.05$, and an extremely significant level was set at $P < 0.01$.

Results and discussion

Effect of refrigerated on the collagen content of yak meat

Connective tissue is the main factor affecting meat texture. The main component of connective tissue is collagen, and changes in the content and characteristics of collagen are important markers

133 affecting the strength of connective tissue (Anton F et al., 2013). Collagen can be divided into
134 soluble collagen and insoluble collagen, and both have important influences on meat quality. The
135 effects of refrigeration on the contents of total collagen and soluble and insoluble collagen in yak
136 meat are shown in Figure 1 a, b and c, respectively. At the end of 7 d, the content of total collagen
137 decreased by 48.65% from the day 0 content. The content of soluble collagen decreased as the
138 refrigeration time increased, and it decreased by 64.15% from day 0 to day 7. Moreover, the
139 content of soluble collagen decreased significantly after day 3 ($P < 0.05$). The content of insoluble
140 collagen decreased significantly with the increase of refrigeration time ($P < 0.05$), and it decreased
141 by 43.24% from day 0 to day 7. According to the data, the contents of total collagen, soluble
142 collagen and insoluble collagen in yak meat decreased significantly during refrigeration, and the
143 decreased rate of soluble collagen was the highest. During refrigerated storage, endogenous
144 enzymes in muscles such as collagenase can promote the degradation of collagen in muscles or
145 transform insoluble collagen to soluble collagen and reduce the content of total collagen and
146 insoluble collagen (Stanton et al., 2005). Soluble collagen can be dissolved in water, and it flows
147 out of muscle as the muscle water holding capacity decreases, which causes a decrease in the
148 soluble collagen content in muscle (Matthew et al, 2009; Eyre et al., 1984; Lu et al., 2013). The
149 present result was supported by the study of Modzelewska-Kapitula & Nogalski (2014), who
150 reported that the content of total collagen and soluble collagen in the *semimilareum* muscle of beef
151 decreased consistently with the increase of refrigeration time.

152 **Effect of refrigeration on the solubility of collagen in yak meat**

153 The main characteristic of collagen is solubility (Bao & Per, 2015). As shown in Figure 2, the
154 solubility of collagen in yak meat showed a significant decrease with the increase of refrigeration
155 time ($P<0.05$), and the decrease rate was 29.86%. A significant decrease occurred after day 3. This
156 was consistent with the significant decrease in total collagen, soluble collagen and insoluble
157 collagen content in yak meat during refrigerated storage. However, the soluble collagen loss rate
158 was the highest, and the overall solubility of collagen decreased. This result is consistent with the
159 report of Stanton & Light (2005), who reported that the collagen solubility of beef decreased
160 during storage.

161 **Effect of refrigeration on the myofibrillar fragmentation index of yak meat**

162 It is well known that the limited degradation of myofibrils is the main reason for the formation
163 of muscle tenderness, and the myofibril fragmentation index can reflect the degradation of
164 myofibrils (Delgado et al., 2001). As shown in Figure 3, the myofibrillar fragmentation index
165 increased significantly with the increase of refrigeration time ($P<0.05$), and the rate of increase
166 was 102.39%. During refrigerated storage after slaughter, the degradation of myofibrils by
167 endogenous enzymes increased the fragmentation degree of muscle fibers (Huang et al., 2016;
168 Bhat et al., 2018; Cruzen et al., 2014). This result is consistent with the report of Huang et al.
169 (2019).

170 **Effect of refrigerated on the WBSF of yak meat**

Shear force is the main evaluation index that can determine meat tenderness (Lana & Zolla, 2016). According to Figure 4, the WBSF of yak meat decreased significantly as the refrigeration time increased ($P<0.05$), and the change trend was similar to the "straight line" from day 0 to day 5. The WBSF decreased from 55.21 ± 2.36 N at day 0 to 26.78 ± 1.97 N at day 7, and the decrease rate was 51.49%. This result is consistent with the report that the tenderness of yak meat decreased significantly during refrigeration by Wang et al. (2019). During refrigerated storage, endogenous enzymes can promote the weakening of muscle connective tissue and increase the degree of myofibrillar fragmentation, which leads to the destruction of muscle structure and improves the tenderness of muscle (Lepeti, J, 2007; Bhat et al., 2018). After 5 days of refrigerated storage, the WBSF of yak meat reached the standard of tender meat based on Van et al. (2014).

Effect of refrigerated on the texture of yak meat

Texture is an evaluation system that simulates the chewing process of food in the mouth (Van et al., 2014). The effects of refrigeration on yak meat texture are shown in Table 1. The hardness, cohesiveness, springiness, gumminess and chewiness of yak meat decreased significantly with the increase of refrigerated storage ($P<0.05$) time, and the decrease rates were 56.39%, 33.33%, 38.41%, 48.83% and 36.58%, respectively, on day 7 compared with day 0. The results showed that the texture of yak meat changed significantly as the refrigerated storage time increased, and the change trend was consistent with the WBSF of yak meat during refrigerated storage.

Correlation analysis

Correlation analysis of the yak meat texture and refrigerated time

The correlation between yak meat texture and refrigeration time is shown in Table 2. Table 2 shows that the shear force, hardness, cohesiveness, springiness, gumminess and chewiness of yak meat had significantly negative correlations with the refrigeration time ($P<0.01$), and the correlation coefficients were $r = -0.964$, $r = -0.985$, $r = -0.960$, $r = -0.951$, $r = -0.945$, and $r = -0.981$, respectively. The results showed that the texture of yak meat improved as the refrigeration time increased.

Analysis of the correlation between the texture characteristics of yak meat and its MFI and collagen characteristics

The correlation analysis of yak meat texture characteristics with MFI and collagen characteristics is shown in Table 2. The WBSF of yak meat had a significantly negative correlation with MFI ($r = -0.926$; $P < 0.01$) and a significantly positive correlation with the total collagen, soluble collagen, insoluble collagen and collagen solubility ($r=0.902$, $r=0.926$, $r=0.814$, $r=0.623$). Hardness was negatively correlated with MFI ($r = -0.921$; $P < 0.01$) and had a significantly positive correlation with the total collagen, soluble collagen, insoluble collagen and collagen solubility ($r=0.928$, $r=0.970$, $r=0.829$, $r=0.702$; $P<0.01$). Cohesiveness had a significant negative correlation with MFI ($r = -0.915$; $P<0.01$) and a significantly positive correlation with the total collagen, soluble collagen, insoluble collagen and collagen solubility ($r=0.892$, $r = 0.935$, $r=0.796$; $r=0.660$; $P < 0.01$). Springiness had a significantly negative correlation with MFI ($r =$

0.962; $P < 0.01$) and a significantly positive correlation with the total collagen, soluble collagen, insoluble collagen and collagen solubility ($r = 0.907$, $r = 0.927$, $r = 0.820$, $r = 0.639$). Gumminess had a negative correlation with MFI ($r = -0.951$; $P < 0.01$) and a significantly positive correlation with the total collagen, soluble collagen, insoluble collagen, and collagen solubility ($r = 0.928$, $r = 0.910$, $r = 0.858$, $r = 0.572$). Chewiness had a significant negative correlation with MFI ($r = -0.897$; $P < 0.01$) and a significantly positive correlation with the total collagen, soluble collagen, insoluble collagen and collagen solubility ($r = 0.936$, $r = 0.952$, $r = 0.848$, $r = 0.649$; $P < 0.01$).

The results showed that the WBSF, hardness, cohesiveness, springiness, gumminess and chewiness of yak meat had significantly negative correlations with MFI, and the correlation coefficients were above 0.89. The WBSF, hardness, cohesiveness, springiness, gumminess and chewiness of yak meat had positive correlations with the total soluble collagen, insoluble collagen, collagen, and collagen solubility, especially with soluble collagen correlation coefficients above 0.91. The results showed that myofibril and collagen characteristics significantly affected the formation of yak meat during refrigerated storage.

Effects of the refrigeration on the microstructure of yak meat

The histological structure analysis further confirmed that the muscle fiber of yak meat changed from integrity to rupture, the muscle fiber space changed from small to large, and the connective tissue changed from integrity to fragmentation with the increase of refrigeration time. The results showed that myofibrillar and collagen characteristics significantly affected the formation of yak

meat texture during the refrigeration time, which provides a theoretical basis and data to support the deep processing and quality control of yak meat.

The changes in the microstructure of yak meat during refrigeration that were determined by HE staining are shown in Figure 5. The muscle fiber of yak meat changed from having integrity to being ruptured as the refrigerated storage time increased, the muscle fiber space increased from small to large, and the connective tissue changed from having integrity to being fragmented. On day 0, the yak meat muscle fiber structures were intact and dense, and the connective tissue was intact. On the first day, small cracks appeared in the muscle fibers, but the interfibrillar structure was still complete and the connective tissue was weakened. At day 3, the muscle fiber of yak meat began to disintegrate, the degree of fragmentation increased, the muscle fiber space began to increase, and the connective tissue gradually decreased. At day 5, the yak meat degree of muscle fiber disintegration and fragmentation were increased, the muscle fiber space was significantly increased, and the connective tissue was obviously weakened. At day 7, the yak meat muscle fiber was greatly disintegrated, the muscle fiber was fragmented in the whole field, the muscle fiber space was further enlarged and had gradually expanded into the muscle fiber, and the connective tissue was broken. In conclusion, the muscle fiber of yak meat disintegrated and fragmented to a large extent during refrigeration, which was consistent with the increase in MFI and the decrease in the shear force value of yak meat. Furthermore, the change in connective tissue having integrity to being fragmented was consistent with the decrease in collagen content and WBSF of yak meat

as the refrigerated storage time increased. The results showed that the myofibrillar fragmentation and collagen characteristics significantly affected the formation of yak meat texture during refrigerated storage.

Conclusion

The content of total collagen, soluble collagen and insoluble collagen and the solubility of collagen in yak meat decreased significantly with the increase of refrigerated storage times. The myofibril fragmentation index of yak meat was significantly increased with the increase of refrigerated storage times. The shear force, hardness, cohesiveness, springiness, gumminess and chewiness of yak meat were significantly decreased as the refrigerated storage times increased. The correlation analysis confirmed that the texture characteristics of yak meat were significantly correlated with the refrigerated time, myofibrillar fragmentation index and collagen characteristics, which indicated that myofibrillar fragmentation and collagen characteristics significantly affected the formation of yak meat texture during refrigerated storage. In conclusion, myofibrillar degradation and connective tissue weakening had significant effects on yak meat texture formation during refrigerated storage, and after 5 days of refrigerated storage, yak meat had a better quality. The results provide a theoretical basis and data to support the deep processing and quality control of yak meat.

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276 **Conflict of Interest**

277 Shengsheng Li declares that he has no conflict of interest. Yawei Zhang declares that he has no
278 conflict of interest. Shujie Liu declares that he has no conflict of interest.

279 **Ethical Approval**

280 This article does not contain any studies with human participants or animals performed by any
281 of the author.

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362 longissimus lumborum during postmortem aging[J]. Meat Science, 121:409-419.

Table

Table 1 Effect on the texture profile analysis of yak meat during refrigerated storage

Storage	Hardness/		Springiness/		Chewiness/
time/D	(N • cm ⁻²)	Cohesiveness	mm	Gumminess	mJ
0	51.64±1.44 ^e	0.63±0.03 ^e	2.76±0.12 ^e	27.24±1.75 ^d	70.31±1.33 ^e
1	46.32±2.17 ^d	0.57±0.02 ^d	2.36±0.08 ^d	21.99±1.53 ^c	65.36±1.05 ^d
3	39.35±2.83 ^c	0.55±0.03 ^c	2.18±0.09 ^c	19.66±0.90 ^b	61.60±0.94 ^c
5	30.95±1.07 ^b	0.46±0.02 ^b	1.95±0.07 ^b	15.62±0.68 ^a	52.90±2.45 ^b
7	22.52±2.67 ^a	0.42±0.01 ^a	1.70±0.10 ^a	13.94±0.14 ^a	44.59±2.01 ^a

Note: Means with different superscripts letters are significantly different (*P* <0.05).

Table 2 Correlation analysis of the texture, Storage time, MFI, collagen content and characteristics of yak meat

Index	Storage time	MFI	TCC	SCC	ICC	CS
WBSF	-0.964**	-0.926**	0.902**	0.926**	0.814**	0.623*
Hardness	-0.985**	-0.921**	0.928**	0.970**	0.829**	0.702**
Cohesiveness	-0.960**	-0.915**	0.892**	0.935**	0.796**	0.660**
Springiness	-0.951**	-0.962**	0.907**	0.927**	0.820**	0.639*
Gumminess	-0.945**	-0.951**	0.928**	0.910**	0.858**	0.572*
Chewiness	-0.981**	-0.897**	0.936**	0.952**	0.848**	0.649**

Note: WBSF means Warner-Bratzler shear force; MFI means myofibril fragmentation index; TCC means total collagen content; SCC means soluble collagen content; ICC means insoluble collagen content; CS means collagen solubility.

* means correlation is significant at $P<0.05$; ** means correlation is significant at $P<0.01$.

Figure

Note: Different letters means significant difference and $P<0.05$, which is the same below.

Fig. 1 Effect on the total, soluble and insoluble collagen contents

Fig. 2 Effect on the collagen solubility (CS) of yak meat during refrigerated storage

Fig. 3 Effect on the myofibril fragmentation index (MFI) of yak meat during refrigerated storage

Fig. 4 Effect on the Warner-Bratzler shear force (WBSF) of yak meat during refrigerated storage

Fig. 5 Effect on the microstructure of yak meat during refrigerated storage