

# Effects of Thawing Temperature on the Physicochemical and Sensory Properties of Frozen Pre-Rigor Beef Muscle

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**Abstract** Pre-rigor bovine *sternomandibularis* muscles were frozen at 3 hr postmortem thawed at various temperatures (18, 2, and -2°C), and then meat quality and sensory properties were compared with those in chilled muscle (control). The meat thawed at 18°C had lower ultimate pH, water holding capacity, and sensory scores and higher muscle shortening, thaw drip loss, and shear values than those of the other samples. The samples thawed at -2°C had significantly lower muscle shortening and higher sensory scores in tenderness and juiciness than those thawed at 18 and 2°C. Muscle shortening, pH, WHC, shear values, and sensory properties were not significantly different between control and sample thawed at -2°C. By holding at -2°C, thaw shortening was prevented and tender meat comparable to the chilled meat was obtained. These results indicate that thaw shortening can be largely eliminated if the frozen pre-rigor muscle is thawed at -2°C.

Keywords: thawing temperature, muscle shortening, thaw drip, shear force, sensory property

#### Introduction

The hot boning of pre-rigor muscle has many potential advantages, such as reduced chilling costs, refrigeration space, and increased yields, over cold boning (1, 2). The process of hot boning involves removing muscle from the bone while it is at near body temperature. When pre-rigor muscle is frozen at temperatures of below about -15°C immediately after hot boning, the development of rigor mortis is arrested and the ATP concentration in frozen pre-rigor muscle remains almost unchanged. However, thaw rigor occurs when the frozen pre-rigor muscle is rapidly thawed in chill, at ambient or elevated temperatures (3-6). The thaw rigor meat normally shows severe muscle contraction, unacceptable tenderness, and extreme drip loss (4, 7, 8).

The thawing temperature of frozen pre-rigor muscle is an important matter since it determines rates of glycolysis and contraction in pre-rigor frozen muscle (9). Several studies about reactions representing the progress of glycolysis in frozen pre-rigor muscle have reported that there exists a temperature zone (at -2 to -5°C) just below the freezing point where the rate of ATP depletion reaches a maximum. Behnke et al. (10) reported that rates of ATP depletion and lactate accumulation in beef sternomandibularis muscle were faster at -35°C (frozen) than at 10 or 0°C (unfrozen). March and Thompson (3) and Koohmaraie et al. (11) also reported that glycolysis in lamb longissimus muscle continues and ATP is depleted at -5 to -35°C; thus shortening does not occur during thawing since the presence of ice precludes contraction. It has been demonstrated in lamb (3, 11) and beef (9) that the undesirable

tenderness and high thaw drip loss accompanying thaw rigor might be prevented if the muscle is stored at just a few degrees below its freezing point for several hours prior to thawing.

The effect of temperature at the onset of rigor on muscle contraction and tenderness has been the subject of many studies. However, there are few reports about the effects of thawing condition on meat quality and the sensory properties of frozen pre-rigor beef muscle. Thus, the objective of the present study was to investigate the effects of thawing temperatures on the physicochemical and sensory properties of frozen pre-rigor bovine muscle.

### Materials and Methods

Muscle sample preparation Eight hanwoo (Korean native cattle) cows (36-48 months old) were slaughtered at a local abattoir. After exsanguinations, sternomandibularis (neck) muscles from the left and right sides of carcasses were removed within 10 min postmortem (PM) and immediately transported to the Konkuk University Meat Laboratory. Neck samples were promptly trimmed of all fat and visible connective tissue so that only the completely trimmed sternomandibularis muscle was left. At 3 hr PM, about 20 g was removed from each sample for determination of sarcomere length, initial pH, and water holding capacity (WHC) of pre-rigor muscles.

Sternomandibularis muscles from each carcass were cut into 4 pieces of approximately equal size (each muscle from the left and right sides was divided into 2 portions). The 4 pieces of each muscle, designated A, B, C, and D, were weighed and a subjective rest length (reference muscle length) was rapidly established for each muscle by attaching clip to both end of sample that across the muscle fibers. After the muscle length was measured, the samples were packaged in polyethylene bags. Piece A sample (the

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'chilled' control for pieces B, C, and D) was immersed in 15°C water bath until 24 hr PM and then stored at 4°C cooler for 3 days. Pieces B, C, and D samples from each carcass were placed separately in a polyethylene bag and frozen at -15°C methanol bath until 24 hr PM. On the following day, piece B samples were thawed at 18°C methanol bath for 6 hr (18°C thaw) and piece C samples were thawed at 2°C for 12 hr (2°C thaw). Piece D samples were immersed at -2°C methanol bath for 12 hr, and then thawed completely at 4°C refrigerator (-2°C thaw). Thawed pieces B, C, and D samples were stored at 4°C cooler for additional 3 days and the samples were analyzed for various parameters as described below.

Sarcomere length Sarcomere length of each pre-rigor, chilled, and thawed sample was determined by neon laser diffraction method (12, 13). Briefly, about 300 mg of muscle samples were carefully cut with scissors and inserted in 2% glutaraldehyde solution with 2% glucose in 0.1 M phosphate buffer, pH 7.0. The samples were fixed at 4°C for 30 min, and then sarcomere length was measured using Helium-Neon-Laser (model No. 212-2; Spectra-Physics, Stratford, CT, USA).

**Thaw, cook, and total muscle shortening** After thawing or cooking, the length between the clips, which were inserted in the muscle at sample preparation, was remeasured. Percent thaw shortening was defined as the net reduction in muscle length (cm) after thawing divided by the original muscle length (cm)  $\times$  100. Muscle lengths were measured before and after cooking, and then difference was recorded as cook shortening. Total shortening for each treatment was determined as:

Total shortening (%)
= (cooked sample length)/(original muscle sample length) × 100

**pH** Five g of pre-rigor, chilled, and thawed muscle samples were homogenized in 20 mL of distilled water using an Ultra Turrax (model No. T 25; Janken and Kunkel, Staufen, Germany). The pH of the homogenate was determined using a pH meter (moel 340; Mettler-Toledo, Manchester, UK) calibrated at pH 4.0 and 7.0.

Water holding capacity (WHC) WHC was estimated by determining expressible juice using modified filter paper press method described by Grau and Hamm (14) as follows. A 300 mg sample of muscle was placed in a filter-press device and compressed for 2 min. The outline area of the expressible juice and the meat film was traced, and the two areas were determined using a compensating polar planimeter. WHC was calculated from duplicate samples as a ratio of the meat film area to the total area; hence, a larger value suggests a higher WHC.

**Thaw drip, cook loss, and total loss** Thaw drip loss was determined by weighing of samples before and after thawing or chilling. After determinations of thaw drip loss, the samples were repacked and cooked as described by Kim and Lee (15). Cook loss was determined by weighing the meat before and after cooking. Also, total moisture

loss for each treatment was determined as:

Total loss = (cooked sample weight) /(original muscle sample weight) × 100

Shear force For determination of shear force, samples were individually cooked in polyethylene bags in a water bath of 75°C for 30 min. Cooked samples were cooled at room temperature for 30 min, and then 4 to 6 cores (11 mm) from each sample were removed parallel to the longitudinal orientation of the muscle fibers. Shear tests were conducted using a Texture Analyser (TA-XT2*i*, Stable Micro Systems, Surrey, England) equipped with a single-blade Warner-Bratzler shear attachment. Each core sample was oriented perpendicular to the shear blade and sheared. Cross head speed was 2 mm/sec with a 25 kg load cell and force to shear the sample was recorded in Newtons (N).

**Sensory evaluation** Cooked meat was evaluated immediately after cooking by 7-member trained descriptive attribute sensory panel. Panelists scored each sample for flavor, tenderness, and juiciness on 9-point scales (9 = desirable flavor, extremely tender and juicy to 1 = undesirable flavor, extremely tough and dry, respectively).

**Statistical analyses** All data were analyzed using the ANOVA procedure of  $SAS^{\circledast}$  software (16). When a significant effect of thawing temperature was found (p< 0.05), means were compared using the Duncan's multiple range test.

### **Results and Discussion**

**Sarcomere length and muscle shortening** Muscle contraction is induced by the release of Ca<sup>2+</sup> ions from the sarcoplasmic reticulum into the myofibrillar space. Sarcomere is the contractile unit of the myofibrils and sarcomere lengths shorten during muscle contraction (17). The rate and extent of movement of Ca<sup>2+</sup> ions within the frozen pre-rigor muscle may depend on thawing temperature.

In this study, sarcomere length was determined in order to evaluate the contractile state of the pre-rigor, chilled, and thawed bovine *sternomandibularis* muscles (Fig. 1). At 3 hr PM, *sternomandibularis* muscles exhibited a resting sarcomere length of  $2.13\pm0.05~\mu m$ . The muscle thawed at  $18^{\circ}$ C had significantly shorter sarcomeres (0.92  $\pm0.04~\mu m$ ) than all other samples (p<0.05), on the other hands, the neck muscles subjected to the sample thawed at  $-2^{\circ}$ C ( $1.79\pm0.05~\mu m$ ) had sarcomere length similar to that ( $1.91\pm0.05~\mu m$ ) of chilled meat (p>0.05).

It is well known that extreme shortening (thaw rigor) occurs when pre-rigor muscle is frozen and subsequently thawed rapidly (9, 18, 19). In the present study, the neck muscle in the sample thawed at 18°C showed severe sarcomere shortening of about 55-60% as a result of thaw rigor. This result is consistent with those of Hamm (5) who reported that sarcomeres of bovine *sternomandibularis* muscle shortened to about 0.7 μm for thaw rigor samples from the original length of about 1.9 μm.

Rigor shortening can be minimized if the pre-rigor muscle is stored at 10-15°C during rigor development (17,

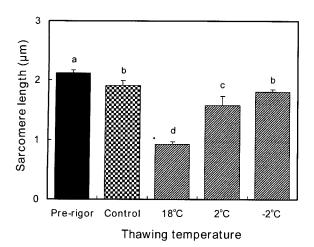


Fig. 1. Sarcomere length of frozen pre-rigor bovine sternomandibularis muscles thawed at various temperatures. Control, Muscle samples were stored at 15°C up to 24 hr PM and then stored at 4°C; 18, 2, and -2°C, muscle samples were frozen at -15°C within 3 hr PM, maintained at -15°C for 24 hr and then either thawed at 18°C for 6 hr, at 2°C for 12 hr or held -2°C for 12 hr and subsequently thawed at 4°C. a-dMeans with different letters are significantly different (p < 0.05).

19-21). Rigor shortening of about 7-13% was observed in control of our study. This agrees with results of Honikel et al. (17), who reported that pre-rigor bovine neck muscles shortened less than 10% in the prerigor state between 6 and 18°C.

When per-rigor muscle is stored at just sub-zero temperature, glycolysis proceeds in the frozen pre-rigor muscle but the muscle does not shorten, presumably due to the restricting effect of the ice (9). In the present study, the sample thawed at -2°C showed sarcomere shortening of about 12-18%, but it was not statistically different when compared to chilled samples. Based on these results, we conclude that holding at -2°C can effectively prevent thaw shortening on frozen pre-rigor muscle.

Red meat is more susceptible to thaw shortening than white meat (18, 21). In previous study, Yu et al. (8) reported that sarcomere shortening of 19-21% was observed when frozen pre-rigor chicken breast muscle was thawed at 18°C. The results of these studies clearly indicate that thaw shortening of red meat is severer than that of white meat.

The thaw, cook, and total muscle shortening in frozen pre-rigor sternomandibularis muscle thawed at various temperatures are summarized in Table 1. Muscle shortening of thawed meat was increased as thawing temperature increased and the sample thawed at 18°C had the highest thaw shortening (58-68%). On the other hands, the sample held at -2°C had a slightly higher muscle shortening than control, but it was not significantly different. These results were in agreement with our sarcomere length shortening results and confirmed previous findings on thaw shortening in beef muscle (20, 22). Also, Xiong and Blanchard (6) reported that muscle shortening of thaw rigor muscle thawed at 20°C was 51%, and muscle incubated at 15°C showed 5% muscle shortening.

As shown in Table 1, in contrast with result in thaw shortening, cook shortening were significantly declined as the thawing temperature increased. The cook shortening of the sample thawed at 18°C had the lowest (5.64%) and control showed the highest cook shortening (p<0.05). The cook shortenings of the samples thawed at 2 and -2°C were 8.65 and 13.1%, respectively. The lowest cook shortening in the sample thawed at 18°C could be due to the severe thaw shortening from the sample thawed at 18 °C.

When thaw shortening and cook shortening were combined, the sample thawed at 18°C had higher total reductions in muscle length than those of the other thawed samples (p<0.05), but total shortening was not significantly different between control and the samples thawed at -2°C (p>0.05). These results indicate that holding at -2°C before thawing has a clearly beneficial effect on muscle shortening characteristics of frozen pre-rigor bovine muscle.

Thaw drip, cooking, and total loss In the present study, thaw drip was collected during the thawing and chilling periods. Thaw drip, cooking, and total fluid loss for chilled or thawed bovine neck muscles are presented in Table 2. Thawing temperatures affected the amount of exudates (thaw drip) from the raw muscles. Thaw drip collected from thawed muscles was greater than drip collected from chilled muscles. The thaw drip loss among thawing treatments was greater at the sample thawed at 18°C than at the samples thawed at 2 and  $-2^{\circ}$ C (p < 0.05). On the other hands, the thaw drip loss showed no significant difference between chilled (control) and the samples thawed at -2°C.

In cooking loss, the samples thawed at 18°C had more cooking loss than that of control muscles (p < 0.05), but

Table 1. Thaw, cook, and total muscle shortening of frozen pre-rigor bovine sternomandibularis muscle thawed at various temperatures

Traits	Control <sup>1)</sup> -	Thawing temperature <sup>2)</sup>		
rians	Coluio	18°C	2°C	-2°C
Thaw shortening (%)	4.85±1.44 <sup>c3)</sup>	63.72±3.81 <sup>a</sup>	26.33±4.90 <sup>b</sup>	9.05±3.56°
Cook shortening (%)	$17.60\pm2.00^{a}$	$5.64\pm0.94^{d}$	8.65±0.92°	13.10±2.87 <sup>b</sup>
Total shortening (%)	20.97±2.32°	65.76±3.62 <sup>a</sup>	32.70±4.60 <sup>b</sup>	21.58±3.34°

1) Muscle samples were stored at 15°C up to 24 hr PM and then at 4°C.
2) 18, 2, and -2°C, Muscle samples were frozen at -15°C within 3 hr PM, maintained at -15°C for 24 hr and then either thawed at 18°C for 6 hr, at 2°C for 12 hr or held -2°C for 12 hr and subsequently thawed at 4°C.
3) All values are the mean±SD; a-d Means in the same raw with different letters are significantly different (p<0.05).

Table 2. Thaw drip loss, cooking loss, and total fluid loss of frozen pre-rigor bovine sternomandibularis muscle thawed at various temperatures

Traits	Control <sup>1)</sup> –			
Traits		18°C	2°C	-2°C
Thaw drip loss (%)	0.84±0.33 <sup>c3)</sup>	18.47±1.96 <sup>a</sup>	2.61±1.16 <sup>b</sup>	1.94±0.54 <sup>bc</sup>
Cooking loss (%)	23.03±3.35 <sup>b</sup>	$26.96 \pm 1.72^{a}$	$26.02\pm2.49^{ab}$	25.66±1.88ab
Total loss (%)	23.67±3.51°	$40.45\pm1.97^{a}$	27.94±2.92 <sup>b</sup>	27.10±2.08 <sup>b</sup>

significant differences in cooking loss were not detected among control and the samples thawed at 2 and  $-2^{\circ}$ C (p> 0.05). When comparing the effects of thawing temperature on cooking loss, the sample thawed at -2°C had a slightly lower cooking loss than those thawed at 18°C, but it was not statistically different (p>0.05).

When thaw drip and cooking loss were combined, the total fluid losses of samples thawed at -2 and 2°C were significantly lower than that of at  $18^{\circ}$ C (p<0.05), but slightly higher than control. Our results clearly indicate that thawing temperature affects fluid loss of frozen prerigor muscle.

The amount of exudates that occurs in beef has been related to the extent of muscle shortening (17). The contraction of myofibrils results in a greater proportion of free water that can be lost from the meat (23). It is thought that the thaw drip from thawed muscle originates from volume changes of the myofibrils during thawing.

As evident from Table 1 and 2, the higher muscle shortening of thawed muscle resulted in the higher thaw drip loss. Especially, the highest thaw drip loss for sample thawed at 18°C may be due to extreme thaw shortening. This finding is in agreement with the results of Marsh and Leet (24), who observed a relationship between drip formation and shortening in pre-rigor frozen and rapidly thawed muscle.

pH, WHC, and shear force The effect of thawing temperature on pH, WHC, and Warner-Bratzler shear force of pre-rigor bovine muscles are shown in Table 3. At 3 hr PM, the initial pH values of pre-rigor muscles were 6.69±0.18. The pH of thawed muscle was slightly increased

as thawing temperature increased and the control samples had higher ultimate pH (5.54±0.13) than those of thawed meat. The samples thawed at 18°C had the lowest ultimate pH (5.35±0.11) and the sample thawed at -2°C and control were had a little higher pH than that thawed at 18°C (p<0.05). However, there were no significant differences in ultimate pH among the control and sample thawed at 2 and  $-2^{\circ}$ C (p>0.05). The small differences in pH among thawing treatments could be due to differences in the rate and extent that glycolysis is completed at these temperature. These results are similar to results of Xiong and Blanchard (6), who reported that thaw rigor bovine muscle had a lower pH than cold-shortened and chilled bovine muscles. Also, the lower ultimate pH of thaw rigor (the sample thawed at 18°C) meat may be related to the denaturation of myofibrillar and sarcoplasmic proteins and increased muscle contrac-tion during thawing.

The pH of meat is one of the major factors affecting WHC (25). It is well known that pH, sarcomere length, and development of rigor influence the water holding ability by altering the cellular and extracellular components (17, 26). The WHC of raw meat is important because it influences meat yield and sensory properties. In this experiment, WHC was determined on pre-rigor, chilled, and thawed samples and WHC of pre-rigor muscle at 3 hr PM was significantly higher (58.71%) than those observed from chilled or thawed meat (p<0.05). The control had a slightly higher WHC (34.24%) than those thawed at -2 and  $2^{\circ}$ C, but there were no significant differences (p>0.05) (Table 3). The samples thawed at 18 °C had the lowest WHC (25.08%) and this result is likely due to extreme muscle shortening and low pH.

Table 3. Ultimate pH, water holding capacity (WHC), and shear force values of frozen pre-rigor bovine sternomandibularis muscle thawed at various temperatures

Traits	Pre-rigor	Control <sup>1)</sup> —	Thawing temperature <sup>2)</sup>		
	(3 hr PM)		18°C	2°C	-2°C
Ultimate pH	6.69±0.18 <sup>a3</sup> )	5.54±0.13 <sup>b</sup>	5.35±0.11°	5.46±0.11 <sup>bc</sup>	5.52±0.10 <sup>b</sup>
WHC (%)	58.71±8.85°	$34.42 \pm 4.53^{b}$	25.08±3.87°	29.42±5.37bc	29.59±3.81 <sup>bc</sup>
Shear force (N)	-	$68.22\pm2.82^{b}$	90.19±11.20 <sup>a</sup>	76.36±13.59 <sup>b</sup>	$69.12 \pm 9.08^{b}$

<sup>&</sup>lt;sup>1)</sup>Muscle samples were stored at 15°C up to 24 hr PM and then at 4°C.
<sup>2)</sup>18, 2, and -2°C, Muscle samples were frozen at -15°C within 3 hr PM, maintained at -15°C for 24 hr and then either thawed at 18°C for 6 hr, at 2°C for 12 hr or held -2°C for 12 hr and subsequently thawed at 4°C.
<sup>3)</sup>All values are the mean±SD; <sup>a-c</sup>Means in the same raw with different letters are significantly different (*p*<0.05).

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All values are the mean±SD; acceptable as were frozen at -15°C within 3 hr PM, maintained at -15°C for 24 hr and then either thawed at 18°C for 6 hr, at 2°C for 12 hr or held -2°C for 12 hr and subsequently thawed at 4°C.

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In present study, shear values of cooked meat was measured as an object measurement of meat tenderness (Table 3). The control samples had the lowest shear values (68.22 N) and the samples thawed at 18°C exhibited significantly higher shear values (90.19 N) as compared to the control (p < 0.05). Shear force values of thawed meat were increased as thawing temperature increased. The samples thawed at -2°C had lower shear values (69.12 N) than that thawed at  $18^{\circ}$ C (p<0.05), but there were no significant differences between at -2 and 2°C samples. Although the samples thawed at 2 and -2°C had a slightly higher shear values than the chilled meat, there were no statistical differences among control and samples thawed at 2 and -2°C (p>0.05). The results obtained in this study are in agreement with the early findings, which thaw rigor meat is usually tough (9, 24). Davey et al. (27) also reported that shortening up to 20% did not appear to affect shear force values, but further shortening significantly increased shear force values.

As shown in Table 2 and 3, the severe contraction of muscle induced by thaw shortening appears to be the main contributing factor for the textural toughening of thaw rigor meat. In addition to, the results of this study clearly indicate that toughening in thaw rigor muscle occurs due to excessive muscle shortening and thaw drip loss during thawing. This finding is in agreement with the results of previous studies (8, 9, 28). Dransfield (9) and Behnke et al. (10) reported that the holding of frozen pre-rigor muscle at just sub-zero temperatures not only allows rigor mortis to proceed but also causes some tenderization. These results indicate that thaw toughness of thaw rigor meat can be effectively controlled by thawing rate.

Sensory properties The content of water and its distribution within cooked meat extremely influence tenderness and juiciness. If thaw drip loss of frozen and thawed muscle is extensive, it may adversely influence sensory properties.

Table 4 presents the results of sensory evaluation of chilled and thawed meat. At flavor sensory evaluation, although no significant differences among the control and thawing treatments were observed (p>0.05), control and sample thawed at -2°C had higher flavor score than those thawed at 18 and 2°C. Tenderness is the organoleptic trait

Table 4. Sensory properties of frozen pre-rigor bovine sternomandibularis muscle thawed at various temperatures

Traits <sup>1)</sup>	Control <sup>2)</sup> -	Thawing temperature <sup>3)</sup>			
		18°C	2°C	-2°C	
Flavor	6.23±0.43 <sup>4)</sup>	5.95±0.46	5.92±0.33	6.08±0.36	
Tenderness	$5.89 \pm 0.32^a$	4.59±0.39 <sup>b</sup>	$4.97\pm0.23^{b}$	5.47±0.42a	
Juiciness	$6.13\pm0.30^{a}$	4.77±0.39°	5.50±0.54 <sup>b</sup>	6.11±0.41 <sup>a</sup>	

<sup>4)</sup>All values are the mean±SD; <sup>a-c</sup>Means in the same raw with different letters are significantly different (p<0.05).

that is mostly affected consumer acceptance. The main factors influencing the tenderness of meat are muscle shortening, aging, and WHC. In this study, control and sample thawed at -2°C generally showed higher tenderness scores than those either thawed at 18 or 2°C (p<0.05) and no significant differences were found between control and sample thawed at  $-2^{\circ}$ C (p>0.05).

For juiciness, cooked meat resulted in significantly lower juiciness as thawing temperature increased and sample thawed at -2°C had higher juiciness score than those thawed at 18 and  $2^{\circ}$ C (p < 0.05). The juiciness of control was significantly higher compared to those thawed at 18 and  $2^{\circ}$ C (p<0.05), but there was no significant difference in juiciness between control and sample thawed at  $-2^{\circ}$ C (p>0.05).

The control and sample thawed at -2°C showed approximately the same sensory scores as those of the chilled meat (p>0.05). On the other hands, sample thawed at 18°C showed the lowest score in sensory parameter of tenderness and juiciness. These results for tenderness and juiciness may be due to extreme muscle contraction and lower water binding capacity associated with the significantly lower pH of sample thawed at 18°C. The beneficial effect of holding the frozen pre-rigor bovine muscle at sub-zero temperature on the quality characteristics of meat clearly observed. On the basis of the results of our study, we might conclude that meat qualities determined by physicochemical and sensory properties of frozen pre-rigor muscle are influenced by thawing temperature, and the holding the frozen pre-rigor muscle at -2°C before thawing can effectively mitigate the negative effect of thaw rigor on meat quality.

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<sup>&</sup>lt;sup>1)</sup>Scale from 1 to 9, 1 = undesirable flavor, extremely tough, and extremely dry; 9 = desirable flavor, extremely tender, and extremely juicy.

<sup>2)</sup>Muscle samples were stored at 15°C up to 24 hr PM and then at 4°C.

<sup>3)</sup>18, 2, and -2°C, Muscle samples were frozen at -15°C within 3 hr PM, maintained at -15°C for 24 hr and then either thawed at 18°C for 6 hr, at 2°C for 12 hr or held -2°C for 12 hr and subsequently thawed

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