Early Postmortem Processing Conditions on Meat Quality of *Hanwoo* (Korean Native Cattle) Beef during Storage

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ABSTRACT : The combined effects of low voltage electrical stimulation (ES) and early short-term temperature conditioning on meat quality of Hanwoo beef (Korean native cattle) during storage were investigated. Shear force was influenced by ES and aging. Combination of ES and the 30°C conditioning resulted in higher myofibril fragmentation index and improved lightness. There was no substantial difference in drip loss among treatments but ES samples showed higher cooking loss than control. Negative effect on shelf-life was not found by early short-term high temperature conditioning. Therefore, the meat quality of Korean native cattle was effectively improved by the combination of ES and the 30°C conditioning. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 12 : 1763-1768)*

Key Words : *Hanwoo* Beef, Electrical Stimulation, Temperature Conditioning, Meat Quality, Storage

INTRODUCTION

Extensive studies have been done to elucidate factors affecting meat tenderness during past 50 years. Although it is not conclusive, extent of proteolysis and rigor shortening have been suggested as major factors influencing change of meat tenderness during postmortem (PM) storage (Taylor et al., 1995; Koohmaraie et al., 1996).

Meat quality is a complex concept because it is influenced by a multivariate system of physicochemical parameters. Meat quality affects appearance, mouthfeel, color and finally consumer preference. For the improvement of meat quality, electrical stimulation (Cross, 1979), temperature conditioning (Whipple et al., 1990), H-bone suspending (Hostetler et al., 1972), calcium activated tenderization (Koohmaraie et al., 1988), and hydrodyne process (Solomon et al., 1997) have been developed. Although lots of studies regarding PM processing conditions have been conducted, the most effective type of treatment and its' optimal conditions remain to be elucidated. Furthermore, the effects of treatments on each quality trait are still controversial and variations likely exist among breeds.

The characteristics of Korean native cattle (Hanwoo) were described in a previous study (Rhee et al., 2000). The combination of electrical stimulation (ES) and short-term temperature treatments was used to create variation in rigor development and short-term conditioning was chosen to reduce risk of microbial contamination by long-term temperature treatment. The objective of this study was to examine the combined effects of low voltage ES and early short-term temperature conditioning on meat quality of *Hanwoo* beef during storage.

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MATERIALS AND METHODS

Reagents

Glutaraldehyde was obtained from Fischer Scientific Co. (Springfield, NJ, U.S.A.) and plate count agar was supplied by Difco Laboratories (Detroit, MI, U.S.A.). The rest of the chemicals used for this study were ACS grade and obtained from Sigma Chemical Co. (St Louis, MO, U.S.A.).

Animals and treatments

Twelve *Hanwoo* bulls, 24 to 26 month old were randomly assigned to two groups. Six were treated with low voltage electrical stimulation (50 V, 60 Hz, 20 s, impulse duration of 200 μs: ES) immediately after bleeding within 3 min of death while the other six were non-treated (NS).

Longissimus muscles from loin and rib section were removed within 30 min of slaughter and cut into three pieces. In order to avoid positional bias in tenderness, the pieces were assigned in equal numbers to one of three temperature conditioning in ES and NS. Each piece was temperature conditioned until 3 h PM at 2, 16 and 30°C, respectively. The samples were stored for 24 h at 2°C, cut into 2.5 cm steaks, and vacuum packaged. The steaks were randomly assigned to one of five times PM and were stored either 1, 2, 3, 7 or 14 d at 2°C. One steak was assigned to meat color, cooking loss and shear force, and one was assigned to the other analyses. At the end of each aging period, the following traits were measured.

Shear force

Warner-Bratzler shear force was determined using an Instron Universal Testing Machine (1011, Instron, Canton, MA, U.S.A.) equipped with a Warner-Bratzler shearing device (cross-head speed of 100 mm/min). The steaks (150±10 g) were placed in a polyethylene bag and cooked in a 75°C waterbath (1901W, Vision Scientific Co., Puchon, Korea) until the internal temperature of the steak reached 70°C. The cooked samples were chilled in running tap water for 40 min. Six cores (1.27 cm diameter) were removed from each steak parallel to the longitudinal orientation of muscle fibers.

Myofibril fragmentation index

Myofibril fragmentation index (MFI) was determined according to the procedures of Culler et al. (1978) with slight modifications. Four-gram minced muscle samples were homogenized for 30 s in 10 volumes (v/w) of 2°C isolating medium (100 mM KCl, 20 mM K-phosphate, 1 mM EDTA, 1 mM $MgCl_2$, 1 mM NaN_3). The homogenates were centrifuged for 15 min at 1,000 × g at 2°C, and the pellet was resuspended in the same volume of isolating medium as before by using a stirring rod. After the centrifuge step was repeated, the pellet was resuspended in 2.5 volumes (v/w) of isolating medium by vortexing, and the suspension was filtered through a polyethylene strainer to remove connective tissue and debris. The strainer was rinsed with 2.5 volumes of isolating medium, and the protein concentration of the final suspension was determined by using the biuret method (Gornall et al., 1949). The myofibril suspension was diluted with isolating medium to a protein concentration of 0.5±0.05 mg/ml and the determination was repeated. The diluted myofibril suspensions were then vortexed, and absorbance was measured at 540 nm with a spectrophotometer (Du-64, Beckman Instruments, San Ramon, CA, U.S.A.). The absorbance was multiplied by 200 to give a MFI value.

Sarcomere length

Three muscle cubes (3×3×3 cm) were removed from the medial, central, and lateral positions of steaks and fixed by the procedure of Howard and Judge (1968). Sarcomere length of fiber in each cube was measured by an eyepiece micrometer using a phase-contrast microscope (BH-2, Olympus Camera Co., Tokyo, Japan) at a magnification of 1,000. Average of 10 measurements was expressed.

Water holding capacity (WHC)

Drip loss was determined by two different methods, Honikel (1987) and vacuum packaging. Cooking loss was calculated by weight difference of samples before and after cooking. The cooking and chilling was done as described in Warner-Bratzler shear force.

Meat color

Color of muscle (CIE L*, a*, b*, chroma, and hue) was measured after exposing surface to the air for 30 min at 2°C. A Minolta chromameter (CR-300, Minolta Camera Co., Osaka, Japan) was used for measurement and average of triplicate measurements was recorded.

2-Thiobarbituric acid value (TBA)

2-Thiobarbituric acid value was determined by the method of Witte et al. (1970) with slight modifications. Samples (10 g) were homogenized at 5,000 rpm for 1 min in the solution containing 20% trichloroacetic acid (25 mL) and distilled water (20 mL). The homogenate was filtered through Whatman #1 (Whatman Instrument Ltd., Maidstone, England) at 2°C and the equal volume of 0.02 M thiobarbituric acid was added to the filtrate. The resultant was mixed and allowed to stay for 24 h at 22°C. The absorbance of sample at 532 nm was measured using spectrophotometer (Du-64, Beckman Instruments, San Ramon, CA, U.S.A.). TBA values were expressed as mg of malonaldehyde/kg of meat.

Total plate count

Samples were prepared according to the core sample technique of Bala et al. (1977) and total plate count was carried out in accordance with the standard plate count method of AOAC (1990).

Statistical analysis

Data were analyzed by mixed model analysis of variance with PROC MIXED of SAS (Inst. Inc., Cary, NC) for a completely randomized design, with a split-plot treatment arrangement. The whole-plot treatments were ES and NS. Subplot treatment was temperature conditioning and sub-, subplot treatment was day of storage. Means were separated using the probability option (PDIFF; a pair-wise t-test) of the least squares means procedures (SAS Inst, Inc., Cary, NC).

RESULTS AND DISCUSSION

Shear force, myofibrillar fragmentation index and sarcomere length

ES treated samples had lower shear force than those of NS at 1 ($p<0.01$), 2 and 7 d ($p<0.05$) PM, respectively (table 1). The lowered shear force consistently found in ES suggests that ES applied in this experiment improved tenderness. Simmons et al. (1997) and Koh et al. (1987) also reported that beef tenderness was increased by the application of low voltage ES. Significant difference was found in MFI only at 1 d ($p<0.01$) PM (table 2). Although substantial differences in MFI were not observed by ES except 1 d during storage, the combination of ES and the

Table 1. Effects of electrical stimulation and aging time on shear force (kg) of *M. longissimus*

Postmortem time (day)	Shear force (kg)		Standard error
	ES[1]	NS[2]	
1	7.40^{av}	9.27^{bv}	0.28
2	7.89^{av}	9.04^{bv}	0.28
3	6.99^{avw}	7.91^{avw}	0.33
7	5.63^{awx}	6.81^{bwx}	0.35
14	4.61^{ax}	5.21^{ax}	0.34

[1] Electrical stimulation after bleeding.
[2] Non-electrical stimulation.
[a,b] Least-square means in the same row lacking a common superscript differ ($p<0.05$).
[v,w,x] Least-square means in the same column lacking a common superscript differ ($p<0.05$).
Means of standard error for each treatment were 0.43 and 0.46, respectively.

Table 2. Effects of electrical stimulation and aging time on myofibril fragmentation index of *M. longissimus*

Postmortem time (day)	Myofibril fragmentation index[1]		Standard error
	ES[2]	NS[3]	
1	49.64^{av}	44.44^{bv}	1.58
2	49.96^{av}	49.05^{avw}	1.63
3	53.67^{av}	58.00^{aw}	1.98
7	72.59^{aw}	71.42^{ax}	2.51
14	88.12^{ax}	83.08^{ay}	3.38

[1] Absorbance at 540 nm × 200.
[2] Electrical stimulation after bleeding.
[3] Non-electrical stimulation.
[a,b] Least-square means in the same row lacking a common superscript differ ($p<0.05$).
[v,w,x,y] Least-square means in the same column lacking a common superscript differ ($p<0.05$).
Means of standard error for each treatment were 3.50 and 2.91, respectively.

30°C conditioning (ES-30°C) showed higher MFI than ES-2°C, NS-16°C and NS-30°C (table 3). As expected, shear force and MFI of *longissimus* decreased as storage time increased (tables 1 and 2). This tenderization continued through 14 d of aging but there was no significant difference in shear force between 7 and 14 d.

The mechanism of meat tenderization is highly complicated and various intricate factors are interacted each other. Olsson et al. (1994) reported that low voltage ES could not prevent cold-shortening when the meat was placed at low temperature during the rigor mortis. They suggested that shear force of *longissimus dorsi* was mainly affected by PM temperature.

Temperature conditioning during early PM influences glycolytic metabolism as reflected by a more rapid drop in pH (Koohmaraie et al., 1988). Tenderization is also closely related to the pH of muscle during aging since calpains activity and autolysis is dependent upon pH of the system. Thus, Tornberg (1996) suggested that the extent of tenderization is decided by the net proteolysis from the calpain activity and inactivation of calpains, especially μ-calpain. The previous report of Rhee and Kim (2000) demonstrated that lower pH and activated μ-calpain and calpastatin were obtained by ES-30°C compared to other treatments. Therefore, faster glycolysis and improved enzyme activity of the samples resulted in faster proteolysis and increased tenderness during storage.

Table 3. Two-way interaction between electrical stimulation treatment and temperature conditioning on myofibril fragmentation index for whole aging times

Temperature conditioning	Myofibril fragmentation index[1]		Standard error
	ES[2]	NS[3]	
2°C[4]	59.27^{ab}	66.01^{ac}	2.02
16°C[5]	61.24^{abc}	57.15^{b}	
30°C[6]	66.87^{c}	59.50^{ab}	

[1] Absorbance at 540 nm × 200.
[2] Electrical stimulation after bleeding.
[3] Non-electrical stimulation.
[4] Storage at 2°C.
[5] Temperature conditioning at 16°C until 3 h postmortem.
[6] Temperature conditioning at 30°C until 3 h postmortem.
[a,b,c] Least-square means lacking a common superscript differ ($p<0.05$).

ES and temperature conditioning did not significantly vary in sarcomere length. This result indicated that significant cold-shortening had not occurred even in the 2°C treatment (figure 1), and the improved tenderness might be occurred by activation of calpain system. This hypothesis was supported by faster degradation of nebulin, desmin, titin and troponin-T by ES-30°C (Rhee et al., 2000).

Water-holding capacity

There were no significant differences in drip loss measured by Honikel (1987) (data not shown) but significant difference were found in vacuum packaging between ES and NS at 3 d PM (table 4). Offer and Knight (1988) suggested that drip loss occurs by the migration of fluid from intracellular space to extracellular space and meat surface. This fluid migration was induced by the shrinkage of myofibrils. Therefore, this result suggests that substantial differences were not produced in terms of protein denaturation and structural damage that related to extent of myofibril shrinkage.

Application of ES showed higher cooking loss than NS at 1 ($p<0.01$) and 14 d ($p<0.05$), respectively (table 5). This result was consistent with Geesink et al. (1994) that cooking loss of ES was higher than that of NS at 2 and 14 d

Table 4. Effects of electrical stimulation and aging time on drip loss (%) for vacuum package of *M. longissimus*

Postmortem time (day)	Drip loss (%)		Standard error
	ES[1]	NS[2]	
2	0.76[av]	0.73[av]	0.06
3	0.78[av]	0.99[bv]	0.06
7	1.45[aw]	1.77[aw]	0.14
14	2.37[ax]	2.55[ax]	0.20

[1] Electrical stimulation after bleeding.
[2] Non-electrical stimulation.
[a,b] Least-square means in the same row lacking a common superscript differ ($p<0.05$).
[v,w,x] Least-square means in the same column lacking a common superscript differ ($p<0.05$).
Means of standard error for each treatment were 0.12 and 0.15, respectively.

Table 5. Effects of electrical stimulation and aging time on cooking loss (%) of *M. longissimus*

Postmortem time (day)	Cooking loss (%)		Standard error
	ES[1]	NS[2]	
1	26.14[av]	23.19[bv]	0.43
2	26.43[av]	26.10[avw]	0.76
3	26.47[av]	25.94[avw]	0.42
7	27.78[avw]	27.07[aw]	0.51
14	29.40[aw]	27.34[bw]	0.57

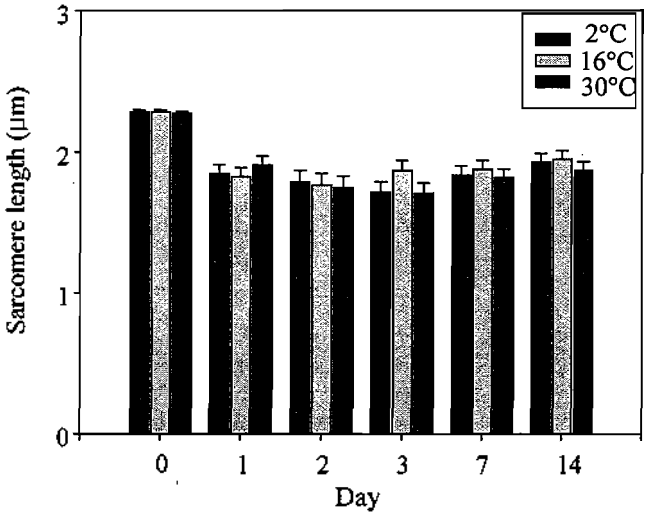
[1] Electrical stimulation after bleeding.
[2] Non-electrical stimulation.
[a,b] Least-square means in the same row lacking a common superscript differ ($p<0.05$).
[v,w] Least-square means in the same column lacking a common superscript differ ($p<0.05$).
Means of standard error for each treatment were 0.73 and 0.76, respectively.

Table 6. Two-way interaction between electrical stimulation treatment and temperature conditioning on CIE L* of *M. longissimus* for whole aging times

Temperature conditioning	CIE L*		Standard error
	ES[1]	NS[2]	
2°C[3]	33.35[a]	33.26[a]	0.24
16°C[4]	33.39[a]	33.19[a]	
30°C[5]	34.50[b]	33.15[a]	

[1] Electrical stimulation after bleeding.
[2] Non-electrical stimulation.
[3] Storage at 2°C.
[4] Temperature conditioning at 16°C until 3 h postmortem.
[5] Temperature conditioning at 30°C until 3 h postmortem.
[a,b] Least-square means lacking a common superscript differ ($p<0.05$).

PM. Although the exact mechanisms for cooking loss is still uncertain, denaturation and solubilization of collagenous system by heating was postulated as a main factor (Purslow, 1989). The subtle structural changes that were not differentiated by the measurement of drip loss could be detected by cooking loss since heating provide much higher driving force to cause shrinkage of endomysium.



2°C: Storage at 2°C.
16°C: Temp. conditioning at 16°C until 3 h postmor tem.
30°C: Temp. conditioning at 30°C until 3 h postmortem.

Figure 1. Effect of temperature conditioning on sarcomere length (μm) of *M. longissimus* during postmortem time.

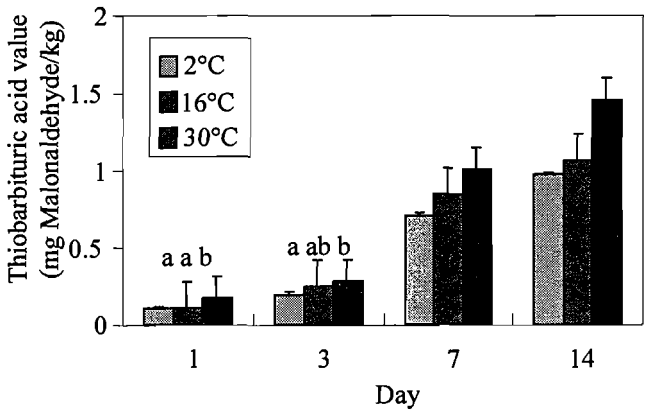
Color

A significant ($p<0.05$) interaction was found in lightness between ES and the temperature treatment (table 6). ES-30°C resulted in significantly brighter color compared with other treatments. The reason for this result might be related to the accelerated metabolic rate. The chance for denaturation of myofibrillar proteins could be increased by hastened glycolysis and higher denaturation of myoglobin by low pH and high temperature might result in lighter appearance. However, there was no critical difference in other color characteristics during storage (data not shown).

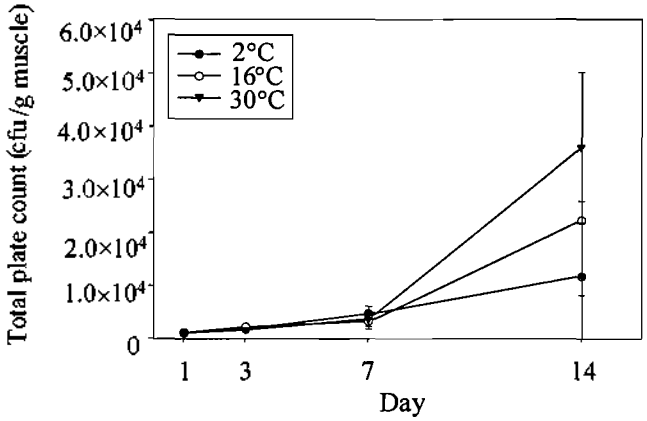
2-Thiobarbituric acid value and total plate count

Temperature conditioning resulted significant differences in TBA value until 3 d PM ($p<0.05$; figure 2). Although the 30°C treatment showed higher TBA value than that of 2°C treatment, the differences disappeared after 3 d PM storage.

Brewer et al. (1992) reported that rancid flavor for inedible meat was not detected when TBA value was above 4.0 mg MA/kg in beef. The highest TBA value in this study was less than 1.45 at 14 d suggesting it was below the sensory threshold for recognizing rancidity.



2°C: Storage at 2°C.
16°C: Temp. conditioning at 16°C until 3 h postmortem.
30°C: Temp. conditioning at 30°C until 3 h postmortem.
Figure 2. Effect of temperature conditioning on 2-thiobarbituric acid value of *M. longissimus* during storage.



2°C: Storage at 2°C.
16°C: Temp. conditioning at 16°C until 3 h postmortem.
30°C: Temp. conditioning at 30°C until 3 h postmortem.
Figure 3. Effect of temperature conditioning on total plate count of *M. longissimus* during storage

There was no difference in total plate count by temperature treatments and the total plate count in 30°C treatment was less than 4.0×10^4 at 14 d storage (figure 3). These results suggest that the application of short-term temperature treatment used in this study (about 2 h 15 min) did not produce any undesirable effect on flavor and microbiological safety of the meat.

As it was considered with our previous results of protein degradation (Rhee et al., 2000) and changes in glycolysis and calpains (Rhee and Kim, 2001), ES-30°C for 3 h PM was the best treatment to improve the meat quality. ES-30°C caused faster degradation of calpain substrates including nebulin, desmin, titin and troponin-T (Rhee et al., 2000), and lower shear force, which resulted in shortening of aging period by more than 7 d. Although ES resulted in the increased cooking loss, it improved lightness and meat tenderness. Furthermore, early short-term temperature treatment did not produce a negative effect on rancidity and total plate count. Thus, it is suggested that meat quality was affected more by metabolic rate like pH decline, ATP and glycogen depletion than by any other factor.

CONCLUSIONS

ES and aging were effective on shear force but MFI and meat color were further improved by the combination of ES and 30°C conditioning. Therefore, Electrical stimulation combined with 30°C conditioning was optimal to improve meat quality of *Hanwoo* (Korean native cattle).

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