ANTIOIXDANT ENZYME ACTIVITY, LIPID OXIDATION AND PROTEIN OXIDATION IN BEEF FROM HANWOO (KOREAN CATTLE) COWS BY SLAUGHTER AGE

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Abstract – This study was conducted to investigate the antioxidant enzyme activity, lipid oxidation and protein oxidation in beef (striploin) from Hanwoo (Korean cattle) cows by slaughter age (1.9-3.7, 4.0-4.8, 5.0-5.7, 6.0-6.9, and 7.5-11.5 yr). The activities of catalase, glutathione peroxidase, and superoxide dismutase were significantly ($P<0.05$) increased by high slaughter age. During chill storage, lipid oxidation (TBARS) and protein oxidation (carbonyl) were promoted by increasing slaughter age. These findings suggest that high slaughter age negatively affects the oxidative stability in Hanwoo cow beef.

Key Words – slaughter age, antioxidant enzyme, lipid oxidation, protein oxidation, cow, Hanwoo.

I. INTRODUCTION

Korean native yellow cattle named Hanwoo, is the most famous for branded beef cattle in Korea. Korean consumer prefers Hanwoo beef to beef from other national breeds or imported beef because it is considered as the highest grade of beef. In Korea, nearly half of Hanwoo beef are being produced from cows among three genders (bulls, steers, and cows). Usually, Hanwoo cows have different slaughter ages unlike other genders due to their different infertile times.

Several studies have reported that slaughter age influences the quality of beef (1, 2, 3, 4). In a previous study on cow beef of Xiong *et al*. (5), the decreased oxidative stability with high age was observed. It is considered that this is because the development of free radicals is promoted by increase of age (6, 7). In addition, slaughter age may change the endogenous antioxidant systems, particularly antioxidant enzymes (8, 9, 10), closely related to the oxidative stability (11, 12).

We therefore conducted this research to investigate the antioxidant enzyme activity, lipid oxidation and protein oxidation in beef from Hanwoo cows by slaughter age.

II. MATERIALS AND METHODS

A. Sample preparation and experimental design

The fresh striploins from 48 heads of Hanwoo (Korean cattle) cows were divided into five different age groups: 1.9-3.7 yr (10 heads; AG1), 4.0-4.8 yr (11 heads; AG2), 5.0-5.7 yr (12 heads; AG3), 6.0-6.9 yr (8 heads; AG4), and 7.5-11.5 yr (7 heads; AG5). Following trimming, the lean beef were sliced into 1 cm thickness and stored for 12 days at 4℃.

B. Analysis of antioxidant enzyme activity

For analyses of activities of catalase, glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD), samples were prepared following the procedure reported by Renerre *et al*. (13). Catalase activity was analyzed with the method developed by Aebi (14). Immediately after mixing meat extracts with 29 mM H_2O_2, the scavenging rate of H_2O_2 was spectrophotometrically monitored at 240 nm for 30 sec at 25℃. GSH-Px activity was analyzed with the enzymatic protocol established by Flohé & Günzler (15). Following mixing meat extracts with enzyme medium (1 mM EDTA-1 mM NaN_3-0.5 units/ml GSH reductase-1 mM GSH-0.15 mM β-NADPH-0.15 mM H_2O_2), the oxidation rate of NADPH to $NADP^+$ was monitored at 340 nm for 3 min at 25℃. SOD activity was analyzed with the pyrogallol

autooxidation method reported by Marklund (16). The inhibition rate of autooxidation of pyrogallol with meat extracts in tris-cacodylate-DTPA buffer (pH 8.2) was monitored at 420 nm for 2 min at 25℃.

C. TBARS content measurement

Lipid oxidation measurement was performed with 2-thiobarbituric acid reactive substances (TBARS) method developed by Sinnhuber & Yu (17). Briefly, 0.5 g of samples were mixed with 0.1 g of antioxidant mixture (propylene glycol-Tween 20-BHT-BHA), 1% (w/v) TBA-0.3% (w/v) NaOH, and 2.5% (w/v) TCA-36 mM HCl, and then heated in a 100℃ water bath for 30 min. Following combination with chloroform and centrifugation for 30 min at 4℃/3,000 g, upper solutions were spectrophotometrically measured at 532 nm. The results were expressed as mg of malonaldehyde (MA) per kg of meat.

D. Carbonyl content determination

Protein oxidation determination was carried out with carbonyl method reported previously by Mercier *et al*. (18). One hundred microliters of meat extracts were transferred into two microtubes, reacted with 0.2% (w/v) DNPH (in 2 N HCl) and 2 N HCl for 1 hour under the dark, and then combined with 20% (w/v) TCA. Sample sediments by centrifugation for 5 min at 2℃/3,000 g were rinsed with ethyl acetate-ethyl alcohol (1:1), dried in a hood, and then mixed with 6 M guanidine-HCl (pH 6.5). Absorbance values of mixtures were read at 370 (DNPH-added) and 280 (HCl-added) nm. The results were calculated as nmol of carbonyl per mg of protein with standard curve of BSA and millimolar extinction coefficient (22.0 $mM^{-1}cm^{-1}$; 19) of protein hydrazones.

E. Statistical analysis

All data were analyzed by SPSS (20) program. Duncan's multiple range tests were conducted to compare significant differences among means of five slaughter age groups when $P<0.05$.

III. RESULTS AND DISCUSSION

The activities of antioxidant enzymes in beef from Hanwoo cows by slaughter age were presented in Figure 1, 2, and 3. In these results, slaughter age had significant influences on the activities of antioxidant enzymes in cow beef. Catalase activity was significantly ($P<0.05$) higher in AG5 compared with AG1, AG2, and AG3 (Figure 1). As well, AG4 had significantly ($P<0.05$) higher catalase activity than both AG1 and AG2. GSH-Px activity was significantly ($P<0.05$) higher in AG5 than other age groups (Figure 2). SOD activity showed the same result to catalase activity (Figure 3). These are similar to previous findings of Gianni *et al*. (7) and Radák *et al*. (8) who reported the increased activities of antioxidant enzymes in human and rat skeletal muscles by high age. Xu *et al*. (9) also similarly observed that increasing age activated antioxidant enzymes in pig muscle.

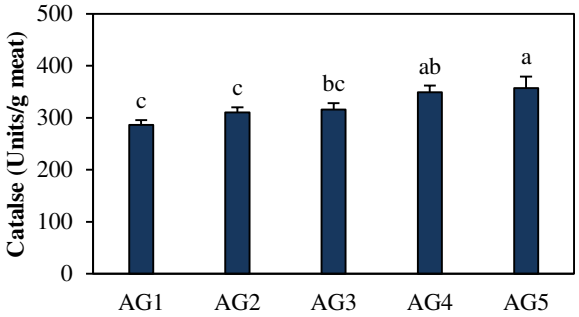

Figure 1. Catalase activity in beef from Hanwoo (Korean cattle) cows by slaughter age. These values are means±S.E. [a-c]Different letters indicate significant differences among slaughter age groups ($P<0.05$). AG1: 1.9-3.7 yr; AG2: 4.0-4.8 yr; AG3: 5.0-5.7 yr; AG4: 6.0-6.9 yr; AG5: 7.5-11.5 yr.

For 12 days of storage at 4℃, high slaughter age accelerated the development of TBARS, an index of lipid oxidation, in beef from Hanwoo cows (Figure 4). TBARS content was significantly ($P<0.05$) higher in AG5 from 8 days of storage compared with other slaughter age groups. From the same times, AG4 had significantly ($P<0.05$) higher TBARS content than AG1. This result is similar to a finding of Xiong *et al*. (5) who reported that beef from older age of cows had higher TBARS content during chill storage. As well, in a study of Ïnal *et al*. (21), the increased TBARS level in human plasma by high age was similarly reported.

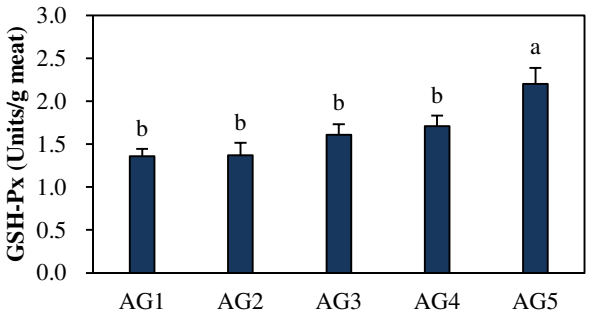

Figure 2. Glutathione peroxidase (GSH-PX) activity in beef from Hanwoo (Korean cattle) cows by slaughter age. These values are means±S.E. [a-b]Different letters indicate significant differences among slaughter age groups (P<0.05). AG1: 1.9-3.7 yr; AG2: 4.0-4.8 yr; AG3: 5.0-5.7 yr; AG4: 6.0-6.9 yr; AG5: 7.5-11.5 yr.

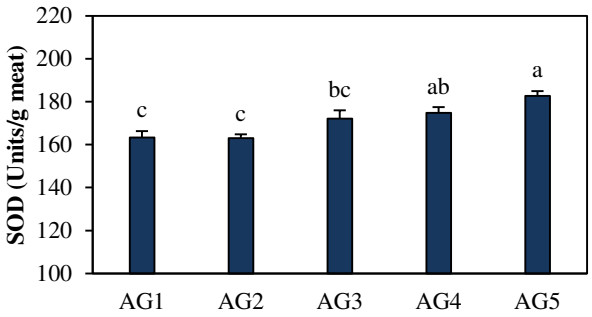

Figure 3. Superoxide dismutase (SOD) activity in beef from Hanwoo (Korean cattle) cows by slaughter age. These values are means±S.E. [a-c]Different letters indicate significant differences among slaughter age groups (P <0.05). AG1: 1.9-3.7 yr; AG2: 4.0-4.8 yr; AG3: 5.0-5.7 yr; AG4: 6.0-6.9 yr; AG5: 7.5-11.5 yr.

In Hanwoo cow beef during chill storage, high slaughter age promoted the generation of carbonyl, a biomarker of protein oxidation (22) (Figure 5). From 8 days of storage, carbonyl content was significantly (P<0.05) higher in AG5 compared with AG1. AG4 also had significantly (P<0.05) higher carbonyl content than AG1 at day 12 of storage. Our finding is similar to a report of Tian *et al*. (23) who found high carbonyl level in brain and liver from old rat. Also, Gautam *et al*. (24) similarly reported the elevated carbonyl level in human neutrophil by increasing age.

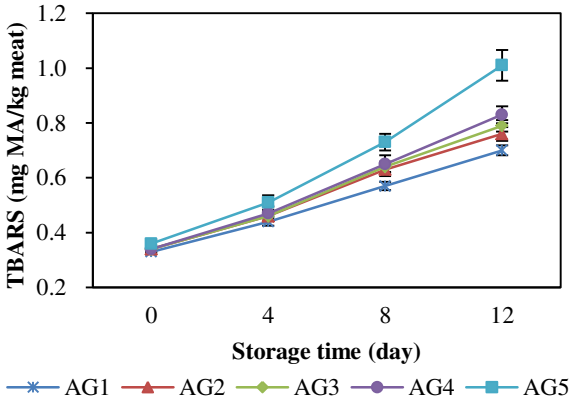

Figure 4. TBARS content in beef from Hanwoo (Korean cattle) cows by slaughter age. These values are means±S.E. AG1: 1.9-3.7 yr; AG2: 4.0-4.8 yr; AG3: 5.0-5.7 yr; AG4: 6.0-6.9 yr; AG5: 7.5-11.5 yr.

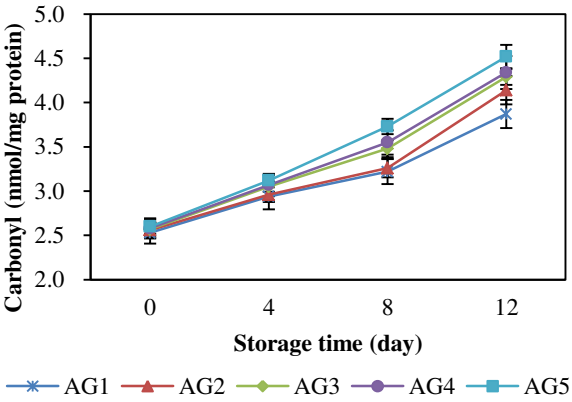

Figure 5. Carbonyl content in beef from Hanwoo (Korean cattle) cows by slaughter age. These values are means±S.E. AG1: 1.9-3.7 yr; AG2: 4.0-4.8 yr; AG3: 5.0-5.7 yr; AG4: 6.0-6.9 yr; AG5: 7.5-11.5 yr.

IV. CONCLUSION

Our research investigated the antioxidant enzyme activity, lipid oxidation and protein oxidation in beef from Hanwoo cows by slaughter age. High slaughter age induced the activities of catalase, GSH-Px, and SOD. Increasing slaughter age decreased the oxidative stability with promotion of lipid oxidation and protein oxidation.

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