ANTIOXIDANT ENZYME ACTIVITY, FERRIC REDUCING /ANTIOXIDANT POWER AND ABTS RADICAL CATION SCAVENGING ACTIVITY IN HANWOO (KOREAN CATTLE) BEEF MUSCLES

Sun Moon Kang, Geunho Kang, Pilnam Seong, Seokgeun Jung, Hyunsup Kim, Beomyoung Park and Soohyun Cho*

National Institute of Animal Science, Rural Development Administration, Suwon 441-706, Republic of Korea

Abstract – The objective of this research was to investigate the effect of muscle type on antioxidant enzyme activity, ferric reducing/antioxidant power (FRAP) and ABTS radical cation scavenging activity (trolox-equivalent antioxidant capacity, TEAC) in Hanwoo (Korean cattle) beef muscles. FRAP and TEAC were the highest ($P < 0.05$) in Guri (chuck tender) and Udoon (top round), respectively. Catalase and glutathione peroxidase activities were the highest ($P < 0.05$) in Guri. Glutathione reductase activity was the highest ($P < 0.05$) in Hongduke (eye of round). Glutathione *S*-transferase activity was the highest in Cheggt (striploin) and Udoon (top round).

Key Words – antioxidant enzyme, FRAP, TEAC, beef muscles, Hanwoo

I. INTRODUCTION

In beef muscles *post-mortem*, free radicals are continuously developed by oxidative processes and create the lipid oxidation provoking myoglobin oxidation, protein oxidation, off-flavor and loss of nutritional components (Monahan, 2000). However, muscle tissues possess intrinsic antioxidants which protect against the attacks of hydrogen peroxides, superoxides, hydroperoxy and hydroxyl radicals and lipid hydroperoxides (Chan & Decker, 1994). Their defense system performs antioxidant work with enzymatic or non-enzymatic ways. Catalase, superoxide dismutase and glutathione peroxidase, which are species of muscle enzymes, eliminate individual target free radicals. Besides, vitamins, proteins, peptides, amino acids and nucleotides scavenge free radicals or chelate metal irons (Decker et al., 2000).

In previous studies on beef (Renerre et al., 1996), it has been reported that some of muscles showed different antioxidant enzyme activity and oxidation stability. But few studies have been conducted to measure the effect of muscle type on antioxidant enzyme (particularly, glutathione reductase and glutathione *S*-trans -ferase), free radical scavenging activity and metal ion chelating ability. Therefore, we carried out this research to investigate the effect of muscle type on antioxidant enzyme activity, ferric reducing/antioxidant power and ABTS radical cation scavenging activity in Hanwoo (Korean cattle) beef.

II. MATERIALS AND METHODS

A. Animals and samples

Seven-heads of Hanwoo (Korean cattle) heifers were indoor-reared with a concentrate and a rice straw until 40 months-old. Dngsim (loin), Cheggt (striploin), Udoon (top round), Hongduke (eye of round) and Guri (chuck tender) were collected 24 hr *post-slaughter* from left carcasses, trimmed and stored at -80℃ until chemical analysis.

B. Preparation of samples for antioxidant activity measurements

Five grams of pulverized beef was mixed with 25 mL of ice-cold 50 mM phosphate buffer (pH 7.0) using a homogenizer (Ultra-Turrax T25 Digital, Ika Werke GmbH & Co., Germany) for 15 sec at 15,000 rpm. The homogenate was centrifuged for 15 min at 2℃, 10,000 g (SCR-20BA Himac Centrifuge, Hitachi Ltd., Japan) and filtered with a Whatman filter paper No. 1. The protein concentration of filtrate was measured by biuret method of Gornall et al. (1948).

C. Antioxidant enzyme activity measurements

In beef extract, antioxidant enzyme activity was analyzed by a spectrophotometer (DU-800, Beckman Coulter Inc., USA) at 25℃ and expressed as milliunits mg^{-1} protein. Catalase activity (Aebi, 1983) was determined by the

decomposition rate of H_2O_2 (29 mM) at pH 7.0 for 30 sec. Total superoxide dismutase (CuZn-SOD+Mn-SOD) activity (Marklund, 1986) was determined by the inhibition rate of pyrogallol autooxidation in 50 mM tris-cacodylate-DTPA buffer (pH 8.2; Zhang et al., 2002) for 2 min. Glutathione peroxidase (GSH-Px) activity (Flohé & Günzler, 1984) was determined by the recovery of GSH coupled to the generation of $NADP^+$ by glutathione reductase (GSH-R) in a reaction mix (1 mM EDTA-1 mM NaN_3-0.5 units/mL GSH-R-1 mM GSH-0.15 mM NADPH-0.15 mM H_2O_2-35 mM phosphate buffer, pH 7.0) for 3 min. GSH-R activity (Mavis & Stellwagen, 1968) was determined by the reduction rate of oxidized glutathione (GSSG) in a reaction mix (3 mM EDTA-1 mM GSSG-0.1 mM NADPH-0.13% BSA-36 mM phosphate buffer, pH 7.6) for 3 min. Glutathione *S*-transferase (GSH-ST) activity (Habig et al., 1974) was determined by the conjugation of GSH (1 mM) with 1-chloro-2,4-dinitrobenzene (1 mM) at pH 6.5 for 3 min.

D. Ferric reducing/antioxidant power (FRAP) measurement

FRAP was performed as described by Benzie & Strain (1999). The Fe^{3+} reduction rate of sample extract was measured in a FRAP reagent (250 mM acetate buffer, pH 3.6-0.83 mM 2,4,6-tris(2-pyridyl)-*s*-triazine-1.67 mM $FeCl_3 \cdot 6H_2O$) for 3 min at 37℃ at 593 nm. The results were calibrated with FRAP value of standard (1000 μM L-ascorbic acid) and calculated as μM Fe^{2+}.

E. ABTS radical cation scavenging activity measurement

According to Re et al. (1999), ABTS radical cation scavenging activity was measured by the decolorization of ABTS solution (in phosphate buffered saline, pH 7.4) with sample extract for 5 min at 30℃ at 734 nm. The results were represented as trolox-equivalent antioxidant capacity (TEAC; μM trolox) using the inhibition rate (%) and a trolox (0-20 μM) standard curve.

F. Statistical analysis

Data was analyzed by ANOVA (analysis of variance) of SPSS (2009) program. Significant differences among means were determined by Duncan's multiple range tests at $P < 0.05$.

III. RESULTS AND DISCUSSION

The effect of muscle type on FRAP in Hanwoo (Korean cattle) beef is represented in Figure 1. FRAP is the assay to measure the ability reducing ferric ion to ferrous ion. In Hanwoo beef muscles it ranged from 202-352 μM Fe^{2+} and was higher than that (less than 150 μM Fe^{2+}) of *M. psoas major* from indoor-reared Argentina steers reported by Descalzo et al. (2007). Guri showed the strongest ($P < 0.05$) FRAP among five muscles of Hanwoo beef. Additionally, Dngsim, Cheggt and Udoon had significantly ($P < 0.05$) stronger FRAP than Hongduke. Fe^{2+} strongly accumulates the myoglobin oxidation through the generation of hydroxyl radical ($OH^{\cdot}$) by Fenton reaction. Thus, high power of Fe^{3+} reduction in meat may promote the discoloration with the persistent supply of $OH^{\cdot}$. This chemical phenomenon has been observed in a experiment of meat homogenate model system by Hayes et al. (2009). However, it didn't has still been revealed that what level of FRAP is harmful to the color stability in meat. TEAC (Figure 2) was significantly ($P < 0.05$) higher in Udoon, when compared with other muscles. But Dngsim and Hongduke had the lowest ($P < 0.05$) TEAC. Among antioxidant enzymes in beef muscles (Figure 3), catalase breaks down a hydrogen peroxide into water and superoxide anion. SOD reduces superoxide anion and is composed of mitochondrial SOD (CuZn-SOD) and cytosolic SOD (Mn-SOD) in organisms. In our data, Hanwoo muscles indicated 2,584-7,025 and 2,125-2,393 milliunits mg^{-1} protein for catalase and SOD, respectively. Particularly, catalase and SOD activities (4,485 and 2,279) of Dngsim among all muscles were higher than those (about 2,600 milliunits mg^{-1} protein and less than 1 IU) of *M. longissimus dorsi* from Charolais heifers finished with a mixed diet reported by Gatellier et al. (2004). In following the order : Guri > Dngsim and Cheggt > Udoon and Hongduke, the activity of catalase showed significant ($P < 0.05$) differences. For SOD activity, there were no significant differences among all muscles in Hanwoo. GSH is an important tripeptide that plays as an antioxidant in animal tissue cells. Its mechanisms are connected with ubiquitous enzymes, such as GSH-Px, GSH-R and GSH-ST. GSH-Px, a selenoenzyme, decreases the oxidative stress by promoting the reduction of hydrogen

peroxide in the presence of GSH. GSH-R, catalyzing the reduction of GSSG to GSH, is an important enzyme for sustaining the constant level of GSH in cell tissues. GSH-ST, is one of multifunctional detoxification enzymes in mammals, defenses cell tissues from a great variety of toxicants, such as electrophilic xenobiotics, carcinogens and mutagenics, by the conjugation with –SH group of GSH. In results of this research, GSH-Px, GSH-R and GSH-ST of Hanwoo muscles ranged from 21.18-36.18, 3.28-7.49 and 81.98-92.42 milliunits mg^{-1} protein, respectively. Among all muscles, Dngsim (22.83) had lower GSH-Px activity than *M. longissimus dorsi* (about 190) from Charolais heifers finished with a mixed diet reported by Gatellier et al. (2004). But GSH-R and GSH-ST activities in beef didn't have been reported in previous studies. GSH-Px activity showed the highest ($P < 0.05$) in Guri but did the lowest ($P < 0.05$) in Udoon. GSH-R activity indicated significant ($P < 0.05$) differences in following the order : Hongduke > Guri > Dngsim, Cheggt and Udoon. GSH-ST activity was significantly ($P < 0.05$) higher in Cheggt and Udoon, when compared with Dngsim.

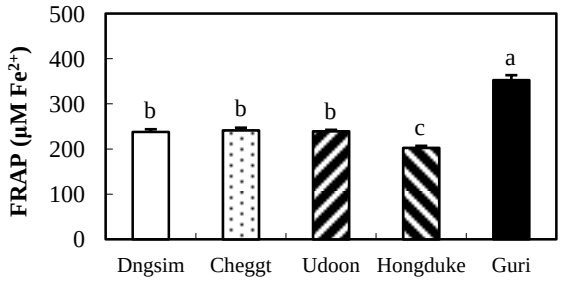

Figure 1. FRAP in Hanwoo (Korean cattle) beef muscles. Values are means±SE. [a-c]Different letters indicate significant differences ($P < 0.05$).

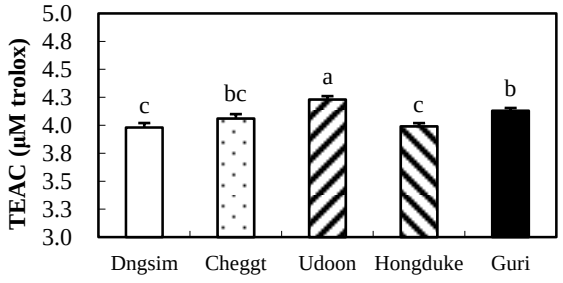

Figure 2. TEAC in Hanwoo (Korean cattle) beef muscles. Values are means±SE. [a-c]Different letters indicate significant differences ($P < 0.05$).

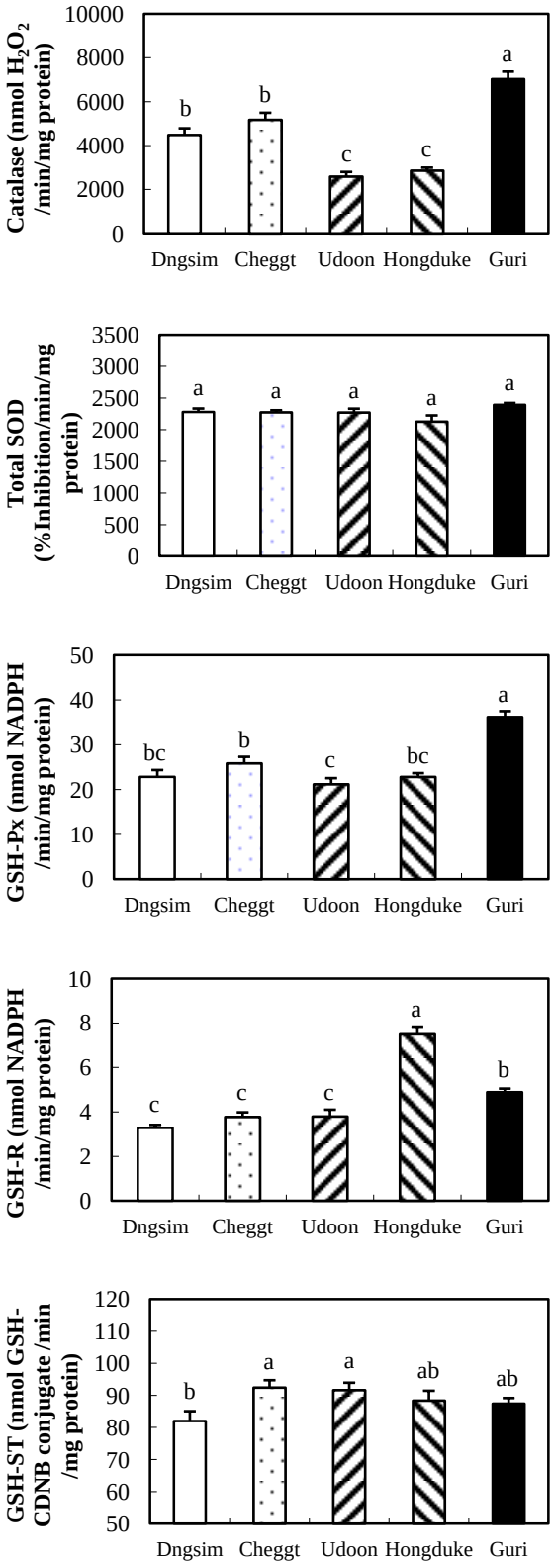

Figure 3. Antioxidant enzyme activity in Hanwoo (Korean cattle) beef muscles. Values are means±SE.

[a-c]Different letters indicate significant differences ($P < 0.05$).

IV. CONCLUSION

This research investigated antioxidant enzyme activity, ferric reducing/antioxidant power and ABTS radical cation scavenging activity in Hanwoo (Korean cattle) beef muscles (Dngsim, Cheggt, Udoon, Hongduke and Guri). Each muscle has its own powerful enzyme activity and antioxidant capacity. Therefore, muscle type may influence the oxidation stability of Hanwoo beef.

ACKNOWLEDGEMENTS

This study was supported by 2012 Postdoctoral Fellowship Program (PJ9070002012) of National Institute of Animal Science, Rural Development Administration, Republic of Korea.

REFERENCES

1. Monahan, F. J. (2000). Oxidation of lipids in muscle foods: fundamental and applied concerns. In E. A. Decker, C. Faustman & C. J. Lopez-Bote, Antioxidants in muscle foods: nutritional strategies to improve quality (pp 3-23). New York: John Wiley & Sons, Inc.
2. Chen, K. M & Decker, E. A. (1994). Endogenous skeletal muscle antioxidants. Critical Reviews in Food Science and Nutrition 34: 403-426.
3. Decker, E. A., Livisay, S. A. & Zhou, S. (2000). Mechanisms of endogenous skeletal muscle antioxidants: chemical and physical aspects. In E. A. Decker, C. Faustman & C. J. Lopez-Bote, Antioxidants in muscle foods: nutritional strategies to improve quality (pp 25-60). New York: John Wiley & Sons, Inc.
4. Renerre, M., Dumont, F. & Gatellier, Ph. (1996). Antioxidant enzyme activities in beef in relation to oxidation of lipid and myoglobin. Meat Science 43: 111-121.
5. Gornall, A. G., Bardawill, C. J. & David, M. M. (1948). Determination of serum protein by means of the biuret reaction. Journal of Biological Chemistry 177: 751-766.
6. Aebi, H. E. (1983). Catalase. In H. U. Bergmeyer, J. Bergmeyer & M. Graβl, Methods of enzymatic analysis (pp 273-286). Weinheim: Verlag Chemie GmbH.
7. Marklund, S. L. (1986). Pyrogallol autooxidation. In R. A. Green, CRC handbook of methods for oxygen radical research (pp 243-247). Boca Raton: CRC Press.
8. Zhang, H., Kamendulis, L. M. & Klaunig, J. E. (2002). Mechanisms for the induction of oxidative stress in Syrian hamster embryo cells by acrylonitrile. Toxicological Sciences 67: 247-255.
9. Flohé, L. & W. A. Günzler. (1984). Assays of gluta -thione peroxidase. In L. Packer, Methods in enzymology (pp 114-121). London: Academic Press, Inc.
10. Mavis, R. D. & Stellwagen, E. (1968). Purification and subunit structure of glutathione reductase from Baker's yeast. Journal of Biological Chemistry 243: 809-814.
11. Habig, W. H., Pabot, M. J. & Jarkoby, W. B. (1974). Glutathione *S*-transferase. The first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry 249: 7130-7139.
12. Benzie, F. F. & J. J. Strain. (1999). Ferric reducing /antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In L. Packer, Methods in enzymology (pp 15-23). London: Academic Press, Inc.
13. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26: 1231-1237.
14. SPSS. (2009). PASW Statistics 18, SPSS Inc., Illinois, USA.
15. Descalzo, A. M., Rossetti, L., Grigioni, G., Irurueta, M., Sancho, A. M., Carrete, J. & Pensel, N. A. (2007) Antioxidant status and odour profile in fresh beef from pasture or grain-fed cattle. Meat Science 75: 299-307.
16. Hayes, J. E., Stepanyan, V., Allen, P., O'Grady, M. N., O'Brien, N. M. & Kerry, J. P. (2009) The effect of lutein, sesamol, ellagic acid and olive leaf extract on lipid oxidation and oxymyoglobin oxidation in bovine and porcine muscle model systems. Meat Science 83: 201-208.
17. Gatellier, P., Mercier, Y. & Renerre, M. (2004) Effect of diet finishing mode (pasture or mixed diet) on antioxidant status of Charolais bovine meat. Meat Science 67: 385-394.