CHARACTERISTICS OF ENZYMATIC ANTIOXIDANT CAPACITY OF LONGISSIMUS DORSI MUSCLES IN YAK

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Abstract – Color stability and myoglobin oxidation of muscles are highly influenced by the antioxidant status of muscles. The objective of the present study was to characterize the enzymatic antioxidant capacity of longissimus dorsi muscles in yak (Bos grunniens). Initial metmyoglobin formation (IMF), an indicator of metmyoglobin reducing activity (MRA), and antioxidant enzyme activities of yak and bovine were determined. The results showed that IMF values for yak and bovine were 55.90 ± 1.68 % and 66.60 ± 3.16 %, respectively ($p<0.05$), suggesting that MRA in yak was significantly higher than that in bovine. Total antioxidant capacities, determined by ferric ion reducing capacity (FRC), cupric reducing antioxidant capacity (CUPRAC) and ABTS.+ radical scavenging ability, were all significantly higher in yak than those in bovine muscles. Furthermore, catalase activity of yak was 517.57 ± 32.55 U/mg protein, while that was 116.62 ± 8.95 U/mg protein in bovine muscles ($p<0.05$). Glutathione peroxidase (GPx) activity in yak meat was 5.99 ± 0.24 U/mg protein, significantly higher than that of 3.14 ± 0.33 U/mg protein in bovine muscles ($p<0.05$). In conclusion, metmyoglobin reducing activity and overall antioxidant status in yak muscles were both higher than those in bovine muscles, which indicated higher antioxidant capacities in yak to counteract meat oxidation.

Key Words –metmyoglobin reduction capacity, antioxidant enzymes, oxidative stability

I. INTRODUCTION

Myoglobin (Mb), a heme-containing protein, is critical to oxygen transportation and storage for live animals, as well as the acceptable color in muscle foods. Yak (Bos grunniens) inhabits steppes of the Himalayan highlands, usually at an altitude of 2000-5000 m, and is the only cattle species adapted to the cold and hypoxia environment of the Qinghai-Tibetan Plateau. Yak shared 100 % similarity in amino acid sequences with bovine Mb and the Mb concentrations in skeletal muscles of yak and bovine were similar ($p>0.05$), however, Mb oxidation and lipid oxidation rates were slower in yak than those in bovine [1]. Yang et al. [2] also reported that the discoloration rate of yak meat in air was lower compared with bovine. The exact mechanism by which yaks maintain Mb and lipid in a more stable status than bovine is not fully clear. Furthermore, since animals living on highland are confronted with greater oxidation stress caused by more free radicals produced under hypoxia environment compared with plains, this mechanism is also essential to understand the living animals' defense against oxidation at hypoxia environment.

Endogenous antioxidant factors, such as antioxidant enzymes, can counteract meat oxidation [3]. Therefore, the objective of this study was, with comparison with bovine, to characterize yak enzymatic antioxidant capacity in order to, at least partially, elucidate the fundamental basis of the observed varied color stability in yak and bovine meats.

II. MATERIALS AND METHODS

Longissimus dorsi were obtained from three adult male Songpan yaks from Ruoergai Prairie, and three adult male bovines from Chengdu Plain of Sichuan (n = 3). The muscles were removed quickly from slaughtered animals within 1 hour and kept frozen at -20°C until use.

Metmyoglobin reducing activity (MRA). MRA was determined by the initial metmyoglobin formation (IMF) methods described by Mancini *et al.* [4]. Slices (2 cm diameter, 0.5 cm thickness) were cut from the frozen muscles,

packaged with polyethylene and allowed to thaw at 4 ℃. Then samples were submerged in 50 ml of 0.3% sodium nitrite solution to oxidize myoglobin. After incubation at 25 ℃ for 20 min, the longissimus dorsi slices were immediately blotted dry. Reflectance spectra at the meat surface were recorded, using a Shimadzu UV/VIS spectrophotometer (UV-2450).

Determination of antioxidant capacity. Ferric ion reducing capacity (FRC) was measured by the method of Min *et al.* [5]. Cupric reducing antioxidant capacity (CUPRAC) was measured by the method described by Apak *et al.* [6]. ABTS.+ radical scavenging ability was measured according to Re *et al.* [7].

Determination of antioxidant enzymes activity. The activity of catalase was measured following the method of Terevinto *et al.* [8]. One unit (U) of catalase activity was defined as the amount of extract needed to decompose 1 umol H2O2 per min. Catalase activity was expressed as U/mg protein. GSH-Px activity was determined using kits (Jiangong Corporation, Nanjing, China). One unit (U) was defined as the amount of extract needed to decompose 1 umol GSH per min. GPx activity was expressed as U/mg protein. Superoxide dismutase (SOD) activity was determined using kits (Jiangong Corporation, Nanjing, China). One unit (U) was defined as the SOD activity that inhibits the reaction by 50 %. SOD activity was expressed as U/mg protein.

Statistical analysis. Data were reported as mean ± SEM for each experiment. Data between breeds were analyzed with Paired sample t- test using Statistical Package for the Social Science (SPSS Inc., version 13.0). For the analysis of iron-induced lipid oxidation, ANOVA tests were performed to identify differences among means. Statistical significance was declared at $P < 0.05$.

III. RESULTS AND DISCUSSION

Metmyoglobin reducing activity. As shown in Figure 1, a significant difference was found between initial metmyoglobin formation (IMF) values in yak and bovine (55.90 ± 1.68 % vs. 66.60 ± 3.16 %, respectively; $p<0.05$). Lower IMF values in yak muscles indicated higher metmyoglobin reducing activity which could partially explain color stability observed in yak than in bovine.

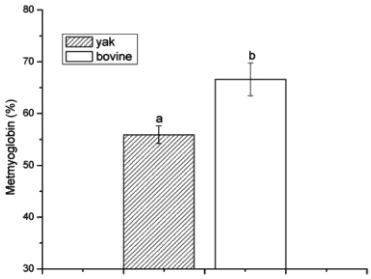

Figure 1. Nitrite-induced metmyoglobin formation in yak and bovine muscles.

Mancini et al. (2008) analyzed 4 metmyoglobin reducing activity surface measurements, in which IMF was most correlated with color-stability in meats ($p<0.05$).

Antioxidant capacity. The FRC value in yak was 62.91 ± 9.72 ug ascorbic acid equivalent/g of muscle, which was almost three times of that in bovine (Table 1, $p<0.05$). The CUPRAC and ABTS.+ radical scavenging values in yak were 5815.53 ± 62.78 ug ascorbic acid equivalent/g meat and 66.44 ± 2.44 % inhibition, nearly 25 % and 15 % higher than those in bovine, respectively (Table 1, $p<0.05$). The results of FRC, CUPRAC and ABTS.+ radical scavenging values showed a higher overall antioxidant capacity in yak muscles compared to their counterpart, which may partly elucidate higher oxidative stability in yak.

Antioxidant enzymes activities. As shown in Table 1, catalase activity in yak muscles was 517.57 ± 32.55 U/mg protein, which was almost 4 times as high as that in bovine muscles ($p<0.05$). GPx activity was 5.99 ± 0.24 U/mg protein in yak muscles, while it was 3.14 ± 0.33 U/mg protein in bovine muscles, only half of the value in yak. SOD activity in yak muscles was similar to that in bovine. Our data showed that catalase and GPx activities in yak muscles were significantly higher than those in bovine muscle. The dramatic differences of antioxidant enzymes, especially catalase, between bovine and yak suggested that antioxidant capacity in yak muscle is higher than that in bovine muscle,

resulting in higher oxidative stability in yak muscle.

Table 1 Antioxidant status in yak and bovine muscles.

	Yak	Bovine
Overall antioxidant capacity		
FRC[A]	62.91 ± 9.72[a]	23.46 ± 4.41[b]
CUPRAC[B]	5815.53 ± 62.78[a]	4349.41 ± 66.32[b]
$ABTS^{\cdot+}$ scavenging ability[C]	66.44 ± 2.44[a]	56.44 ± 3.66[b]
Antioxidant enzymes activity[D]		
Catalase	517.57 ± 32.55[a]	116.62 ± 8.95[b]
GPx	5.99 ± 0.24[a]	3.14 ± 0.33[b]
SOD	10.19 ± 0.52[a]	10.99 ± 0.76[a]

Values are means ± standard error; A Expressed as ug ascorbic acid equivalent/g of muscle; B Expressed as ug ascorbic acid equivalent/g of muscle; C Expressed as inhibition % of the $ABTS^{\cdot+}$ radical; D Expressed as U/mg protein.

IV. CONCLUSION

From our study, metmyoglobin reducing activity and overall antioxidant status in yak muscles were both higher than that in bovine muscles, which indicated higher antioxidant capacities in yak to counteract meat oxidation. The exact mechanisms of varied Mb and lipid oxidation in yak and bovine need further study.

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