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Post-mortem oxidative stability of three yak (*Bos grunniens*) muscles as influenced by animal age

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ABSTRACT

The influence of animal age and muscle source on the oxidative stability of yak steaks was examined. Longissimus thoracis (LT) muscles from yaks of different age groups (0.5, 1.5, 2.5, and 3.5 years), and three muscle sources of LT, Psoas major (PM), and Biceps femoris (BF) from yaks of 0.5, 1.5, and 2.5 years, were evaluated for metmyoglobin content, activity of antioxidant enzymes, and antioxidant capacity. Oxidative stability was influenced ($P < 0.05$) by muscle source and animal age. LT steaks from 0.5, 1.5, and 2.5 year old yaks exhibited lower ($P < 0.05$) metmyoglobin content than their PM and BF counterparts. Furthermore, LT steaks from 3.5 year old yaks demonstrated lower ($P < 0.05$) metmyoglobin content and greater ($P < 0.05$) activities of antioxidant enzymes than LT steaks from other age groups. These results indicated the necessity to develop muscle- and age-specific processing strategies to improve color and oxidative stability of yak meat.

1. Introduction

Yak (*Bos grunniens*) is the predominant large ruminant species in the high altitude Qinghai–Tibetan Plateau, China, which is characterized by year-round low temperatures and hypoxic atmosphere. Yaks play an important role in Tibetans' life by providing them with meat, milk, fiber, and fuel (Wiener, Han, & Long, 2003). Yak meat has been increasingly becoming popular among the consumers in China in recent years for its game-like flavor. The semi-open farming system and the lack of sufficient feed stuff in rangelands in winter and spring lead to yaks being slaughtered at an age of 3.5 years or even 5 years (depending on the body weight), which is much longer than the typical slaughtering age (1.5–2 years) for beef cattle. Such a long raising time for livestock may lead to increased oxidation and quality deterioration in meat (Hilton et al., 1998).

Oxidative stability is critical to quality of fresh and processed meats (Decker, Livisay, & Zhou, 2000; Zhang, Xiao, & Ahn, 2013). Recent genomic comparisons between yak and cattle identified an expansion of gene families related to hypoxic response and energy metabolism in yaks, and the related genes were different or expressed at varied levels in yaks and cattle (Hu et al., 2012; Qiu et al., 2012). This suggests that the oxidative stability of yak meat may be possibly influenced by genetic as well as environmental factors. Previous studies reported that color of yak meat was preferred by consumers to cattle of the same age (Gu, Chen, Chen, Yang, & Sun, 2007; Yang et al., 2009). Furthermore, myoglobin oxidation, lipid oxidation, and discoloration were lower in yak meat than in beef (Gu, Chen, Chen, et al., 2007; Yang et al., 2009), despite the similar myoglobin concentrations in skeletal muscles and the 100% similarity in amino acid sequences of myoglobin shared by these two species (Gu, Chen, Yin, Tang, & Sun, 2007). It is possible that the high oxidative stability of yak meat could be potentially due to the endogenous antioxidant capacity, which is determined by the expression of genes related to hypoxic response and energy metabolism in order to defend the yaks against reactive oxygen species and free radicals.

Myoglobin oxidation and oxidative stability in fresh meats are governed by multitude of extrinsic and intrinsic factors (Faustman & Cassens, 1990; Faustman, Sun, Mancini, & Suman, 2010; Suman & Joseph, 2013). Several researchers reported that catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) retard lipid oxidation and discoloration in beef (Lauridsen, Nielsen, Henckel, & Sørensen, 1999; Pastsart, De Boever, Claeys, & De Smet, 2013). Moreover, these antioxidant enzymes were negatively related to lipid oxidation (Chen, Zhou, Xu, Zhao, & Li, 2010; Jin, He, Yu, Zhang, & Ma, 2013). Two important intrinsic factors influencing metmyoglobin formation and lipid oxidation are muscle source and animal age (Mancini & Hunt, 2005; Suman, Hunt, Nair, & Rentfrow, 2014). Several previous investigations highlighted muscle-specificity (Hunt & Hedrick, 1977; Joseph, Suman, Rentfrow, Li, & Beach, 2012; McKenna et al., 2005;

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Pastsart et al., 2013; Seyfert, Mancini, Hunt, Tang, & Faustman, 2007) and critical influence of animal age (Du Plessis & Hoffman, 2007; Girard, Aalhus, Basarab, Larsen, & Bruce, 2012; Tuma, Henrickson, Stephens, & Moore, 1962) on beef color stability.

While myoglobin oxidation and lipid stability in beef have been extensively investigated, scientific information on oxidative stability of fresh yak meat is non-existent. Therefore, the objective of the present study was to examine the influence of animal age and muscle source on oxidative stability of fresh yak meat.

2. Materials and methods

2.1. Animals and meat samples

Twenty-four male yaks purchased from local producers were used in this study. These yaks represented four stages of growth (0.5 ± 0.1 years, 80.5 ± 5.5 kg body weight; 1.5 ± 0.1 years, 120.5 ± 8.7 kg body weight; 2.5 ± 0.2 years, 160.5 ± 7.6 kg body weight; and 3.5 ± 0.2 years, 185.8 ± 13.2 kg body weight) with six animals ($n = 6$) in each group. The experimental unit was an animal. Based on the research guidelines of the Sichuan Academy of Grassland Science, all yaks were placed on the same pasture at an altitude of 3500 m for 3 months to attain a similar nutritional background before slaughter. Animals were humanely harvested according to the Operating Procedures of Cattle Slaughtering (GB/T 19477-2004, China) at a commercial abattoir in Hongyuan, Sichuan Province. After evisceration, carcasses were immediately split and cooled to 4 °C. Carcasses were fabricated 24 h postmortem, and 2.5-cm steaks were sliced from Longissimus thoracis (LT) at the 7th–8th rib, Psoas major (PM) at the central portion, and Biceps femoris (BF) at the thickest part of the hind leg. Due to unanticipated reasons beyond our control, PM and BF samples were not available from the 3.5 year old yaks. From each steak, 10 g samples were removed for antioxidant enzymes activity assays and were immediately frozen at −20 °C until analyses. The steaks were stored at 4 °C for analyses of metmyoglobin formation.

2.2. Metmyoglobin content

Steaks were placed on polystyrene trays, over-wrapped with oxygen-permeable polyvinyl chloride film, and stored in the dark at 4 °C for analyses of myoglobin oxidation. Reflectance spectra were measured at the surface on day 7 during refrigerated storage by using a diffuse reflectance measurement (ISR-240A Integrating Sphere Attachment, Shimadzu Corporation, Kyoto, Japan). For each steak, average values were calculated from triplicate readings made on non-overlapping zones of the surface. Metmyoglobin (MetMb) percentage was estimated according to the method of Krzywicki (1979).

2.3. Activity of antioxidant enzymes

Muscle sample (5 g) was homogenized with 45 mL ice cold phosphate buffer (100 mM Na_2HPO_4, pH 7.0), and the homogenate was centrifuged at 6000 ×g for 10 min at 4 °C. The supernatant was used for determination of activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx).

The activity of catalase was measured according to the method of Terevinto, Ramos, Castroman, Cabrera, and Saadoun (2010). The muscle extract supernatant (90 μL) and extraction buffer (2820 μL) were mixed in a quartz cuvette (1 cm path length) at room temperature (22–25 °C), and 90 μL 5.65 mM H_2O_2 solution was added. The reaction was spectrophotometrically (Shimadzu UV-2450 UV/VIS spectrophotometer, Shimadzu Corporation, Kyoto, Japan) monitored by the decrease in absorbance at 240 nm during 3.5 min incubation. The activity was calculated using the molar extinction coefficient of H_2O_2 (43.6 M^{-1} cm^{-1}). One unit (U) of catalase activity was defined as the amount of extract needed to decompose 1 μM H_2O_2 per minute. Catalase activity was expressed as U/mg protein.

Assay kits were used to determine SOD and GPx activities. SOD activity was measured by hydroxylamine method using total superoxide dismutase assay kit (A001-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Muscle extract supernatant (0.1 mL) was incubated with 1 mL reagent 1 (0.067 M phosphate buffer, pH 7.8), 0.1 mL reagent 2 (10 mM hydroxylamine hydrochloride), 0.1 mL reagent 3 (7.5 mM xanthine), and 0.1 mL reagent 4 (0.023 U/mL Xanthine oxidase). The control contained all the reagents and an equivalent volume of distilled water instead of muscle extract supernatant. After incubation at 37 °C for 2 min, 2 mL chromogenic agent (3.3 g/L sulfanilic acid, 10 g/L alpha naphthylamine) was added. The absorbance at 550 nm was recorded spectrophotometrically (Shimadzu UV-2450 UV/VIS spectrophotometer, Shimadzu Corporation, Kyoto, Japan). One unit of SOD activity was defined as the amount of enzyme in 1 mL of the reaction solution at 50% SOD inhibition at 37 °C.

GPx activity was measured by the colorimetric method using glutathione peroxidase assay kit (A005, Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Muscle extract supernatant (0.2 mL) was incubated with 0.2 mL 1 mM reduced glutathione (GSH), 0.1 mL reagent 1 (1.25 mM H_2O_2), and 2 mL reagent 2 (1.67% HPO_3, 0.05% EDTA, 28% NaCl); the control contained all the reagents and an equivalent volume of distilled water instead of muscle extract supernatant. After incubation at 37 °C for 5 min, the mixture was centrifuged at 4000 ×g for 10 min. One milliliter supernatant was mixed with 2.5 mL chromogenic agent (0.04% DNTB (5,5′-dithiobis-(2-nitrobenzoic acid)), 1% sodium citrate). The absorbance was recorded at 412 nm spectrophotometrically (Shimadzu UV-2450 UV/VIS spectrophotometer, Shimadzu Corporation, Kyoto, Japan). One unit of GPx activity was defined as the amount of the enzyme capable of decomposing 1 μM glutathione per minute at 37 °C.

2.4. Antioxidant capacity

The antioxidant capacity was evaluated by three assays, including ferric ion reducing capacity (FRC), cupric reducing antioxidant capacity (CUPRAC), and ABTS.+ radical scavenging ability (ABTS). Muscle sample was homogenized with 3 volumes of 50 mM acetate buffer (pH 5.6) in a Waring blender (Waring Co., Torrington, CT, USA) and was centrifuged at 10,000 ×g for 20 min at 4 °C. The supernatant was separated and used for the following assays.

Ferric ion reducing capacity was measured by the method of Min and Ahn (2009). One milliliter supernatant was mixed with 1 mM ferric chloride solution and placed at room temperature (22–25 °C) for 10 min, and 1 mL 11.3% TCA solution was added. The mixture was centrifuged at 10,000 ×g for 10 min. The supernatant (2 mL) was mixed with 0.8 mL 10% ammonium acetate and 0.2 mL ferrozine color reagent for 10 min. The absorbance was read spectrophotometrically (Shimadzu UV-2450 UV/VIS spectrophotometer, Shimadzu Corporation, Kyoto, Japan) at 562 nm against a reagent blank. FRC was expressed as ascorbic acid equivalents in mg/g muscle.

Cupric reducing antioxidant capacity (CUPRAC) was measured by the method described by Apak, Güçlü, Özyürek, and Karademir (2004) with modification. Ten microliter supernatant was reacted with 1 mL 10 mM copper(II) chloride, 1 mL 1 M ammonium acetate, and 1 mL 7.5 mM neocuproine in 96% ethanol. After incubating at room temperature (22–25 °C) for 1 h, the absorbance was read at 450 nm against a blank. The CUPRAC was expressed as ascorbic acid equivalents in mg/g muscle.

ABTS.+ radical scavenging ability was determined by the discoloration assay (Re et al., 1999). Equal volumes of 14 mM ABTS [2,2-azinobis-(3-ethylbenzothiazoline 6-sulphonate)] solution and 4.9 mM potassium persulfate solution were mixed to produce ABTS radical cation (ABTS.+). The mixture was kept in the dark at room temperature for 16 h. The ABTS.+ solution was diluted with PBS (phosphate buffered saline, pH 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm and was

equilibrated at 30 °C. A blank reading at 734 nm (A_0) was taken immediately after mixing 4.0 mL of diluted ABTS.+ solution with 40 μL of water. For addressing possible spontaneous discoloration in ABTS.+ solution, another reading was recorded in the blank after 6 min of incubation at 30 °C. Supernatant of muscle extract (40 μL) was mixed with 4.0 mL of ABTS.+ diluted solution, and the absorbance at 734 nm was read after 6 min of incubation at 30 °C. The percentage of inhibition of the ABTS.+ was calculated as follows.

$$\text{Inhibition } \% = 100 \text{ x } [(A_0 - A_6)/A_0]$$

A_0 represents the absorbance measured immediately at 734 nm wavelength after mixing 4.0 mL of diluted ABTS.+ solution with 40 μL of distilled water. A_6 represents the absorbance measured after 6 min of incubation either in the blank or in the supernatant of muscle extract. The true percentage of inhibition was obtained by subtracting the spontaneous inhibition% of the ABTS.+ blank solution from that of muscle extract supernatant.

2.5. Statistical analysis

Six replications were used for data analyses ($n = 6$). All Data were analyzed using the Statistical Package for the Social Science (SPSS Inc., version 19.0) and reported as means and standard error of means (SEM). Data of LT from yaks of 0.5, 1.5, 2.5 and 3.5 years were analyzed using one-way analysis of variance (ANOVA) to examine the effect of animal age on oxidative stability. Data of muscle sources of LT, PM, and BF from yaks of 0.5, 1.5 and 2.5 years were analyzed using a model with fixed effects of age, muscle source, and age × muscle source interaction in a two-way ANOVA. The ANOVA tables obtained were further analyzed for the comparison of means by least significant difference (LSD) procedures. Pearson correlations were used for testing the correlations between MetMb and antioxidant capability of muscles (activity of antioxidant enzymes and antioxidant capacity).

3. Results and discussion

3.1. Evolution of oxidative stability with age

The results of the evolution of oxidative stability in Longissimus thoracis (LT) from yaks of 0.5, 1.5, 2.5 and 3.5 years of age are presented in Table 1. Age may influence ($P < 0.05$) MetMb levels in steaks. Overall, in LT, MetMb contents were not different ($P > 0.05$) among the 0.5, 1.5, and 2.5 year age groups (Table 1). These results were in agreement with those of Xiong et al. (2007) in beef patties prepared from semimembranosus and semitendinosus muscles of mature cows at 2–4 years, 6–8 years, and 10–12 years of age. These authors observed that MetMb formation was similar among different age groups during refrigerated storage for 7 days. On the other hand, in the present study LT steaks in the 3.5 year group demonstrated lower ($P < 0.05$) MetMb formation than the LT counterparts from other age groups, indicating a muscle-specific effect of animal age. In contrast to the present results, other researchers observed greater MetMb formation in beef semimembranosus and gluteus medius steaks from 1.5 year old animals than those from 1 year old animals (Girard et al., 2012). This difference may be attributed to the species-specificity in meat color (Suman & Joseph, 2013).

The activities of CAT, SOD and GPx in LT exhibited a pronounced influence ($P < 0.05$) of age (Table 1). In LT steaks, the activities of the three antioxidant enzymes (CAT, SOD and GPx) were highest in the 3.5 year group, which exhibited the lowest MetMb formation (Table 1). The recommended age for slaughtering yaks is 3.5 years, and animals at this age have desirable nutritional status (Xie, Luo, Yang, & Zheng, 2005). In this point of view, the findings on LT steaks suggested the possible advantage of 3.5 years as the suitable slaughter age for yaks since steaks from such animals are relatively stable against oxidative deterioration. The influence of age on activities of antioxidant enzymes has been reported in several meat species, although the results were not conclusive. Xu, Wang, Guo, Liu, and Feng (2007) observed that the CAT, SOD and GPx activities in pig muscles steadily increased as the animals grew from day 1 to day 168. However, Gatellier, Mercier, and Renerre (2004) reported no effect of animal age on CAT, SOD, and GPx activities in 2.5–8 years and 8–15 year old cows.

No age-dependent difference was observed in FRC capacity ($P > 0.05$), whereas CUPRAC in LT decreased ($P < 0.05$) with age (Table 1). ABTS radical scavenging ability demonstrated overall greater ($P < 0.05$) in LT steaks from older animals than in their counterparts from younger yaks. Based on these results, the antioxidant capacity (FRC, CUPRAC, and ABTS) in yak steaks appeared not to be associated with MetMb formation. Very limited information is available on the effect of animal age on the antioxidant capacity of fresh meats. On the other hand, previous studies reported an influence of species on antioxidant capacity of fresh meats. Min and Ahn (2009) reported that chicken breasts exhibited greater FRC and CUPRAC activities than beef loins. Serpen, Gokmen, and Fogliano (2012) observed that the ABTS scavenging capacity of chicken was greater than that of pork, beef and fish.

3.2. Oxidative stability of three muscle sources

3.2.1. MetMb content

The results of MetMb formation in LT, PM, and BF from yaks of 0.5, 1.5 and 2.5 years are presented in Table 2. There was an effect of muscle source ($P < 0.05$), but no effect of age and age × muscle source interaction ($P > 0.05$). LT demonstrated lower MetMb content than PM and BF. In addition, in the 2.5 year age group, BF exhibited lower MetMb content than PM. The results of the present study are in agreement with the findings of previous investigations in beef muscles. McKenna et al.

Table 1
Percentage metmyoglobin formation, activity of antioxidant enzymes and antioxidant capacity (mean ± standard error of the mean) in Longissimus thoracis (LT) from yaks of 0.5, 1.5, 2.5, and 3.5 years of age.

	0.5 year	1.5 years	2.5 years	3.5 years
MetMb	52.3 ± 2.4^{b}	50.0 ± 1.5^{b}	47.7 ± 2.2^{b}	40.4 ± 1.4^{a}
CAT	3.2 ± 0.3^{a}	3.9 ± 0.3^{ab}	4.6 ± 0.4^{b}	5.9 ± 0.4^{c}
SOD	60.7 ± 2.6^{a}	61.8 ± 2.0^{a}	72.3 ± 2.7^{b}	81.2 ± 1.2^{c}
GPx	24.6 ± 2.2^{c}	12.3 ± 1.4^{a}	17.7 ± 0.6^{b}	37.7 ± 0.6^{d}
FRC	8.4 ± 0.5^{a}	8.8 ± 0.3^{a}	8.7 ± 0.3^{a}	8.6 ± 0.3^{a}
CUPRAC	4205.4 ± 70.5^{b}	3761.6 ± 138.1^{a}	3822.8 ± 69.2^{a}	3893.2 ± 114.8^{a}
ABTS	17.7 ± 0.3^{b}	15.5 ± 0.6^{a}	17.0 ± 0.8^{ab}	18.3 ± 0.4^{b}

[a–d] Means in the same row without a common superscript letter differ significantly from left to right ($P < 0.05$).
MetMb = percentage metmyoglobin formation after storage at 4 °C for 7 days; CAT = catalase; SOD = superoxide dismutase; GPx = glutathione peroxidase; FRC = ferric ion reducing capacity; CUPRAC = cupric reducing antioxidant capacity; ABTS = ABTS.+ radical scavenging ability.

Table 2
Percentage metmyoglobin formation (mean ± standard error of the mean) in Longissimus thoracis (LT), Psoas major (PM), and Biceps femoris (BF) from yaks of 0.5, 1.5 and 2.5 years after storage at 4 °C for 7 days.

	Muscle		
Age group	LT	PM	BF
0.5 year	52.3 ± 2.4^{ax}	59.9 ± 2.2^{bx}	58.9 ± 1.6^{bx}
1.5 years	50.0 ± 1.5^{ax}	69.8 ± 3.1^{by}	56.6 ± 2.2^{ax}
2.5 years	47.7 ± 2.2^{ax}	66.5 ± 2.4^{cxy}	55.7 ± 3.6^{bx}
Effect	$P_{age} = 0.516$	$P_{muscle} < 0.05$	$P_{age \times muscle} = 0.057$

[a–c] Means in the same row without a common superscript letter differ significantly from left to right ($P < 0.05$).
[x–y] Means in the same column without a common superscript letter differ significantly from above to below ($P < 0.05$).
P_{age}, P_{muscle} and $P_{age \times muscle}$ mean the effect of age, muscle source and age × muscle source interaction on percentage metmyoglobin formation, respectively.

(2005) compared the color stability of 19 beef muscles and categorized longissimus as a muscle with high color stability, BF as low color stability, and PM as very low color stability, which was similar to our observation on the 1.5 and 2.5 year group. In addition, Joseph et al. (2012) reported that beef Longissimus lumborum steaks demonstrated greater color stability and redness than PM after 9 days of refrigerated storage, and these authors attributed the superior color stability of longissimus to the over-abundance of antioxidant proteins in sarcoplasm. Several other investigations also highlighted that beef Longissimus lumborum is a color-stable muscle, whereas PM is a color-labile muscle (Seyfert et al., 2006), which supported the findings in yak muscles. Our results on MetMb formation also indicated that yak LT is a color-stable muscle, whereas PM is color-labile.

3.2.2. Activity of antioxidant enzymes

The results of activity of antioxidant enzymes in LT, PM, and BF from yaks of 0.5, 1.5 and 2.5 years are presented in Table 3. There was an effect of age and muscle source in CAT and SOD ($P < 0.05$), but no effect of age and age × muscle source interaction ($P > 0.05$). On the other hand, in GPx, a two-way age × muscle source interaction ($P < 0.05$) was observed. The activities of CAT, SOD and GPx in LT exhibited a pronounced influence ($P < 0.05$) of age, whereas in PM such a trend was observed in CAT and SOD. On the other hand, in BF, only SOD was influenced by age (Table 3). In general, steaks from older animals exhibited greater levels of activities of the aforementioned enzymes. Muscle source influenced CAT, SOD, and GPX activities in yak steaks ($P < 0.05$; Table 3). For CAT, there was no difference between the three muscles in the 0.5 year age group, whereas in the 1.5 and 2.5 year groups BF exhibited lower values than LT and PM. In partial agreement, Pastsart et al. (2013) documented that the longissimus muscle of Belgian double-muscled cattle demonstrated greater CAT activity than the inner BF region after 48 h post-mortem. With respect to SOD, in all three age groups, yak LT demonstrated greater activity than PM and BF, while PM and BF had similar values. In agreement, Pastsart et al. (2013) also observed that after 10 days of retail display, beef longissimus exhibited greater SOD activity than the inner and outer BF regions. In the present study, for the 0.5 year age group yaks, LT demonstrated greater GPx values than BF and PM, whereas in the 2.5 year group there was no effect of muscle. Our results in the 0.5 year age yaks agreed partially with the previous report (Pastsart et al., 2013) on beef muscles that GPx activities were lower in the inner BF region than in the longissimus (Pastsart et al., 2013).

Table 3
Activity of antioxidant enzymes (mean ± standard error of the mean) in Longissimus thoracis (LT), Psoas major (PM), and Biceps femoris (BF) muscles of yaks at 0.5, 1.5 and 2.5 years of age.

Parameter	Age group	Muscle		
		LT	PM	BF
CAT	0.5 year	3.2 ± 0.3^{ax}	2.7 ± 0.4^{ax}	2.4 ± 0.3^{ax}
	1.5 years	4.0 ± 0.3^{bx}	3.6 ± 0.3^{abxy}	2.9 ± 0.2^{ax}
	2.5 years	4.6 ± 0.4^{by}	3.9 ± 0.5^{by}	2.8 ± 0.4^{ax}
	Effect	$P_{age} < 0.05$	$P_{muscle} < 0.05$	$P_{age \times muscle} = 0.664$
SOD	0.5 year	60.7 ± 2.6^{bx}	26.4 ± 2.4^{ax}	27.2 ± 2.4^{ay}
	1.5 years	61.8 ± 2.0^{bx}	25.0 ± 1.6^{ax}	20.1 ± 2.1^{ax}
	2.5 years	72.3 ± 2.7^{by}	44.0 ± 2.4^{ay}	38.1 ± 2.7^{az}
	Effect	$P_{age} < 0.05$	$P_{muscle} < 0.05$	$P_{age \times muscle} = 0.184$
GPx	0.5 year	24.6 ± 2.2^{bz}	16.9 ± 1.3^{ax}	18.4 ± 1.5^{ax}
	1.5 years	12.3 ± 1.4^{ax}	17.6 ± 1.9^{bx}	14.7 ± 1.6^{abx}
	2.5 years	17.7 ± 0.6^{ay}	17.0 ± 1.2^{ax}	16.4 ± 1.0^{ax}
	Effect	$P_{age} < 0.05$	$P_{muscle} = 0.354$	$P_{age \times muscle} < 0.05$

[a–b] Means in the same row without a common superscript letter differ significantly from left to right ($P < 0.05$).
[x–z] Means in the same column without a common superscript letter differ significantly from above to below ($P < 0.05$).
P_{age}, P_{muscle} and $P_{age \times muscle}$ mean the effect of age, muscle source and age × muscle source interaction on activity of antioxidant enzymes, respectively.
CAT = catalase; SOD = superoxide dismutase; GPx = glutathione peroxidase.

Table 4
Antioxidant capacity (mean ± standard error of the mean) in Longissimus thoracis (LT), Psoas major (PM), and Biceps femoris (BF) muscles of yaks at 0.5, 1.5 and 2.5 years of age.

Parameter	Age group	Muscle		
		LT	PM	BF
FRC	0.5 year	8.4 ± 0.5^{ax}	8.1 ± 0.5^{ax}	7.7 ± 0.5^{ax}
	1.5 years	8.8 ± 0.3^{ax}	8.3 ± 0.5^{ax}	8.0 ± 0.4^{ax}
	2.5 years	8.7 ± 0.3^{ax}	8.2 ± 0.5^{ax}	8.0 ± 0.3^{ax}
	Effect	$P_{age} = 0.686$	$P_{muscle} = 0.126$	$P_{age \times muscle} = 0.997$
CUPRAC	0.5 year	4205.4 ± 70.5^{ay}	4055.4 ± 88.5^{ay}	4147.2 ± 157.5^{ax}
	1.5 years	3761.6 ± 138.1^{ax}	3960.5 ± 162.6^{by}	4144.2 ± 129.5^{bx}
	2.5 years	3822.8 ± 69.2^{abx}	3590.2 ± 59.6^{ax}	3948.3 ± 86.9^{bx}
	Effect	$P_{age} < 0.05$	$P_{muscle} = 0.074$	$P_{age \times muscle} = 0.203$
ABTS	0.5 year	17.7 ± 0.3^{by}	12.6 ± 0.7^{ax}	12.3 ± 0.5^{ax}
	1.5 years	15.5 ± 0.6^{bx}	13.1 ± 0.7^{ax}	14.1 ± 0.6^{abxy}
	2.5 years	17.0 ± 0.8^{bxy}	12.9 ± 0.7^{ax}	15.7 ± 1.0^{by}
	Effect	$P_{age} = 0.114$	$P_{muscle} < 0.05$	$P_{age \times muscle} < 0.05$

[a–b] Means in the same row without a common superscript letter differ significantly from left to right ($P < 0.05$).
[x–y] Means in the same column without a common superscript letter differ significantly from above to below ($P < 0.05$).
P_{age}, P_{muscle} and $P_{age \times muscle}$ mean the effect of age, muscle source and age × muscle source interaction on antioxidant capacity, respectively.
FRC = ferric ion reducing capacity; CUPRAC = cupric reducing antioxidant capacity; ABTS = ABTS.+ radical scavenging ability.

The correlation between antioxidant enzyme activity and MetMb formation was analyzed to determine the possible contribution of antioxidant enzymes to oxidative stability. Only SOD was negatively correlated with MetMb ($r = -0.48$), while CAT ($r = -0.08$), and GPx ($r = 0.16$) did not exhibit any meaningful correlation. These findings suggested that SOD contributed to myoglobin redox stability and oxidative stability in yak muscles.

3.2.3. Antioxidant capacity

The results of antioxidant capacity in LT, PM, and BF from yaks of 0.5, 1.5 and 2.5 years are presented in Table 4. There was an effect of age in CUPRAC ($P < 0.05$), but no effect of muscle source and age × muscle source interaction ($P > 0.05$). On the other hand, in ABTS, a two-way age × muscle source interaction ($P < 0.05$) was observed. No muscle-dependent differences were observed for FRC capacity ($P > 0.05$). In contrast, in the 0.5 year age group, there was no effect ($P > 0.05$) of muscle source, whereas in the 1.5 and 2.5 year groups BF demonstrated the highest ($P < 0.05$) CUPRAC activity. With respect to ABTS, PM demonstrated the lowest values ($P < 0.05$) in the three age groups (0.5, 1.5, and 2.5 years), and LT exhibited the greatest ($P < 0.05$) values in the 0.5 year yaks.

The correlation between the antioxidant capacity and MetMb formation was analyzed to determine the possible contribution of antioxidant capacity to oxidative stability. Only ABTS was negatively correlated with MetMb ($r = -0.43$), while FRC ($r = -0.11$), and CUPRAC ($r = -0.03$) did not exhibit any meaningful correlation. These findings suggested that ABTS contributed to myoglobin redox stability and oxidative stability in yak muscles.

4. Conclusions

The findings of the present study indicated that muscle source and animal age critically influence myoglobin oxidation, antioxidant enzyme activity, and antioxidant capacity in fresh yak steaks. Furthermore, LT steaks from 3.5 year old yaks demonstrated the lowest MetMb formation and highest antioxidant enzyme activities, indicating the possible suitability of this age group for color stability of retail yak meat. These results indicated the necessity to develop muscle- and age-specific processing strategies to improve color stability and marketability of fresh yak steaks.

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