EFFECT OF MUSCLE AND RATE OF PH AND TEMPERATURE FALL ON ANTIOXIDANT ENZYME ACTIVITIES IN BEEF

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***Abstract*— Beef from double-muscled Belgian Blue cattle is characterized by fast glycolysis and slow temperature fall postmortem (pm), which may induce heat shortening and affect meat quality. Particularly the colour and colour stability is affected in deeper muscles. The aim of this study was to investigate the effect of different muscle types; inner *biceps femoris* (IBF), outer *biceps femoris* (OBF) and *longissimus* (LD), and the rate of pH and temperature fall pm on the activities of superoxide dismutase (SOD), catalase (Cat) and glutathione peroxidase (GSH-Px) as the major antioxidant enzymes in muscle. Fourty young bulls were sampled for this purpose. The results showed that the activities of Cat and GSH-Px in the IBF were lower than in the OBF and LD, while the SOD activity was lower in the IBF and LD compared to the OBF ($P<0.01$). The activities of GSH-Px and Cat had a negative correlation with the temperature at 5 hours pm, the duration of heat shortening (pH <6 and temperature >35°C), the L^* value at day 0 ($P<0.01$) and the metmyoglobin formation ($P<0.05$), whereas lipid oxidation (TBARS) was negatively correlated with Cat activity ($P<0.01$).**

***Index Terms*— Beef, *biceps femoris*, *longissimus*, Superoxide dismutase, Catalase, Glutathione peroxidase**

I. INTRODUCTION

Double-muscled Belgian blue cattle have a more glycolytic muscle fiber type and heavier carcasses compared to conventional animals resulting in faster pH decline and slower temperature fall postmortem compared to carcasses of non double-muscled animals (Clinquart et al., 1998; De Smet, 2004). Particularly in the deeper laying muscles of the hindquarter, this may induce heat shortening and increase protein denaturation, resulting in aberrant meat quality (Sammel et al., 2002; De Boever et al., 2009). Protein denaturation may also affect the postmortem activity of endogenous enzymes.

Oxidative deterioration in meat leads to loss of nutritional value and reduced sensory quality. In muscle, there are several mechanisms to protect oxidative processes including the endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (Cat) and glutathione peroxidase (GSH-Px). Cat and GSH-Px are considered to be hydrogen peroxide and fatty acid hydroperoxides decomposing enzymes in the cytosol, while SOD plays an important role in protecting against damage by superoxide anion radicals. The contribution of the postmortem activity of these enzymes in retarding the oxidative deterioration of muscle post mortem is not well established. However, it may be argued that a reduced activity of these enzymes negatively affects postmortem muscle oxidative stability.

The objective of this work was to investigate the effect of muscle type; outer *biceps femoris* (OBF), inner *biceps femoris* (IBF) and *longissimus* (LD) on antioxidant enzyme activities (GSH-Px, SOD and Cat) in relation to the rate of pH and temperature fall and quality parameters such as color, lipid oxidation, metmyoglobin formation and protein denaturation in double-muscled Belgian blue beef.

II. MATERIALS AND METHODS

A. Animals and meat samples

A total of 40 double-muscled Belgian Blue young bulls with mean age at slaughter and mean carcass weight of 21.6 ± 2.7 months and 502.4 ± 42.2 kg respectively were used. At 48 hours pm, the *longissimus* (LD), outer *biceps femoris* (OBF) and inner *biceps femoris* (IBF) were sampled, vacuum packed and frozen at -20°C until enzyme activity determination.

For the simulated retail display, samples of the LD, IBF and OBF were taken at 48 hours pm, wrapped in oxygen permeable foil and displayed at 4°C under fluorescent light (approximately 1200 lux).

B. pH and temperature measurements

The temperature and pH were measured at 1, 3 and 5 hours pm in the LD muscle at 8 cm depth and in the IBF at 10 cm. Conditions for heat shortening were defined as pH <6.0 and temperature >35°C (Thompson, 2002).

C. Colour measurements and other analyses

Beef colour L* value and a* value was determined with a Hunterlab Miniscan color meter (D65 light source, 10° standard observer, 45°/0° geometry, 1-inch light surface, white standard) and the metmyoglobin formation (%MetMb) was determined using reflectance values at specific wavelengths (AMSA, 1991) at 0, 1, 7 and 10 days of display.

In addition, lipid oxidation and protein denaturation were determined on samples at 0 and 10 days of display. Lipid oxidation was assessed spectrophotometrically by the thiobarbituric acid reactive substances (TBARS) method based on Tarladgis et al. (1960) and is expressed as µg malondialdehyde (MDA) / g meat. Protein denaturation was evaluated by measuring the protein solubility in a low ionic strength solution (mg protein/g meat). More protein denaturation is assumed to result in a lower protein solubility (Claeys et al., 2002).

D. Antioxidant enzyme activity assays

After thawing, the muscle samples were kept on ice during the procedure. A 5 g muscle sample was homogenized in 10 ml of 0.05 M phosphate buffer (pH 7.0) and centrifuged at 4^{o}C for 20 min at 7000g. The supernatant fraction was filtered through glass wool before determining superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (Cat) activities.

The SOD activity assay was performed as described by Marklund and Marklund (1974) by measuring the inhibition of pyrogallol autoxidation. A unit of enzyme activity was defined as the amount of sample needed to inhibit the reaction by 50%. The activity of GSH-Px was determined by measuring the oxidation of NADPH. One unit of GSH-Px activity was defined as the amount of extract required to oxidize 1 µmol of NADPH per min at 25 ^{o}C (Hernandez et al., 2004).

The Cat activity was determined according to the method of Aebi (1983). One unit of Cat activity was defined as the amount of sample required to decompose 1 µmol of H_2O_2 per min at room temperature.

E. Statistical analysis

The experimental data were analyzed with the analysis of variance (ANOVA) procedure of SAS for windows, followed by DUNCAN multiple comparison means tests in case of significance. Pearson correlations were used for testing the correlations between antioxidant enzyme activities and other parameters.

III. RESULTS AND DISCUSSION

Table 1 Comparison of pH, temperature, heat shortening time, color L* and a* values, thiobarbituric acid reactive substances (TBARS), %metmyoglobin (MetMb), and protein denaturation (mean ±SE) in inner *biceps femoris* (IBF), outer *biceps femoris* (OBF) and *longissimus* (LD) of young double-muscled Belgian Blue bulls (n=40)

	IBF	OBF	LD	P
pH 1h pm	6.61 ± 0.03^{a}		6.47 ± 0.04^{b}	*
pH 3h pm	5.64 ± 0.04		5.69 ± 0.04	
pH 5h pm	5.42 ± 0.01^{b}		5.55 ± 0.02^{a}	**
Temperature 1h pm	39.88 ± 0.10		39.87 ± 0.09	
Tempeature 3h pm	39.35 ± 0.17^{a}		36.00 ± 0.23^{b}	**
Temperature 5h pm	36.70 ± 0.14^{a}		30.12 ± 0.27^{b}	**
Heat shortening (hours)	3.88 ± 0.18^{a}		1.20 ± 0.13^{b}	**
L* d0 pm	49.92 ± 0.48^{a}	37.43 ± 0.40^{c}	39.22 ± 0.63^{b}	**
a* d0 pm	20.40 ± 0.39^{b}	21.29 ± 0.29^{ab}	21.80 ± 0.27^{a}	**
Δa* (d1-d7; /day)	-1.32 ± 0.04^{c}	-0.56 ± 0.06^{a}	-0.76 ± 0.06^{b}	**
TBARS d0 pm	0.35 ± 0.02^{a}	0.32 ± 0.01^{a}	0.23 ± 0.01^{b}	**
TBARS d10 pm	3.29 ± 0.23^{a}	2.05 ± 0.18^{b}	0.99 ± 0.13^{c}	**
%MetMb d0 pm	22.91 ± 0.57^{a}	15.26 ± 0.35^{b}	16.09 ± 0.54^{b}	**
%MetMb d10 pm	60.74 ± 1.38^{a}	40.86 ± 2.35^{c}	48.40 ± 2.41^{b}	**
Δ%MetMb (d1-d7; /day)	3.26 ± 0.16^{a}	0.92 ± 0.07^{b}	1.22 ± 0.13^{b}	**
Protein solubility d0 pm	57.85 ± 0.55^{c}	76.95 ± 0.79^{a}	72.84 ± 1.38^{b}	**
Protein solubility d10 pm	51.06 ± 0.56^{c}	67.80 ± 0.73^{a}	61.27 ± 0.95^{b}	**
Δ protein solubility (d0-d10)	6.79 ± 0.51^{c}	9.15± 0.47^{b}	11.66 ± 0.89^{a}	**

$^{a, b, c}$ Within a row, mean values with different superscripts differ significantly at P<0.05; * = P< 0.05, ** = P<0.01
TBARS: mg/kg meat; protein solubility: mg protein/g meat

The mean values according to the muscle types are shown in Table 1. Compared with the LD muscle, the IBF muscle had a lower pH at 5 hours pm and a higher temperature at 3 and 5 hours pm (P<0.01). Furthermore, IBF had a longer heat shortening time than LD. The average L^* value of the IBF at day 0 was significantly higher than in the LD and OBF (P<0.01), which illustrates the paler color in the deeper muscles and the resultant two-toning in the BF muscle.

The IBF also decreased more in red colour (Δ a* value) during the first 7 days of display (P<0.01) and there was a higher formation of metmyoglobin, which indicates a reduced colour stability. TBARS values at day 0 were higher in the IBF and OBF compared to the LD muscle, while the value at day 10 of display was higher in the IBF than the OBF and LD (P<0.01).

The protein soubility was lower in the IBF < LD < OBF (P<0.01) suggesting more protein denaturation in the IBF. This might result from the longer duration of heat shortening.

Antioxidant enzyme activities according to muscle type are shown in Table 2. The IBF and LD had a lower activity of Cat and GSH-Px (P<0.01) than the OBF. The OBF had a higher SOD activity (P<0.01) than IBF and LD muscles. The lower antioxidant enzyme activities in the IBF might result from increased denaturation induced during the longer heat shortening time. The activities of the three enzymes were not or very weakly interrelated. Only the SOD activity had a weak negative correlation with the Cat activity (r = -0.21; P<0.05).

Table 2 Activities (mean ± SE) of superoxide dismutase (SOD), catalase (Cat) and glutathione peroxidase (GSH-Px) in inner *biceps femoris* (IBF), outer *biceps femoris* (OBF) and *longissimus* (LD) of young double-muscled Belgian Blue bulls (n=40)

	IBF	OBF	LD	P
GSH-Px (U g-1)	1.33 ± 0.04^{b}	1.51 ± 0.05^{a}	1.57 ± 0.05^{a}	**
Cat (U g-1)	1650 ± 64.03^{b}	2203 ± 84.69^{a}	2065 ± 71.03^{a}	**
SOD (U g-1)	137.20 ± 4.30^{b}	152.80 ± 3.51^{a}	132.58 ± 3.82^{b}	**

[a,b] Within a row, mean values with different superscripts differ significantly at P<0.05; ** = P<0.01

Correlation coefficients between the antioxidant enzyme activities and other parameters are shown in Table 3. Cat activity was negatively correlated with temperature at 3 and 5 hours pm, heat shortening duration, L^* value, TBARS, rate of %MetMb formation (P<0.01) and positively correlated with pH 5 h, protein denaturation (P<0.01), a* value and the decrease in a* value during 7 days of display (P<0.05) with correlation coefficients in the range of |0.2| to |0.5|.

The GHS-Px activity had a negative correlation with temperature at 5 hours pm, heat shortening, L* value, TBARS after 10 days of display, Δ%MetMb between day 1 and day 7 display (P<0.01) with correlation coefficients in the range of |0.25| to |0.30|. The SOD activity was not related to any of the meat quality parameters.

Table 3 Correlation coefficients between the antioxidant enzyme activities and other meat quality characteristics across *biceps femoris* and *longissimus* beef samples (n= 69 – 80)

	SOD activity	GSH-Px activity	Cat activity
pH 1h pm	NS	NS	NS
pH 3h pm	NS	NS	NS
pH 5h pm	NS	NS	0.29^{**}
Temperature 1h pm	NS	NS	NS
Tempeature 3h pm	NS	NS	-0.31^{**}
Temperature 5h pm	NS	-0.28^{**}	-0.37^{**}
Heat shortening (hours)	NS	-0.30^{**}	-0.41^{**}
L* d0 pm	NS	-0.37^{**}	-0.50^{*}
a* d0 pm	NS	NS	0.24^{*}
Δa* (d1-d7; /day)	NS	0.34^{**}	0.26^{*}
TBARS d0 pm	NS	NS	-0.33^{**}
TBARS d10 pm	NS	-0.42^{**}	-0.28^{**}
%MetMb d0 pm	NS	-0.25^{*}	-0.24^{*}
%MetMb d10 pm	NS	NS	NS
Δ%MetMb (d1-d7; /day)	NS	-0.28^{**}	-0.34^{**}
Protein solubility d0 pm	NS	0.29^{**}	0.37^{**}
Protein solubility d10 pm	NS	0.24^{*}	0.44^{**}
Δ protein solubility (d0-d10)	NS	0.23^{*}	NS

*, ** Correlation is significant at the 0.05 and 0.01 level, respectively.

Some of the significant correlation coefficients might only reflect the muscle differences described in Table 1. However, some other correlations also seemed to appear within muscles. Overall, the data suggest that differences in temperature and pH fall between and within muscles result in increased protein denaturation that subsequently affects postmortem enzyme activity. The consequence of these events for the oxidative stability of meat needs further investigation.

IV. CONCLUSION

The activities of SOD, Cat and GSH-Px were lower and lipid oxidation and formation of metmyoglobin was higher in the inner part of the *biceps femoris* compared to the outer part of the *biceps femoris* and the *longissimus* muscle, associated with an increased degree of heat shortening and protein denaturation. Whether the reduced enzyme activities are contributing to the reduced oxidative stability remains to be determined.

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