Effect of Hot Boning on Colour Stability and Antioxidant Enzyme Activities in Beef Inner and Outer *Biceps femoris*

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Abstract— **Meat from double-muscled Belgian Blue cattle is characterized by a fast pH decline and slow temperature fall post mortem (pm), which may provoke heat shortening (pH<6 and temperature>35°C) and negatively affect meat quality, particularly in deeper muscles of the hindquarter. The aim of this study was to investigate the effect of hot boning (within 2 hours pm) on colour and colour stability and on antioxidant enzyme activities (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px)) in inner and outer *Biceps femoris* (IBF and OBF) from 7 Belgian Blue young bulls (mean age and carcass weight 22.7 months and 496 kg respectively). The mean duration of heat shortening was 3.4 hours for the cold-boned IBF, whereas there was no period of heat shortening in the hot-boned IBF and in the cold- and hot-boned OBF. The colour *L**-value was higher in the cold-boned IBF than OBF (49.3 and 37.6 respectively; P<0.05) resulting in two-toning, whereas there was no significant difference between hot-boned IBF and OBF (33.8 and 34.3 respectively). Concomitantly, the decrease in *a**-value and increase in %metmyoglobin over a 7 day display period was higher (P<0.05) for the cold-boned IBF compared to the hot-boned IBF and the cold- and hot-boned OBF. The activities of CAT, GSH-Px and SOD for the IBF and OBF were not significantly different between hot- and cold-boned samples. Hot boning of *Biceps femoris* in beef carcasses has distinct advantages over cold boning, especially for avoiding heat shortening in the inner part of the muscle and for increasing the colour uniformity in the muscle.**

Keywords— **Hot boning, Antioxidant enzymes, Colour stability.**

I. INTRODUCTION

The muscle tissue and meat quality of double-muscled Belgian Blue cattle differs in many aspects from meat of conventional breeds (De Smet, 2004). The more glycolytic fiber type in combination with the larger muscle mass of these animals results in faster glycolysis and leads to a faster pH fall and slower cooling post mortem (pm) compared to carcasses of non double-muscled animals. Particularly in the deeper muscles of the hindquarter, this may induce heat shortening (pH < 6 and temperature > 35 °C) and the occurrence of a pale and two-toned meat colour. In a previous study, we found that heat shortening was at least partly responsible for two-toning in the *Biceps femoris* (BF) muscle of Belgian Blue beef (De Smet et al., 2008). In work of Sammel et al. (2002), partial hot boning improved the colour stability of beef *Semimembranosus* muscle. We therefore hypothesised that complete hot boning of hindquarter muscles in Belgian Blue beef carcasses might avoid heat shortening through a faster cooling rate, thereby resolving the meat colour defects.

During storage, meat is also subject to oxidative deterioration. *In vivo*, there are several mechanisms to protect muscle against oxidative stress including the role of the endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). The contribution of these enzymes in retarding the oxidative deterioration of muscle pm is not well established. Protein denaturation resulting from severe heat shortening may also affect the activity of these endogenous enzymes. Hence, it may be argued that heat shortening conditions result in a reduced activity of these enzymes and thereby negatively affect post-mortem muscle oxidative stability.

The objective of this study was to investigate the influence of hot boning on antioxidant enzyme activities (GSH-Px, SOD and CAT) of beef inner and outer *Biceps femoris* compared to conventional cooling and cold boning. Additionally, the influence of hot boning on the colour and colour stability of this muscle was evaluated.

II. MATERIALS AND METHODS

A. Animal

Seven double-muscled Belgian Blue young bulls with mean age at slaughter and mean carcass weight of 22.7 ± 2.5 months and 495.9 ± 56.3 kg, respectively, were used. The animals originated from commercial farms in Belgium and were slaughtered in two different abattoirs.

B. Hot boning and meat samples

Hot boning was performed on the 7 carcasses within 2 hours after slaughter. The BF muscle of one carcass side was completely cut out to allow faster cooling of the muscle, whereas the other carcass side was left intact and served for cold boning. The hot boned BF muscle was vacuum packed and stored in the dark at 12°C during the first 5 h pm and thereafter at 4°C until 48 h pm. Cold boning was also performed at 48 h pm. At this time, the outer BF (OBF) and inner BF (IBF) were sampled, vacuum packed and frozen at -20°C until enzyme activity determinations. For the simulated retail display, additional samples were wrapped in oxygen permeable foil and immediately displayed at 4°C under fluorescent light (approximately 1200 lux).

C. Temperature and pH measurements

Temperature and pH were measured at 1, 3, 5 and 48 hour pm in the IBF at 10 cm depth and the OBF at 2 cm depth. Conditions for heat shortening were defined as pH < 6.0 and temperature > 35°C (Thompson, 2002).

D. Colour measurements

Colour parameters were measured with a Hunterlab Miniscan colour meter (D65 light source, 10° standard observer, 45°/0° geometry, 1-inch light surface, white standard) at 0, 1, 2, 3, 4, 5, 6, 7 and 10 days of display to assess colour stability (AMSA, 1991). CIE *L**-values (lightness), *a**-values (redness), and spectral reflectance (400-700 nm) data were collected. Spectral data were used to calculate % metmyoglobin (%MetMb) values, as described by Krzywicki (1979). The colour stability of the meat was evaluated from the slope of the linear regression describing the decline in *a** value and the increase in %MetMb during the display from day 1 until day 7, expressed respectively as Δ*a** and Δ%MetMb. Two-toning in BF, expressed as the difference between initial *L**(d0) IBF and *L**(d0) OBF, was also used as an index for colour acceptance.

E. 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°C for 20 min at 7000g. The supernatant fraction was filtered through glass wool before determining enzyme 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°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.

F. Statistical analysis

The data were analyzed by a 2-way analysis of variance procedure to test for the effect of boning method (cold-boned *vs* hot-boned) and muscle (IBF *vs* OBF) by using the SAS software, followed by DUNCAN multiple comparison means tests in case of significance.

III. RESULTS

There was no significant difference in average pH at 1 h pm between IBF and OBF for both the hot- and cold-boned treatments. At 5 h pm, the pH in cold-boned IBF was lower than cold-boned OBF and hot-boned IBF ($P<0.05$), but there was no significant difference between hot-boned IBF and OBF.

Conversely, the temperature at 3 and 5 h pm was lower in the IBF than OBF when cold boned (P<0.05), whereas there was no significant difference between hot-boned IBF and OBF. The average duration of heat shortening was estimated at 3.4 h for the cold-boned IBF muscle, whereas no heat shortening occurred in the other treatments (Table 1).

Table 1 Temperature (T), pH, heat shortening (HS) time and colour stability parameters for cold-boned and hot-boned inner and outer *Biceps femoris* (IBF and OBF) muscle (mean ± SD, n=7)

Trait	Muscle	Cold-Boned	Hot-Boned
pH (5h)	IBF	$5.41 \pm 0.06^{b,z}$	6.10 ± 0.16^{a}
	OBF	$6.59 \pm 0.29^{a,y}$	6.29 ± 0.37^{b}
T (5h)	IBF	$35.99 \pm 0.80^{a,y}$	12.67 ± 2.35^{b}
	OBF	$21.00 \pm 2.29^{a,z}$	12.46 ± 1.74^{b}
HS (h)	IBF	$3.41 \pm 0.73^{a,y}$	0.00 ± 0.00^{b}
	OBF	0.00 ± 0.00^{z}	0.00 ± 0.00
Δ *a** (/d)	IBF	$-1.63 \pm 0.38^{b,z}$	-0.89 ± 0.53^{a}
	OBF	-0.82 ± 0.72^{y}	-0.93 ± 0.53
Δ%MetMb	IBF	4.41 ± 2.18^{y}	2.79 ± 1.65
(/d)	OBF	2.06 ± 2.17^{z}	2.65 ± 1.33
2-toning	BF	11.66 ± 3.25^{a}	0.40 ± 0.48^{b}

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

[y, z] Within a column and variable, mean values with different superscripts differ significantly at P<0.05

The average lightness (*L**) value at d 0 of display was higher (P<0.05) for the cold-boned IBF (49.3) compared to the cold-boned OBF and hot-boned IBF and OBF which did not differ (37.6, 33.8 and 34.3 respectively) (Fig. 1). This pattern maintained throughout the display period. The average initial redness (*a**) value was higher (P<0.05) for the cold-boned IBF than for the other treatments. On the opposite, the decline in *a** value during the 10 d of display was larger for the cold-boned IBF compared to the other treatments (Fig. 2). After 10 d of display, the average *a** value was lower (P<0.05) for the cold-boned IBF than for the other treatments. Concomitantly, a larger increase in %MetMb was observed in the cold-boned IBF during display compared to the other treatments, indicating the unstable colour in the cold-boned IBF muscle (Table 1).

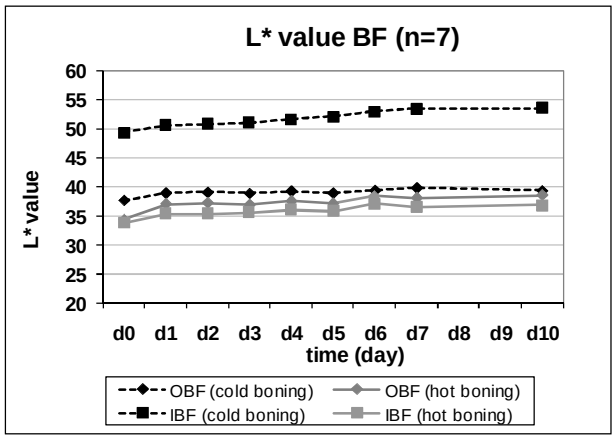

Fig. 1 The change in mean lightness (L*) value for inner and outer *Biceps femoris* (IBF and OBF) muscle

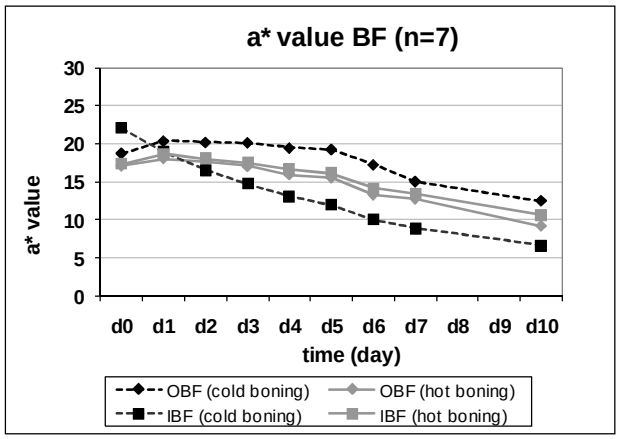

Fig. 2 The change in mean redness (a*) value for inner and outer *Biceps femoris* (IBF and OBF) muscle

The activities of the antioxidant enzymes at d 0 of display are shown in Table 2. The SOD, CAT and GSH-Px activities were not affected by the boning method. On the other hand, the CAT and GSH-Px activities were non-significantly higher for the OBF compared to the IBF, whereas the SOD activity was slightly lower in the OBF compared to the IBF regardless of boning method.

Table 2 Antioxidant enzyme activities (U/g) at 48 h pm (corresponding to d 0 of display) for cold and hot-boned inner and outer *Biceps femoris* (IBF and OBF) muscle (mean ± SD, n=7)

	Muscle	Cold-boned	Hot-boned
CAT	IBF	93.9 ± 25.2	93.0 ± 21.4
	OBF	107.2 ± 43.4	104.4 ± 32.9
GSH-Px	IBF	1.20 ± 0.55	1.21 ± 0.45
	OBF	1.36 ± 0.57	1.30 ± 0.54
SOD	IBF	70.6 ± 8.6	71.0 ± 7.4
	OBF	62.7 ± 9.3	66.4 ± 8.5

IV. DISCUSSION

The faster pH fall and slower temperature decline in the cold-boned IBF induced heat shortening, which resulted in a paler colour compared to the OBF and thus in an unattractive two-toning appearance of this muscle. To tackle this problem, hot boning was applied which allowed a faster cooling. The results showed that the temperature and the pH decline was comparable in the hot-boned IBF and OBF. As a consequence, there was no period of heat shortening in the IBF and OBF muscle when hot boned. Moreover, the large difference in lightness between the IBF and OBF muscle following cold boning was significantly reduced after hot boning and thus also the problem of two-toning. Concomitantly, the colour stability of the IBF muscle was improved when hot boned, as was apparent from the lower decrease in redness and the lower increase in %MetMb during display. Avoiding heat shortening through hot boning does not only reduce the pale meat appearance, it also improves the colour stability. The common underlying mechanism might be a reduced protein denaturation.

The SOD, CAT and GSH-Px activities were remarkably similar for the hot- and cold-boned samples. Although there was a tendency for differences between IBF and OBF, it might thus be concluded that the boning method has no effect on the activities of these three endogenous antioxidant enzymes.

V. CONCLUSION

The fast pH fall, slow temperature decline and the severe heat shortening in the IBF muscle from double-muscled Belgian Blue bulls under normal cooling practices, resulted in a pale colour and two-toning in the BF. Hot boning of the BF muscle can be seen as an appropriate solution for faster chilling and tackling problems with colour stability. Hot boning did not affect the activities of CAT, SOD, and GSH-Px.

REFERENCES

Aebi H E (1983) Catalase, pp. 273-286. *In* Bergmeyer H U, ed. Methods of enzymatic analysis III. Verlag Chemie, Weinheim

AMSA (1991) Guidelines for meat color evaluation. Reciprocal Meat Conference Proc. 44:1-17

De Smet S (2004) Double-muscled cattle. *In* Jensen W K, Devine C, Dikeman M, eds. Encyclopedia of Meat Sciences. Elsevier, Oxford

De Smet S, Lescouhier S, Claeys E (2008) Two-toned colour in biceps femoris muscle in relation to post mortem pH and temperature fall in Belgian Blue beef, ICoMST Proc., 54th International Congress of Meat Science and Technology , Cape Town, South Africa, 2008, Short paper, Session 6

Hernandez P, Zomenno L, Arinno B, Blasco A (2004) Antioxidant, lipolytic and proteolytic enzyme activities in pork meat from different genotypes. Meat Sci 66:525-529

Krzywicki K (1979) Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. Meat Sci 3:1-10

Marklund S, Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47:469-474

Sammel L, Hunt M, Kropf M. C et al. (2002). Influence of chemical characteristics of beef inside and outside semimembranosus. J Food Sci 67:1323-1330

Thompson J (2002). Managing meat tenderness. Meat Sci 62:295-308