MEAT COLOUR STABILITY AS INFLUENCED BY GENOTYPE, POST SLAUGHTER TREATMENT, AND AGEING/PACKAGING COMBINATION

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Abstract – This paper describes the role of metmyoglobin reductase activity in the colour stability of steaks sampled at the *M. longissimus lumborum* in 5 different beef breeds, *Bos indicus* (Brahman), Sanga type (Nguni), British *Bos taurus* (Angus), European *Bos taurus* (Charolais) and the composite (Bonsmara), 10 animals per genotype, n=50. Carcasses were split and the right sides were electrically stimulated and left sides not stimulated. Steaks were aged till 3 and 9 days *post mortem* in cling-wrapped polystyrene trays, or in vacuum bags till 14 and 20 days *post mortem*. Metmyoglobin reductase activity, myoglobin redox derivatives (oxymyoglobin, metmyoglobin and deoxymyoglobin) and meat colour CIE (L*, a*, b*, hue angle and Chroma) were evaluated. Breed and ageing/packaging influenced metmyoglobin reductase activity; post slaughter treatment only had an effect (P<0.05) on metmyoglobin reductase at 3 days *post mortem*. Ageing showed differences (P<0.05) for oxymyoglobin, metmyoglobin and deoxymyoglobin. Differences (P<0.05) were observed between breeds for L* and hue angle. Packaging method had an effect on colour stability.

Key Words – Myoglobin redox derivatives, Metmyoglobin reductase.

- INTRODUCTION

The accumulation of metmyoglobin (Metmb) on the surface of meat leads to meat colour deterioration, this is a concern for producers as it results in consumer discrimination against the product [1]. The enzyme metmyoglobin reductase (MRA) helps in the reduction of Metmb back to myoglobin (Mb). The activity of this enzyme is an important factor to examine in studies on colour stability [2]. The more MRA muscle retains/maintains/preserves, the better its colour stability [3]. Metmb accumulation is affected by factors such as temperature, pH, Metmb reducing systems, lipid oxidation and oxygen consumption rate [4]. The accumulation of Metmb on the meat surface is influenced by the enzymatic ability of MRA to reduce Metmb to Mb. Reduction of Metmb by MRA depends largely on the presence of NADH [3]. Controversy in the research community exist on the role that MRA activity play in determining colour stability in meat [3,4,5,6,7]. The inconsistent results could be due to the type of assay used for MRA [3] determination.

The objective of this study was to evaluate the role of MRA in meat colour stability using five different beef breeds, two post-slaughter treatments and two packageing methods with two ageing periods each by using the suggested method of Sammel *et al*. [3] (reduction of DCPIP) to determine MRA, and compare it to Mb, Metmb and meat colour using instrumental colour measurements.

- MATERIALS AND METHODS

The beef breeds Brahman (Br) *(Bos indicus)*, Nguni (N) (Sanga type), Angus (A) (British *Bos Taurus)*, Charolais (C) (European *Bos Taurus)* and Bonsmara (Bo) (composite) were used, 10 steers per genotype. The animals were finished off on a feedlot diet for a period of between 90-110 days at the ARC-API feedlot and were slaughtered at the ARC-API abattoir when they reached a live weight which would produce a carcass of Class A (no permanent incisors), and

fat class 2 to 3 (1-≤5 mm) (South African Beef Classification System). After exsanguination the carcasses were halved. The right sides were electrically stimulated for 20 s (400 V peak, 5 ms pulses at 15 pulses/s) and entered the cold rooms (± 2 °C) within 60 min after slaughter (ES). The left sides were placed in a room with a controlled temperature of 10 °C for 6 hours, thereafter in cold rooms at ± 2 °C (NS). Four steaks were cut from the *M. longissimus lumborum* and two retail procedures were simulated for ageing of the sample steaks. Two steaks were aged in a display cabinet at 6 °C up to 3 or 9 days *post mortem* on polystyrene trays covered with polypropylene cling wrap (PP). The remaining two steaks were aged up to 14 or 20 d *post mortem* in vacuum bags at 1-4 °C in a cold room. The following analyses were determined on the steaks on 3, 9, 14 and 20 d *post mortem*.

MRA was determined by the method of Sammel [3] using the reduction of dichlorophenol-indophenol (DCPIP). A 2 g meat sample was homogenized in 10 ml of 0.2 mM sodium phosphate buffer (pH 5.6) using an ultra-turrax T25. The homogenate was centrifuged at 1500 x g for 30 min at 4°C. The supernatant was filtered using a filter paper. Reduction of dichloro-phenolindophenol (DCPIP) was measured by a change in absorbance at 600 nm. The DCPIP reagent consisted of 0.56 mg 2,6-DCPIP and 3.7 mg disodium EDTA in 20 ml of 50 mM Tris buffer (pH 8.1). To a plastic cuvette, 2.4 ml of DCPIP reagent was added, the reaction was initiated by simultaneously adding 200 µl NADH, and 400 µl of muscle extract and absorbance was measured at 600nm after 3 minutes at 37 °C. As DCPIP was reduced by the muscle extract, absorbance decreased. Reducing activity was calculated from the linear phase of the assay using Beer's law as nmoles reduced/min/g.

Relative concentrations of myoglobin derivatives were evaluated according to Krywicki [8] as described by Viriyarattanasak *et al*. [9].

Meat colour was measured with a Minolta meter (Model CR200, Osaka, Japan) on the samples after the appropriate ageing on 3, 9, 14 and 20 d *post mortem* after allowing 60 min of blooming at room temperature. The colours components L* (lightness), a* (green to red) and b* (blue to yellow) values [10] were recorded. Three recordings were performed on each steak and the average used in calculations. Chroma (intensity of the red colour) and hue angle (discolouration) were calculated [11].

The data were subjected to analysis of variance for a split plot design [12] with the six beef breeds (A, Bo, Br, C and N) as whole plots and the four ageing periods (3, 9, 14 and 20 d *post mortem*) and treatments (ES and NS) as sub-plots. Means for the interactions between sub-plot and whole-plot were separated using Fisher's protected t-test least significant difference (LSD) at the 5% level of probability [13].

RESULTS AND DISCUSSION

Breed and ageing/packageing effect ($P<0.05$) on MRA activity are represented in Figure 1. Post slaughter treatment had no effect on MRA.

Overall MRA at 9 d *post mortem* polystyrene packaging (longest exposure to oxygen) was higher than at 3 d *post mortem*. C had highest MRA at 3 d, but at 9 d *post mortem* C had the lowest MRA in contrast to the other breeds A, Bo, Br and N that had the highest MRA at that point. The availability of oxygen had a stimulating effect on the MRA in the A, Bo, Br and N breeds, but less so in the C breed. NS treatment at 3 d *post mortem* had higher MRA than in the ES steaks.

At 14 d *post mortem* vacuum packaging with less available oxygen showed overall lower MRA in the A, Bo, Br, and N breeds than in 9 d *post mortem* steaks, but higher than in 3 d *post mortem* steaks. On the other hand the MRA seems to be retained at 14 d *post mortem* in C being more active compared to the other breeds. A and Br seem to have an active MRA system

at 20 d *post mortem* with the other breeds, Bo, N and C having a decreased MRA, but still quite active and able to reduce available MetMb.

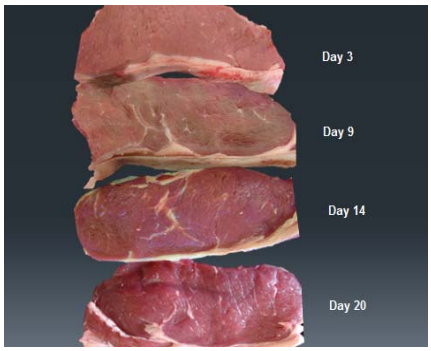

Figure 2: Visual example of how ageing and packaging affect meat colour.

Ageing had an effect on oxymyoglobin (OxyMb), metmyoglobin (MetMb) and deoxymyoglobin (DeoxyMb) (Table 1). At 3 d *post mortem* there were very high levels of OxyMb compared to the other ageing. The steaks packaged in cling-wrapped polystyrene trays had exposure to oxygen and light, advantageous to the formation of OxyMb at 3 d *post mortem*. MetMb levels were the highest at 9 d *post mortem*. The steaks were highly discoloured on the surface (Figure 2) due to oxidation deterioration of myoglobin. Behreds [2] reported that lighting affects the oxidation of myoglobin due to temperature increases on the meat surface. Vacuum packaged 14 d and 20 d *post mortem* steaks also had high levels of DeoxyMb due to a lack of exposure to oxygen. This is also reflected in the brighter colour of the steaks after blooming as more DeoxyMb was available to be oxigenated to OxyMb (Figure 2).

Although only L* and Hue angle were influenced ($P<0.05$) observed by breed (Table 2), ageing/packaging affected L*, a*, b*, Chroma and Hue angle (Tables 1). Steaks from C, Br and Bo had the highest hue angle (less discoloration) compared to A and N. C was the lightest, followed by the Br and A, then Bo and N having the darkest meat colour overall. The lower L*, a*, and Chroma and highest Hue angle measured in the 9 d *post mortem* ageing/packaging steaks corresponded with the discolouration visible obvious in this group, corresponding with higher MetMb levels and highest MRA detected.

According to Sammel *et al*. [3] all these parameters are related to colour stability.

Table 1 The effect of ageing on metmyoglobin reductase activity (MRA), oxymyoglobin (OxyMb), deoxymyoglobin (DeoxyMb) and metmyoglobin (MetMb) ($P<0.05$) and meat colour (L*, a*, b*, Chroma and hue angle)

	Ageing/packaging					
	3 days pm	9 days pm	14 days pm	20 days pm	SEM[1]	P-Value
MRA	8.44E-08^{a}	8.87E-08^{d}	8.69E-08^{c}	8.57E-08^{b}	1.05E-09	P<0.001
OxyMb	69.7^{d}	2.1^{b}	1.8^{a}	3.6^{c}	0.49	P<0.001
DeoxyMb	181.9^{d}	90.4^{a}	100.1^{b}	103.8^{c}	0.84	P<0.001
MetMb	1.84^{a}	15.8^{c}	2.2^{a}	0.6^{a}	0.59	P<0.001
L*	40.2^{c}	38.5^{a}	40.1^{b}	40.4^{d}	0.47	P<0.001
a*	11.8^{b}	8.3^{a}	13.7^{c}	13.5^{c}	0.58	P<0.001
b*	9.4^{c}	8.8^{b}	7.6^{a}	7.7^{a}	0.45	P<0.001
Chroma	15.1^{b}	12.2^{a}	15.7^{c}	15.6^{c}	0.42	P<0.001
Hue angle	38.6^{b}	46.8^{c}	28.7^{a}	29.1^{a}	1.04	P<0.001

[1] Standard error of means; pm = *post mortem*
[a,b,c,d] Means within a row with different superscripts differ significantly (P<0.05)

Table 2 The effect breed on lightness and hue angle

	Cattle breeds						
	Angus	Bonsmara	Brahman	Nguni	Charolais	SEM[1]	P-Value
Lightness (L*)	39.2[b]	38.9[b]	41.4[c]	36.9[a]	42.6[d]	0.52	P<0.001
Hue angle	34.3[a]	35.0[a]	38.0[b]	33.6[a]	38.1[b]	0.77	P<0.001

[1] Standard error of means
[a,b,c,d] Means within a row with different superscripts differ significantly (P<0.05)

O'Keefe & Hood [14] and Madhavi & Carpenter [3] found that MRA was highest in muscles that were colour stable. This was because greater MRA is believed to extend meat colour stability by increasing reduction of MetMb to Mb. In our study the opposite was true. Meat colour is less stable in packaging that allow access of oxygen to the meat surface and these samples seemed to have the highest MRA, except in the C breed. This characteristic could be genotype dependent and not necessarily an indication of colour stability, as indicated by King *et al.* [15].

- CONCLUSION

Although differences were observed for MRA, it is not a measure for colour stability. The enzyme is NADH-dependent and requires the presence of ferrocyanide for in vitro reduction of MetMb. Higher MRA measured do not necessarily mean effective reducing of MetMb to Mb. The study is ongoing, and more evidence will accumulate.

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