Metmyoglobin Reducing Activity and Colour Stability of Ovine *Longissimus* Muscle.

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Background:

The maintenance of a desired meat colour is of crucial importance for the marketability of meat as consumers discriminate against meat with off colour characteristics.[6, 7] The cause of the discolouration is a critical factor for the meat industry especially as there are financial losses due to discolouration.[17] Meat colour depends on the amount and state of myoglobin in meat. The bright red colour is the oxygenated form of myoglobin (Mb) while the tan-brown pigment is the oxidized form metmyoglobin (MetMb). The major factor leading to discolouration is the accumulation of MetMb at the meat surface during storage[9] . MetMb reducing enzymes can reduce MetMb to Mb which, in turn, can be oxygenated to bright red oxymyoglobin. MetMb reducing activities have been reported for muscles from a number of different species. However, the importance of MetMb reducing activity in the maintenance of muscle colour is still a matter of debate. A number of groups have found no or even a negative correlation between MetMb reductase activity and colour stability,[3, 8, 14, 15] while others[2, 10, 18] have reported that the enzyme activity may be the controlling factor retarding the accumulation of MetMb. Hence, the importance of reductase activity in maintaining the colour stability of meat.

Objectives: Since meat discolouration is of great economical importance, further investigation is needed to identify and characterise the metmyoglobin reducing enzyme systems which reduces MetMb to Mb. The MetMb reductase activities in bovine and porcine skeletal muscles have been investigated.[12, 13] However, to our knowledge, there is no data on the MetMb reductase in ovine skeletal muscle or its importance in the maintenance of meat colour. We decided to characterise MetMb reducing activity in ovine muscle. Furthermore, since the temperature at which muscles enter rigor can have a major effect on meat colour and colour stability, we also studied the effect of rigor temperature on MetMb reducing activity and colour stability.

Material and methods :

Characterisation of MetMb reducing activity in ovine muscle: Two lambs (Romney* Coopworth, 9 months old) were slaughtered at Lincoln University facilities and the longissimus muscles from both carcass sides were excised immediately after dressing. The muscles were knife-minced and extracted as described by Mikkelsen et al (1999). MetMb reducing activity was determined according to Mikkelsen et al (1999) using an Unicam UV4 spectrometer (Unicam Ltd , UK). The standard assay mixture contained 0.1 ml 5 mM EDTA; 0.1 ml 50 mM phosphate buffer pH 7.0; 0.1 ml 3.0 mM K_4 Fe $(CN)_6$; 0.1 ml water; 0.3 ml 0.77 mM Mb Fe(III) in 2.0 mM phosphate buffer pH7.0; 0.3 ml muscle extract and 0.25 ml 2.0 mM NADH. The assay mixture pH was 6.4 and the reaction was carried out at room temperature (25 ^{0}C). Blanks contained water instead of NADH. Activity is expressed as nanomoles of metmyoglobin reduced per min. per gram of meat and is the mean of three measurements.

Equine and ovine MetMb: Equine MetMb was prepared according to Mikkelsen et al.(1999) and ovine MetMb was prepared from heart muscle as described by Hagler et al.(1979) using the modification of Livingston et al.(1985). The MetMb preparation was split into two aliquots. One aliquot was heated for 10 min in a water bath of 50 ^{0}C. Precipitated material was pelleted by centrifugation and the supernatant collected. The resulting metmyoglobin preparations were stored(5 ml aliquots) at -100 ^{0}C until used.

Effect of rigor temperature: Fourteen lambs (Romney * Coopworth , 9 months old) were slaughtered at Lincoln University facilities, and longissimus muscles from the right and left carcess-side were excised immediately after dressing. Muscles were divided into anterior and posterior , vacuum packed and stored over night at 5, 10, 15, 20, 25, 30 or 35°C. For each treatment n=8 . The next day the samples were divided into two parts . Measurements were made on one part 1 day postmortem and on the other, 14 days postmortem after vacuum storage at 2°C. Muscle (3g) was homogenized in 10 volumes distilled water and the pH of the homogenate determined at 24 hr postmortem. Colour stability and MetMb activities were determined using equine MetMb.

Colour stability measurements: Steaks (20 mm thick) were cut from each muscle, placed in polystyrene trays, covered with oxygen permeable polyvinylchloride film and stored at 2 ^{0}C in an illuminated "fluorescent" display cabinet. Meat colour was determined using a Minolta Chromameter CR-210 (observer 2^0 , illuminant 65). The chromameter was calibrated with a white tile (L*=98.14 , a*=-0.23 and b*=1.89). Three replicate measurements were taken for each sample after blooming (3 hrs at 2°C) and then every 24 hrs storage at 2°C until the colour was visually unacceptable. Colour was described as L* (lightness), H (Hue angle) and C (Chroma) . The colour stability is expressed as the rate of change in colour parameters.

Statistical analysis: Data analysis was by Minitab version 11.

Results and Discussion: The MetMb reducing activity in the ovine LD muscle extract was indicated from the decrease in 505 and 630 nm peaks which characterise MetMb and the increase in 540 and 580 nm peaks which characterise oxymyoglobin [5]. In accordance with earlier reports on bovine and porcine MetMb reductase, [5, 12, 13] NADH is essential for the initiation of the reduction reaction (Table 1). EDTA was added to the reaction mixture to stabilize enzyme activity.[5] Omission of EDTA, however, resulted in only a slight decrease in activity. .Ferrocyanide was essential for the expression of full activity of ovine MetMb reducing activity (Table 1). In agreement with earlier reports on porcine and bovine MetMb, ovine MetMb was reduced non enzymatically by NADH (Table 1). However, no non-enzymatic reduction of equine metmyoglobin was observed (Table 1). To inactivate any possible MetMb reducing activity in the ovine MetMb preparation, the preparation was heated to 50°C[5]. Heating ovine metmyoglobin resulted in a slight increase in both non enzymatic reducing activity and total activity (Table 1).This may be due to a slight unfolding of MetMb which facilitates the enzyme attack on the heam group. Arihara et al. (1995) characterised the MetMb reducing activity in the microsomal fraction as NADH-cytochrome b5 reductase. Since NADH is essential for its reducing activity and our preparation contained the light microsomal fraction (35,000 x g supernatant), the reducing activity may represent NADH-cytochrome b5 reductase.

Table 2 shows the effect of rigor temperature on the ultimate pH, MetMb reductase activity and colour parameters. Ultimate pH was significantly affected by rigor temperature (p=0.002) which was also reported by Farouk and Swan(1998). Rigor temperatures up to 25°C did not affect MetMb reductase activity. The activity was negatively affected by temperatures above 30°C. In a separate experiment it was observed that 37°C incubations produced a complete loss of activity (data not shown). Rigor temperature, however,

did not have a significant effect on L- or H-values which changed very little during the display period. The increased L-value at 35°C may be due to its low water holding capacity of the meat which was confirmed by the high drip losses (data not shown). It is known that nonbound moisture increase the reflectance of light from the meat surface and results in a lighter appearance [4,16]. Rigor temperature had a significant effect on C-values at 1 day post mortem but little effect at 14 days postmortem. The rate of change in chroma during display was also affected by rigor temperature. However, this effect may be a consequence of the high initial C-values at day 1. The high chroma, which persisted to some extent during the display period, is probably due to a decrease in the mitochondrial oxygen consumption rate (OCR)[13], which allows for a higher oxygen partial pressure. This would delay the formation of metmyoglobin [13]. Given the fact that a large decrease in MetMb reductase activity (35°C) did not have a negative effect on the C-values or clearly affect the change in C-values during display, the activity of this enzyme system is probably less important for colour and colour stability than other factors such as OCR.

Conclusion: The presence of MetMb reductase activity has been confirmed in ovine LD muscle. The activity requires NADH and is dependent on ferrocyanide. However, its activity does not appear to be the principal determinant of colour or colour stability of ovine longissimus dorsi.

Pertinent literature:

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Table 1. The metmyoglobin reducing activity of ovine LD under different assay conditions

EDTA	$K_4Fe(Cn)_6$	Ovine LD muscle extract	NADH	Ovine MbFe(III)	Equine MbFe(III)	Activity (nanomole min^{-1} g^{-1})
+	+	+	+	+	-	423.78 ± 5.49
+	+	+	+	+	-	572.06 ± 9.77 *
-	+	+	+	+	-	385.87 ± 5.51
+	-	+	+	+	-	70.68 ± 0 .57
+	+	-	+	+	-	201.54 ± 3.24
+	+	-	+	+	-	222.68 ± 4.58 *
+	+	+	-	+	-	0
+	+	+	+	-	+	250.48 ± 10.07
+	+	-	+	+	+	0

*** Heated ovine metmyoglobin ($50C^0$ for 10 min) .**

Table (2) . Effect of rigor temperature on metmyoglobin reductase activity and colour stability.

Item			Colour parameters of LD muscles 24 hrs postmortem						Colour parameters of LD muscles vacuum packed for 2 weeks					
Temp.	pH	Met.Mb reductase (nanomole $min^{-1}g^{-1}$)	$L_{day\ 1}$	$L_{day\ 10}$	C_{Day1}	λ_{24hr}	H_{Day1}	H_{Day10}	$L_{day\ 1}$	$L_{day\ 7}$	C_{Day1}	λ_{2weeks}	H_{Day1}	H_{Day7}
5	5.86^a	196.3^a	50.4^a	48.8^a	16.1^a	-0.36^a	21.5^{ab}	20.8	51.0^{ab}	48.6	19.6^a	-1.13	17.8^{ab}	20.3
10	5.75^{ab}	198.5^a	50.5^a	49.3^{ab}	16.2^a	-0.39^a	21.4^a	21.9	51.0^b	48.2	21.0^{ab}	-1.22	17.1^a	18.6
15	5.64^b	172.9^a	50.9^a	50.0^{ab}	16.5^a	-0.47^{ab}	21.5^a	22.4	51.1^b	48.2	21.6^{ab}	-1.30	17.3^{ab}	18.9
20	5.68^b	196.5^a	51.1^a	50.0^{ab}	16.6^a	-0.59^{bd}	21.5^a	23.0	51.2^b	48.7	22.0^b	-0.78	17.3^{ab}	21.1
25	5.74^{ab}	193.2^a	50.3^a	49.3^{ab}	18.1^{ab}	-0.68^{dc}	19.9^b	21.0	51.2^{ab}	48.6	21.7^{ab}	-1.25	17.2^a	19.8
30	5.79^{ab}	167.9^a	50.8^a	49.3^{ab}	19.9^{bc}	-0.84^c	19.7^b	21.6	51.2^{ab}	48.4	21.9^b	-1.25	17.2^{ab}	19.7
35	5.89^a	83.6^b	52.7^b	50.9^b	21.9^c	-0.64^{bdc}	20.6^{ab}	22.4	53.0^a	50.2	22.1^b	-0.97	18.4^b	19.7
SEM	0.06	12.8	0.35	0.66	0.85	0.062	0.44	1.01	0.58	0.48	0.67	.056	0.34	1.13
Muscle														
anter.	5.83^a	176.8	50.6^a	49.2^a	17.7	-0.57	20.4^a	20.9^a	50.9^a	48.3	21.2	-1.20^a	17.1^a	20.7^a
Poster.	5.70^b	166.6	51.4^b	50.1^b	18.1	-0.54	21.3^b	22.9^b	51.9^b	49.1	21.7	-1.05^b	17.8^b	18.7^b
SEM	0.03	9.95	0.22	0.35	0.56	0.045	0.27	0.53	0.31	0.27	0.38	0.11	0.20	0.59

λ = the rate of change in chroma (the slope of the fitted linear trend)

***a, b, c, = Means in same column with different superscript are significantly different at $p \leq 0.05$**