METMYOGLOBIN REDUCING ACTIVITY IN VEAL AND VENISON MEATS

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Background

The colour of fresh meat is an important quality attribute which influence the consumers purchase decision. The failure to have an attractive colour will affect the dynamics of meat sales and have financial consequences[1,2]. Meat discolouration is caused by the accumulation of metmyoglobin (MetMb) during aerobic retail display[3]. The existence of enzymatic system/s capable of reducing MetMb has been reported[4]. Such system/s can reduce MetMb to myoglobin (Mb), hence potentially decrease the degree of discolouration and increase shelf life. While there is agreement on the presence of MetMb reducing systems in meat, their role in maintaining fresh meat colour is more controversial. Metmyoglobin reducing activities were measured and characterized in beef[5], pork[6] and lamb[7]. To our knowledge such information is not available for other economically important meats, depending on availability and cultural preference, such as veal and venison. In normal fresh meat when colour stability is not altered through special dietary or processing regimes, different species exhibits different colour stabilities and consequently different shelf lives. Information on MetMb reducing activities in meat from these species could advance our knowledge on the relationship between MetMb reducing activity and fresh meat colour stability.

Objectives

The current study was undertaken to characterize and measure MetMb reducing activity in veal and venison and to compare the activity in these meats with the reported MetMb reducing activities in beef, pork and lamb meats with the aim of investigating whether a meaningful relationship could be found between MetMb reducing activity and the known shelf life of these meats.

Materials and methods

Venison: samples from longissimus dorsi (LD) muscles of two years old stags (n=8) were obtained from carcasses (average weight 55.3 ± 4.1 kg) and vacuum packed for 3 weeks (standard aging time according to the New Zealand game industry board specifications).
Veal: samples were obtained at 48 h post mortem from LD muscles of 75 (± 2) days old male calves (n=6, average live wt 88 ± 5 kg).
Metmyoglobin reducing activity. A sarcoplasmic and particulate metmyoglobin reducing activity (SMRA and PMRA, respectively) were obtained from the samples as described ealier[8]. The supernatant and the particulate extracts were oxidised with a slight excess of $K_3Fe(CN)_6$, dialysed (10000 MW cut-off membrane) against 2.0 mM phosphate buffer (pH 7.0) at 4 ^{0}C several times. Metmyoglobin reductase activity was determined as described[6,7] using $VERSA_{max}$ microplate reader (Molecular Devices, Sunnyvale, CA, USA) after scaling down the assay final volume to 200µl. The standard assay mixture contained 15 µl 5 mM EDTA; 15 µl 50 mM phosphate buffer (pH 7.0); 15 µL 3.0 mM K_4 Fe $(CN)_6$; 50 µl 0.75 mM Mb Fe(III) in 2.0 mM phosphate buffer (pH7.0); 10 µl 2.0 mM NADH; muscle extract (50 µl for SMRA and 10 µL, of 5x dilution of dialysed samples, for MMRA) and water to a final volume of 20 µl assay mixture. The standard assay mixture pH was 6.8 and the assay was carried out at 25 °C. The reaction was initiated by adding NADH and followed by the change in absorbance at 580 nm. Blanks contained all the additions except NADH, which was replaced by water. The activity was calculated as the mean of three replicates and expressed as nanomoles of MetMb reduced per min per gram of meat.

Results and discussion

High propotions (82- 88 %) of the total MetMb reducing activity, were present in the pellet fraction of LD muscles in veal and venison meat (Table 1). SMRA in veal LD muscle was higher than those reported for pork[6,9] and less than that in beef or lamb meats[7,10]. PMRA in veal LD was less than that in beef, but higher than that in lamb LD muscle. Venison on the other hand, had comparable SMRA and PMRA to those reported in beef [10].
The effects of different assay pH and NADH concentrations on MetMb reducing activities in the different fractions of the muscle is presented in Figure 1. The pellet fractions from veal and venison seems to be more

sensitive to changes in pH and NADH which indicate the possibility of different MetMb reducing system/s from that present in the supernatant. Earlier[10], it was suggested that this activity could be activity remained in the pellet fraction due to the hydrophobic segment that binds NADH cytochrome b_5 metmyoglobin reductase to membranes[11], because the current methodology do not employ a detergent to solubilize the enzyme or unsufficient mechanical release of microsomes and mitochondria by homogennization. However, the behaviour of sarcoplasmic and pellet MetMb reducing activities in regard to the effects of assay pH and NADH concentration indicate to a possible different MetMb reducing systems in these two fractions. The capacity of SMRA, beef= venison > lamb > veal > pork, do not correspond with the expected display shelf-life of these meats, beef > veal > lamb > pork > venison. Moreover, given that beef and venison exhibit similar SMRA and PMRA and the shelf-life of beef >> venison, it is unlikely that MetMb reducing activity is contributing to the colour stability of fresh meat during display storage.

Conclusions

MetMb reducing activity is higher in venison than in veal LD muscles. Since the order of the MetMb reducing activity in beef, lamb, veal , pork and venison do not generate a meaninful relationship with their expected shelf-life display, MetMb reducing activity is unlikely to contribute to fresh meat colour stability. However, from a physiological point of view, differences in SMRA and PMRA presented in this study could be of interest.

References

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Table 1. Metmyoglobin reducing activity in veal and venison LD muscle extracts (SN and Pellet) under different assay conditions.

	Veal						Venison					
	EDTA	$K_4Fe(CN)_6$	NADH	Muscle extract	Equine MbFe(III)	Activity (nmol min^{-1} g^{-1})	EDTA	$K_4Fe(CN)_6$	NADH	Muscle extract	Equine MbFe(III)	Activity (nmol min^{-1} g^{-1})
Supernatant extract	+	+	+	+	+	162 ± 7	+	+	+	+	+	312 ± 5
	+	-	+	+	+	56 ± 3	+	-	+	+	+	135 ± 4
	-	+	+	+	+	164 ± 5	-	+	+	+	+	274 ± 8
Pellet extract	+	+	+	+	+	775 ± 95	+	+	+	+	+	2422 ± 101
	+	-	+	+	+	204 ± 24	+	-	+	+	+	1474 ± 43
	-	+	+	+	+	802 ± 43	-	+	+	+	+	1817 ± 81

(+) = presence (-) = absence

-No MetMb reducing activities were found when NADH, muscle extract or equine MetMb was omitted from the assay mixture. The absence of EDTA from the assay mixture decreased MetMb reducing activities in venison extracts but not veal. The absence of $K_4Fe(CN)_6$ from the assay mixture resulted in decreased MetMb reducing activities and the extend of the effect varied between venison and veal. The present results and earlier reports[5-7,10] indicates that MetMb reduction by various species extracts is influenced by the assay components.

Figure 1. Effects of NADH concentrations and pH on MetMb reducing activity in sarcoplasmic and pellet fraction of veal and venison LD muscles.