THE RELATIONSHIP BETWEEN THE COLOUR STABILITY AND THE FORMATION OF THE METMYOGLOBIN LAYER AT THE SURFACE OF BEEF, PORK AND VENISON MUSCLES.

Key Words: Colour, discolouration, metmyoglobin, myoglobin, autoxidation, oxygen penetration, beef, pork, venison.

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INTRODUCTION

The colour of meat is the most important factor in determining its attractiveness to purchasers. Fresh meat colour is determined by the relative amounts and depths of the three derivatives of myoglobin. Reduced myoglobin or deoxymyoglobin (De-MbO) is the purple pigment of deoxygenated muscle and of the freshly cut meat surface. On exposure to air, reduced myoglobin combines with oxygen to form a surface layer of bright red oxymyoglobin (MbO) which is associated with the colour of fresh meat and considered attractive by the consumer. During subsequent refrigerated storage of meat, the colour changes from the initial bright cherry red or pink colour to a greenish-brown. This change is due to the spontaneous autoxidation of MbO to metmyoglobin (MetMb). The meat then becomes increasingly discoloured and unattractive.

It has been suggested that the rate of discolouration of meat depends on the depth to which oxygen can penetrate into the tissue and oxygenate the myoglobin (Renerre & Labas, 1987). One explanation for the development of MetMb at the surface of fresh muscles involves the formation of a thin MetMb layer at 2-4 mm below the surface of the meat, which increasingly discolours the meat as the oxidised layer moves towards the surface over time (Winstanley, 1979). The movement of the brown layer to the surface of the meat is reported to be related to the autoxidation rate of myoglobin at the surface layers and on the thickness of the MbO layer (Renerre, 1990). In contrast to oxygen uptake in living muscle, uptake in meat has received little attention (Hood, 1980). Therefore, the objective of this research was to determine the relationship between the rate of MetMb formation in the surface layers of meat and the discolouration rate of beef, pork and venison muscles.

MATERIALS AND METHODS

Sample Selection and Preparation: *Longissimus dorsi* (LD) muscles were obtained 24 h *post-mortem* from one side of non-electrically stimulated beef, pork and venison carcases of commercially slaughtered female animals (n=2) of typical market weights. The muscles selected were of normal pH (5.4-5.7) and were not pale, soft, and exudative (Trout, 1992). Each muscle was trimmed and sliced into two 25 mm thick steaks for retail display. Prior to the colour analysis, steaks were packaged in polystyrene trays which were overwrapped with oxygen permeable film and stored at 5°C for < 2 h. The remaining portion of each muscle was used for the squash plate analysis.

Colour assessment: Display-life (ie. time of acceptable meat colour) was assessed on duplicate steaks using a 10-member, trained, sensory panel who scored the meat as being acceptable (ie. would purchase the meat without reservation) or unacceptable (ie. would not purchase the meat). The steaks were evaluated at 2, 6 and 24 h after slicing the meat, and then twice daily for one week under warm white fluorescent light of 1000 lux at the meat surface (Trout & Gutzke, 1995).

Squash plate method: The squash plate method of O'Keeffe and Hood (1982), with some minimal modifications suggested by Powell and MacDougall (1993), was used in the present study to determine the (i) depth of oxygen penetration, and (ii) the thickness of the MetMb layer over time. Triplicate samples (20.0±0.5 mm wide; 20.0±0.5 mm high; and 2.0±0.1 mm thick) of each muscle were prepared by slicing the tissue perpendicular to the fibre direction. These samples were wrapped in oxygen permeable film, and pressed to a thickness of 2 mm between two translucent acrylic plates. Squash plates were stored in refrigerated display (5°C) for one week. Using a micrometer, the thickness of the MbO layer and the MetMb layer were determined at 2, 6, 8, 10, 12, and 24 h after slicing the meat, and then twice daily for one week. Average thickness of the MetMb layer was determined over time, using the following formulae, where: MbO $(mm)_{max}$ = maximum MbO depth (mm); Total $(mm)_x$ = thickness of MetMb + MbO layers (mm) at time x; MetMb $(mm)_x$ = thickness of MetMb layer (mm) at time x :

Thickness of oxidised De-MbO layer (mm) = Total $(mm)_x$ - MbO $(mm)_{max}$

Thickness of oxidised MbO layer (mm) = MetMb $(mm)_x$ - De-MbO $(mm)_{oxidised}$

Experimental design and data analysis: The experiment was replicated twice using a 3 x 6 factorial arrangement (3 species x 6 time intervals) of a randomised complete block design. Data were analysed by analysis of variance using SAS (SAS Institute Inc., 1994).

RESULTS

Depth of oxygen penetration: The results in Fig. 1 show that the initial (ie. 2 h after slicing) depth of oxygen penetration of the pork muscles (2.67 mm) and the beef muscles (2.34 mm) were similar (p>0.05), but two-fold greater than the venison muscles (1.30 mm). Upon continued storage in air, there was further penetration of oxygen into the muscle and this was species and time dependent, indicated by a strong interaction between these two variables (p=0.0001). The maximum depth of oxygen penetration of pork muscle (5.95 mm) was almost four times that of venison (1.58 mm). Moreover, the beef muscle possessed a similar depth (p>0.05) of oxygen penetration as pork initially, but the rate of oxygen uptake was much slower (Fig. 1).

MetMb layer formation: The results in Fig. 1 show that the MetMb layer first formed in the venison muscle at 24.0 h, but was not observed until 72.0 h for beef, and 120.0 h for pork. Shortly after its formation, the rate of increase in the thickness of the oxidised myoglobin layer of muscles over time was species dependent, as indicated by a significant species and time interaction (p=0.0001). Once formed, the rate of increase in the thickness of the MetMb layer over time was very similar for beef and venison muscles, but it was more rapid for the pork muscle (Fig. 1). There was also a difference between species in the way that the MetMb layers formed. The results in Fig. 2 showed that the oxidation of the surface layers of the muscles was caused by oxidation of both the oxygenated (MbO) and deoxygenated (De-MbO) layers, and was different for each of the species studied. With pork, once it was initiated, oxidation occurred rapidly and mainly in the MbO layer. For venison, oxidation occurred much sooner, but the increase in the MetMb layer was more gradual, and the oxidation occurred mainly in the De-MbO layer. The pattern of oxidised layer formation for beef muscles was intermediate between venison and pork.

The relationship between the colour stability and the formation of the MetMb layer at the surface of beef, pork and venison muscles: The results in Table 1 highlight the fact that the colour stability of beef, pork and venison muscles was highly correlated to the formation of the MetMb layer at the surface of the meat (r=0.94). There was a three-fold difference in the display-life between the least colour stable muscle (venison; 50.4 h) and the corresponding beef (143.6 h) and pork (159.7 h) muscles. Significant differences between the species were also

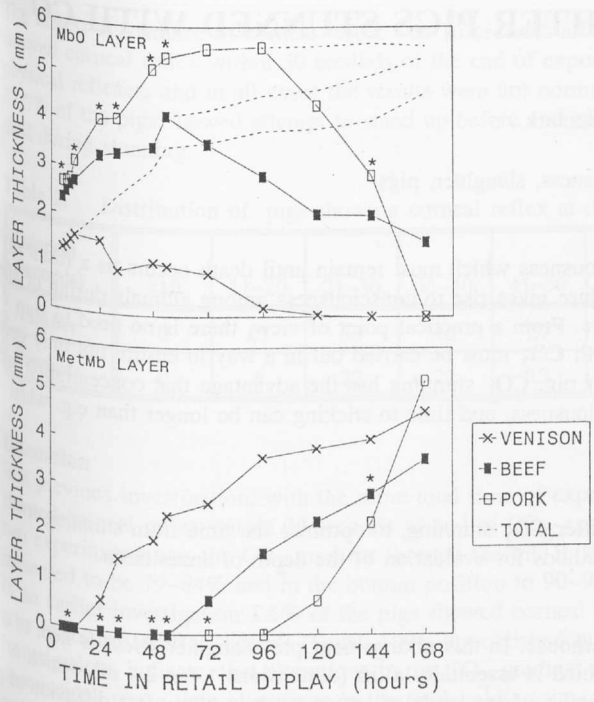

Fig. 1. Average thickness of MbO, MetMb and total (MbO + MetMb) layers of venison, beef and pork *L. dorsi* muscles that were stored for seven days in refrigerated display (5°C), as determined using squash plates. For MbO and MetMb layers, venison was significantly different ($p<0.05$) from beef and pork for the different time intervals, as determined by $LSD_{0.05}$. Similar layer thicknesses ($p>0.05$) between beef and pork are indicated by an asterisk (*).

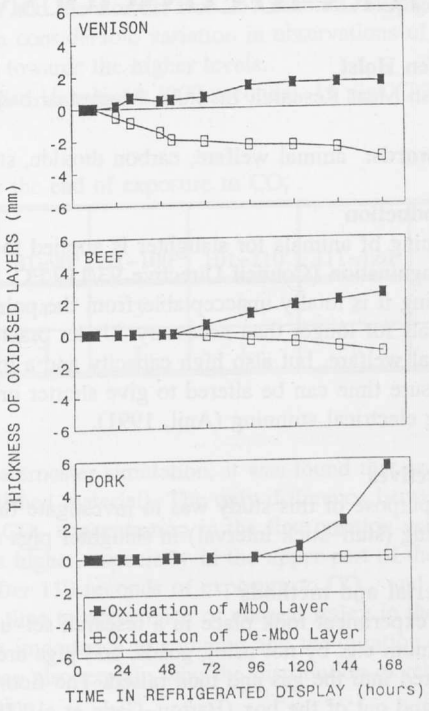

Fig. 2. Change in the thickness of MetMb layer over time, relative to the point of maximum oxygen penetration, as determined using squash plates. Positive values indicate oxidation of the MbO layer, and negative values indicate oxidation of the De-MbO layer.

observed for when the MetMb layer was first formed ($p=0.0001$). Furthermore, formation of the MetMb layer was observed to be different for beef (90.0 h) and pork (144.0) muscles, but a similar trend was not shown for the colour stability of these muscles (pork = beef). Nevertheless, the present results imply that differences in the colour stabilities of meat may be related to the rate of MetMb formation at the surface of muscles.

Table 1. The relationship between the colour stability and the formation of the MetMb layer at the surface of venison, beef and pork *L. dorsi* muscles during refrigerated storage at 5°C. ^{abc}Means in the same column with the same superscripts did not significantly differ ($p>0.05$); terms in brackets are standard errors of least square means.

SPECIES	Colour Stability versus Formation of MetMb Layer in Meat	
	Colour Stability of Meat (ie. display-life; h)	Formation of MetMb Layer (h)
Venison	50.4^{a} (6.2)	28.0^{a} (8.1)
Beef	143.6^{b} (5.1)	90.0^{b} (8.1)
Pork	159.7^{b} (4.4)	144.0^{c} (8.1)

CONCLUSION

It is concluded from this research that differences in the colour stabilities of meat may be related to the rate of MetMb formation at the surface of muscles. In addition, the results of this study clearly showed that the oxidation of the oxygenated and deoxygenated myoglobin layers were different for beef, pork and venison. From these results, it appears that the deeper the MbO layer, the deeper the layer of MetMb development. It follows then that the colour stability of meat may depend on producing a layer of MbO thick enough to prolong the MetMb development underneath. Thus, it is anticipated that the results of this work could be used to increase the retail display-life of meat.

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