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THE RELATIONSHIP BETWEEN DISCOLOURATION RATE DURING REFRIGERATED DISPLAY AND THE AUTOXIDATION RATE OF PURIFIED MYOGLOBIN: THE EFFECTS OF MUSCLE TYPE AND SPECIES.

Key Words: Colour, myoglobin, autoxidation, beef, lamb, pork, venison, *L. dorsi*, *G. medius*, and *B. femoris*

GRAHAM R. TROUT[1] and DEAN GUTZKE[2]

[1] Australian Meat Technology, Rocklea, Brisbane, 4106, AUSTRALIA.

[2] CSIRO Division of Food Science and Technology, Brisbane Laboratory, Cannon Hill, 4170, AUSTRALIA.

INTRODUCTION

Colour is one of the major factors consumers take into consideration when purchasing meat since consumers equate colour with quality. Once meat is cut, it progressively discolours during subsequent refrigerated display and becomes increasingly unacceptable to consumers. The colour changes from the initial bright cherry red or pink colour to a greenish-brown as the result of spontaneous autoxidation of oxy-myoglobin to metmyoglobin.

The rate at which meat discolours during refrigerated display is extremely variable (Hood, 1980) and depends on both display conditions and intrinsic properties of the meat. External factors which have been shown to have the greatest effect on discolouration rate are the gaseous atmosphere in which the meat is displayed and the display temperature and light intensity (Faustman & Cassens, 1990). However, even under standardised conditions there is still variation from muscle-to-muscle and between the same muscle from different species (Renerre & Labas, 1987).

Although it is not fully understood why there is this intrinsic difference in colour stability, several explanations have been suggested. One explanation for this intrinsic difference is the variation in concentration or activity of reducing compounds or reducing enzyme or enzyme systems present in meat (Reddy & Carpenter, 1991). The role of these reducing systems *in vivo* is to reduce any metmyoglobin formed so as to maintain the myoglobin in its reduced form so that it can bind oxygen (Hagler *et al.*, 1979). A second explanation given for the variation in discolouration rate which is based on data from marine animals (Livingston *et al.*, 1986) is that there is inherent differences in the oxidation rate of the myoglobin from different species. A third explanation for this difference is that the muscles contain different levels of anti-oxidative and pro-oxidative compounds which control the oxidation rate myoglobin in the muscle.

To help elucidate the relative importance of these different factors in controlling the discolouration rate of meat, the following experiment was carried out with the objective of determining if the variation in discolouration rate of meat from different muscles and species is due to intrinsic differences in the autoxidation rate of the myoglobin in the muscle.

MATERIALS AND METHODS

Meat selection and preparation. Three muscles of differing colour stability, *Longissimus dorsi* (LD), *Gluteus medius* (GM) and *Biceps femoris* (BF), were obtained 24 hours post-mortem from beef, pork, lamb and venison carcases that were typical market-weight animals. The muscles selected were of normal pH (5.4-5.7) and were not affected by PSE (Trout, 1992). Each muscle was trimmed and sliced from the anterior end into four 25 mm thick steaks for retail display and two 300 g portions for myoglobin extraction and purification.

Colour assessment. The four steaks from each treatment were packaged in polystyrene trays which were over-wrapped with oxygen permeable film and stored at 5°C for visual colour assessment. Surface colour was assessed on duplicate steaks using a 10-member, trained, sensory panel who scored the meat from 1 (extremely brown) to 8 (extremely red). The steaks were evaluated under warm white fluorescent light of 1000 lux at the meat surface. Colour was assessed at 2 and 6 hours after slicing the meat and then daily for one week.

Myoglobin purification. Myoglobin was extracted and purified from the 300 g muscle portions from all muscles using the method of Trout & Gutzke (1995). This method, based on previous similar procedures (Maxwell *et al.*, 1974; Wittenberg & Wittenberg, 1981), allows the extraction and purification of preparative quantities of highly purified (>96% pure), fully reduced myoglobin (>98% reduced) in approximately 24 hours.

Myoglobin oxidation rate. The oxidation rate of the purified myoglobin was carried out using a Cary 3E UV-VIS spectrophotometer fitted with a 12 cell multi-cell holder and a peltier temperature control unit. The purified myoglobin (2.0 mg/ml) was diluted with an equal volume of 20 mM phosphate buffer just prior to analysis. The oxidation rate was determined by monitoring the change in absorbance at 572 nm and the rate constant was calculated using a linear fitting technique (Schwartz, 1978).

Experimental treatments and calculations. *Discolouration rate.* The treatments evaluated were a factorial combination of the four species and the three muscle types which resulted in twelve different muscle combinations being evaluated. From the mean colour scores for each muscle, $t_{0.5}$ was calculated as the time in hours taken to reach half the final colour score.

Myoglobin oxidation rate. The rate constant for myoglobin oxidation was determined for each myoglobin preparation from each muscle and species at three different pH levels (5.50, 6.00 and 6.50) and three different temperatures (20°C, 30°C and 40°C). This data was then used to calculate the rate constant (k) at the meat display temperature (5°C) and at the pH of each muscle (5.5-5.7). To obtain the rate constant at 5°C for each muscle at each pH, log k was plotted versus 1/T (Arrhenius, 1889) which resulted in a straight line. The line was extrapolated to 5°C to obtain the rate constant at 5°C for each pH and each muscle (this plot has been shown to be linear from 5°C to 40°C [Livingston *et al.*, 1986]). To determine k at 5°C at each of the pH values of the different muscles, an equation was developed using linear regression to relate k at 5°C to pH. From the resulting equations, k at 5°C was calculated for each muscle pH. $t_{0.5}$ was calculated from the k value as follows: $t_{0.5} = 0.693/k$.

The experiment was carried out in duplicate and the data was analysed by analysis of variance using SAS.

RESULTS: The results in Table 1 show the discolouration rate and myoglobin oxidation rate of the different species averaged over muscle type expressed as $t_{0.5}$.

TABLE 1. Muscle pH and $t_{0.5}$ values for discolouration rate and myoglobin oxidation rate for venison, lamb, beef and pork muscle, averaged over muscle type.

SPECIES	MUSCLE pH	$t_{0.5}$ (Hours)	
		DISCOLOURATION RATE	MYOGLOBIN OXIDATION RATE
VENISON	5.64	31.0_a	394.4^a
LAMB	5.66	52.3^b	333.8^a
BEEF	5.67	72.6^c	312.5^a
PORK	5.59	107.7^d	207.1^b

These results highlight the fact that there are large significant differences ($p<0.05$) in discolouration rate between muscles from the different species. For example, there was a three-fold difference in discolouration rate between the most colour stable pork muscles ($t_{0.5}$=107.7 hours) and the least stable venison muscles ($t_{0.5}$=31.0 hours).

In contrast, there was no difference ($p>0.05$) in oxidation rate of the different myoglobins, as measured by $t_{0.5}$, between three of the species. Although pork myoglobin had a significantly lower oxidation rate than that of myoglobin from the other three species, the differences in myoglobin oxidation rate do not appear to explain the variation in discolouration rate observed with the meat. This point is illustrated by the fact that there was no significant positive correlation between the $t_{0.5}$ for discolouration rate and $t_{0.5}$ for myoglobin oxidation rate ($p>0.05$). Some of the variation in $t_{0.5}$ for myoglobin oxidation from the different species was due to the different pH values of the muscles (Table 1) since there was a significant correlation between muscle pH and $t_{0.5}$ ($r=0.56$).

The results in Table 2, which show the effect of muscle averaged over species on discolouration rate and myoglobin oxidation rate, are similar to those obtained with the different species. Although there are significant differences in discolouration rate between the three muscles ($p<0.05$), the myoglobin oxidation rate does not show corresponding differences.

TABLE 2. Muscle pH and $t_{0.5}$ values for discolouration rate and myoglobin oxidation rate for the *B. femoris, G. medius* and *L. dorsi* muscles, averaged over species.

MUSCLE	MUSCLE pH	$t_{0.5}$ (Hours)	
		DISCOLOURATION RATE	MYOGLOBIN OXIDATION RATE
B. fermoris	5.66	52.5^a	357.4^a
G. medius	5.62	60.6^b	288.7^b
L. dorsi	5.60	85.6^c	289.8^b

CONCLUSION The main conclusion from this research is that there is no direct relationship between the oxidation of purified myoglobin and the discolouration rate of meat. Moreover, there are large differences between the rate at which meat discolours and the rate at which myoglobin oxidises. Depending on the species and muscle type, meat discolours two to twelve times faster than purified myoglobin oxidises. These observations indicate that in addition to the role of reducing systems in muscle, endogenous oxidative systems may play a significant role in determining the rate of formation of metmyoglobin.

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