Changes in the antioxidant effects on myoglobin of high and low molecular weight fractions extracted from beef, pork and venison muscles after refrigerated display.

Key Words: Myoglobin, autoxidation, antioxidant, beef, pork, venison

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BACKGROUND:

Colour is a major factor consumers consider when purchasing meat since they 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 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. 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 are inherent differences in the oxidation rate of the myoglobin from different species. A third explanation for this difference is that different muscles contain different levels of anti-oxidative and pro-oxidative compounds which control the *in vivo* oxidation rate of myoglobin.

To help determine 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 species is due to differences in antioxidative properties of the muscle.

OBJECTIVES: To 1) determine the *in vitro* antioxidative effects on purified myoglobin of high molecular weight (HMW) and low molecular weight (LMW) soluble fractions extracted from muscles from three species of different colour stability (pork>beef>venison), 2) determine how the antioxidative properties of these fractions change with the time the muscles are in refrigerated aerobic display and 3) identify the compounds responsible for the antioxidative effects.

METHODS:

Meat selection and preparation. The *Longissimus dorsi* muscle was removed 24 hours post-mortem from non-electrically stimulated pork, beef and venison carcases from 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 of fat and connective tissue, sliced from the anterior end into six steaks of standard thickness (20 mm) and weight (100 $\pm$ 10 g), packed in polystyrene trays, overwrapped with PVC and stored in refrigerated display (5°C) for either 0, 1, 2, 3, 5 or 7 days.

Preparation of HMW and LMW soluble muscle fractions. The soluble muscle fractions were prepared using a modification of the procedure of Decker and Hultin (1990)). One steak (100g) from each species was removed from display at one of the six time intervals, cut into 20mm cubes and homogenised with 300ml of 0.25 M sucrose, 0.05 M Tris buffer (pH 7.5) in a Waring blender on high speed for 1 min. To obtain the HMW soluble fraction, the homogenate was centrifuged at 1,000 *g* for 12 min at 0°C, the supernatant was filtered through a single layer of tissue paper to remove fat. The soluble LMW fraction was obtained by filtering an aliquot of the soluble HMW fraction through an ultrafiltration unit (Amicon Model 12) operated at 400 kPa in a 100% oxygen atmosphere and fitted with a 10 kilodalton molecular weight cut-off, low-binding, regenerated cellulose membrane (Millipore). Both the HMW and LMW fractions were filtered through a 0.2 μm sterile cellulose acetate filter (Sartorius) immediately after preparation and stored at 0°C until used (within 30 min).

Myoglobin Preparation. Myoglobin for oxidation kinetics was extracted and purified from fresh beef LD muscle using the method of Trout and Gutzke (1996). This method allows the extraction and purification of preparative quantifies of highly purified (>96% pure), fully reduced myoglobin (>98% reduced) in approximately 24 hours. To chelate any free copper and iron in the myoglobin preparations, DTPA (at a final concentration of 3.0 - 4.0 μM) was added to the myoglobin until there was no change in the rate constant (Trout and Gutzke, 1996).

Inhibition of myoglobin oxidation by muscle extracts. The antioxidative effect of the HMW and LMW fractions extracted from pork, beef, venison muscles at each of the six display periods was determined using a modified method of Yin and Faustman (1993). In brief, this method involved adding aliquots of each fraction to the purified bovine myoglobin and measuring the change in myoglobin oxidation rate. This was carried out by mixing 0.75ml of each fraction with 0.75ml myoglobin (4.0 mg/ml) and 1.5ml 20mM, pH 6.00 phosphate buffer so that the final myoglobin and phosphate concentrations were 1.0mg/ml and 10mM, respectively All reactants were incubated at the reaction temperature of 40°C for 30 minutes before mixing. The oxidation rate of the purified myoglobin both with and without the added fractions was determined using a Cary 3E UV-VIS spectrophotometer fitted with a 12 cell multi-cell holder and a peltier temperature control unit. The rate of oxidation was determined by monitoring, every 15 minutes, the change in absorbance at 572 nm. The rate constant was calculated using a linear fitting technique (Schwartz, 1978). The inhibitory effect on myoglobin of the soluble muscle fractions was calculated as follows: INHIBITION (%) =100 - ((Myoglobin Rate Constant (+fraction)/ Myoglobin Rate constant (control))*100)

Identification of antioxidative compounds. *Carnosine and anserine.* The carnosine and anserine concentrations of the beef, pork and venison steaks which had been stored in refrigerated storage for either 0 or 5 days was determined using a HPLC procedure of Kurth (1994). *Ascorbate.* L-ascorbic acid was determined daily on steaks displayed in refrigerated storage using an enzymic colorimetric kit from Boehringer Mannheim Biochemicals. The assay was carried out on press juice obtained by centrifuging 6.0 g of muscle at 40000 *g* for 30 min at 0°C. *Free Copper + Iron Analysis.* The free copper+iron concentration of the LMW extracts was measured at 0 or 7 days on the LMW extract using the method of Trout and Gutzke (1996)

Data Analysis. The experiment was replicated three times and analysed by Analysis of Variance

RESULTS:

LMW and HMW Fractions. The results in Table 1 show that the LMW fraction from all three species produced similar inhibitory effects on myoglobin oxidation both initially and over the seven days of refrigerated display. The inhibitory effect did not change with time ($p>0.05$) and was between 36% and 43% in all cases. In contrast, with the HMW fractions there was a significant ($p<0.05$) difference in the inhibitory effect between species initially (20% for venison 80% for beef and pork) and the change in inhibitory effect over time. With the beef and pork HMW fractions, there was no difference in the inhibitory effect initially and the inhibitory effect of HMW fractions from both species decreased progressive over the seven days display until they were both zero at day seven. With the venison HMW fraction, the inhibitory effect decreased from 20% at day zero to -40% at day 7.

Antioxidative compounds. Analysis of the muscles for carnosine concentration (Table 1) showed that venison had a lower carnosine level than muscles from the other two species at day zero and the difference were maintained over the five days of storage. However, when the anserine concentration was included in the analysis there was no difference in carnosine plus anserine between species or over the five days display. Analysis of the muscles for ascorbate (Table 1) showed that there was no difference in ascorbate level between species either initially or over the seven days of display ($p>0.05$). In all cases, the ascorbate level dropped to virtually zero after two days display. The free copper + iron concentration increased with both beef and pork from approximately 2.0 uM on day zero to approximately 3.0 uM on day five. In contrast, the level in venison muscle was approximately twice the level in beef and pork initially ($p< 0.05$) but did not change with time.

CONCLUSION:

The results of this study show that both the HMW and LMW extracts from muscle have appreciable antioxidant properties. The antioxidative effects of the LMW fraction do not differ between species or change over time. In contrast, the HMW fractions have higher antioxidative properties initially, but this effect disappears rapidly during refrigerated display. Moreover, the HMW fraction from venison has a much lower antioxidant effect initially and this increases to a pro-oxidant effect over the seven days of display. The difference in antioxidant/pro-oxidant compounds analysed for did not seem to explain the different colour stability of the muscles from the different species.

REFERENCES:

Hagler, L., Coppes, Jr. R.I. and Herman, R.H. 1979. J. Biol. Chem., 254: 6505-6511. **Hood, D.E.** 1980. Meat Sc., 4: 247-265. **Kurth, L.** 1994 "Report on project CS215" MRC, Sydney, Australia. **Livingston, D.J., Watts, D.A. and Brown, W.D.** 1986. Arch. Biochem. Biophys., 249: 106-115. **Reddy, M. and Carpenter, C.E.** 1991. J. Food Sc., 56: 1161-1163. **Steel, R.G.D. and Torrie, J.H.** 1980. Principles and Procedures of Statistics (2nd Ed.). McGraw-Hill Book Co., New York. **Trout, G.R.** 1992. Proc. ICMoST, Clermont, France. 983-987. **Trout, G.R. and Gutzke, D.A.** 1995. Proc. 41st ICoMST., San Antonio, TX. pp C83-C84. **Trout, G.R. and Gutzke, D.A.** 1996. Meat Science (Accepted). **Yin and Faustman, C.** 1993. J. Agri. Food Chem. 41: 853.

TABLE 1. Changes in antioxidant effects of fractions from pork, beef and venison muscle during refrigerated display.

	SPECIES	DAY O	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 7
INHIBITION (%) - LMW	PORK	40	38	37	38	36	35	36
	BEEF	40	43	40	39	38	37	37
	VENISON	34	36	35	34	34	33	34
INHIBITION (%) -HMW	PORK	80^{a}	55^{b}	55^{b}	57^{b}	60^{b}	38^{c}	-5^{e}
	BEEF	80^{a}	55^{b}	53^{b}	57^{b}	47c	38c	-6^{e}
	VENISON	20^{d}	30c	7^{e}	3^{e}	-18^{e}	-20^{e}	-40^{f}
CARNOSINE (mg/100g muscle)	PORK	489^{a}	ND	ND	ND	ND	562^{a}	ND
	BEEF	479^{a}	ND	ND	ND	ND	498^{a}	ND
	VENISON	311^{b}	ND	ND	ND	ND	338^{b}	ND
CARNOSINE + ANSERINE (mg/100g muscle)	PORK	553^{a}	ND	ND	ND	ND	650^{a}	ND
	BEEF	564^{a}	ND	ND	ND	ND	599^{a}	ND
	VENISON	688^{a}	ND	ND	ND	ND	778^{a}	ND
FREE [CU + FE] (uM)	PORK	2.00^{a}	ND	ND	ND	ND	2.80^{b}	ND
	BEEF	1.96^{a}	ND	ND	ND	ND	3.09^{b}	ND
	VENISON	3.99^{c}	ND	ND	ND	ND	3.79^{c}	ND
[ASCORBATE] (uM)	AVERAGED OVER SPECIES	91.0^{a}	7.3^{b}	1.1^{c}	2.9^{c}	2.0^{c}	1.0^{c}	0.9^{c}

ND = Not determined; For a given asssay, means with different superscripts are significantly different ($p<0.05$).