POSTMORTEM METABOLIC ACTIVITY AND SUPEROXIDE GENERATION IN VENISON

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Introduction

The pH-temperature profile during rigor, the onset of the proteolytic events and the changes in ionic strength and balance in fluids that baths muscle cells can trigger and cause changes in cells integrity and viability. Two assays viz: The reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and the reduction of Nitro Blue Tetrazolium (NBT) have been widely used in medical research to investigate the metabolic activity of tissue (Carrier, et al., 1999) and to evaluate antioxidants that scavenge superoxide radicals (Ponti et al., 1978; Robak and Gryglewski, 1988), respectively. Tetrazolium salts acts as hydrogen acceptors to form the water insoluble formazan pigments. These compounds have been used to quantify the reduction-oxidation enzyme systems of muscle (Belkin et al., 1988) including many dehydrogenases (Stoward et al., 1991). The reduction of tetrazolium salts in viable tissue is related to the presence of both enzymes and coenzymes involved in the process (Ferrera et al., 1993). The aim of the present study is to examine rigor temperature, electrical stimulation (ES) and display time effects on the metabolic activity and superoxide anion production in venison postmortem.

Materials and Methods

Meat: The experimental design and meat sampling were reported elsewhere (Bekhit et al., 2007).

The superoxide-mediated reduction of Nitro Blue Tetrazolium (NBT) by NADH/phenazine methosulphate (PMS) system was used to measure the ability of meat to scavenge superoxide radicals. Thin meat cores (30- 40 mg and 0.5 cm in diameter) were prepared using a filleting knife and a cork borer. The method is based on those described by Ponti et al., (1978) and Robak and Gryglewski (1988) with modifications. The assay mixture contained 25µM NBT, 1µM PMS, 100µM NADH in 0.04 M saline phosphate buffer (pH 5.56) containing Cremophor (8%w/v) and water to total assay volume of 1 ml. The assay mixture and the meat core were placed immediately after coring at the bottom of a cuvette. The generation of superoxide anions was started by adding NADH to the assay. The assays were incubated in the dark at room temperature (23 °C) for 10 min and the absorbance was measured at 560 nm. Measurements were carried at 0, 1, 3 and 6 days of retail display at 4°C for 1, 21 and 42 days post-mortem meat. The metabolic activity of venison tissue was determined at 24 h only using procedures described by Ferrera et al. (1993) with modifications. One gram meat and 6 ml homogenization buffer (containing 250 mM sucrose, 1 mM EDTA and 10mM KCl, pH 5.3) was homogenized for 20 s with an Ultra-Turrax at 9000 rpm. A 75 µl homogenate was added to 200 µl of MTT solution (0.6 mM), mixed well and incubated at room temperature for 2 h in the dark. A blank for each sample was run parallel excluding MTT. After the incubation period, the assay mixture was centrifuged for 1 min at 10,000 Xg and the supernatant was discarded. One ml of DMSO was added to the pellet, vortexed and the mixture was incubated at room temperature for 1 h in dark, centrifuged for 1 min at 10,000 Xg and absorbance of the resultant red purple supernatant (formazan) was measured against DMSO at 505 nm (spectrophotometer UV300, Unicam Ltd, U.K.). Measurements were carried out in triplicate. Superoxide anion generation values were analysed as a completely randomised split–split–strip plot design as described earlier (Bekhit et al., 2007). Venison metabolic activity at 24 h post-mortem was analysed using two-way ANOVA to determine the significance of the effect of ES treatment and rigor temperature.

Results and Discussion

Under aerobic conditions, phenazine methosulphate (PMS) can be reduced by NADH and this will lead to reduction of oxygen to yield superoxide anion which is capable of reducing NBT (Nishikimi et al., 1972; Ponti et al., 1978). By comparison with a control (all the assay mixture except of meat), the production of superoxide anions from meat could be determined. Relative superoxide anion production decreased with postmortem time ($P < 0.001$). Venison (1 day post mortem time only) incubated at 35°C had higher ($P < 0.01$) superoxide at 0 display time (Figure 1). There was no interaction between rigor temperature and ES on superoxide production.

The reduction of MTT decreased with the increase in rigor temperature ($P < 0.001$, Figure 2). ES tended to decrease the reduction of MTT which was significant ($P= 0.044$) for first kill in which the kill group and stimulation interacted to affect MTT reduction. The reduction of MTT is generally regarded as an indicator of cell redox activity (Bernas and Dobrucki, 2002) and has been used as a measure of mitochondrial intergrity (McCluskey et al., 1999), cell viability (Ferrera et al., 1993) and in the evaluation of cell cytotoxicity (Olivieri et al., 2000). However, recent studies have demonstrated that mitochondria plays a minor role in MTT reduction (Succinate-dependent mitochondrial MTT reduction) and that MTT reduction occurs mainly in the cytoplasm and involves NADH and NADPH-dependent mechanisms which are insensitive to respiratory chain inhibitors

(Bernas and Dobrucki, 2002). From the above, it is reasonable to suggest that higher rigor temperature probably reduced the activity of mitochondria and/or NADH and NADPH-dependent mechanisms responsible for the reduction (Berridge and Tan, 1993) including the reduction-oxidation enzyme systems of muscle (Belkin et al., 1988; Stoward et al., 1991).

Figure 1. Overall means of relative superoxide anion values produced in venison LD muscles from electrically stimulated and un-stimulated carcasses incubated during rigor at 15 and 35 °C. Superoxide radical generation was determined on venison at 1, 21 and 42 days postmortem time using NBT assay as described in the material and method section.

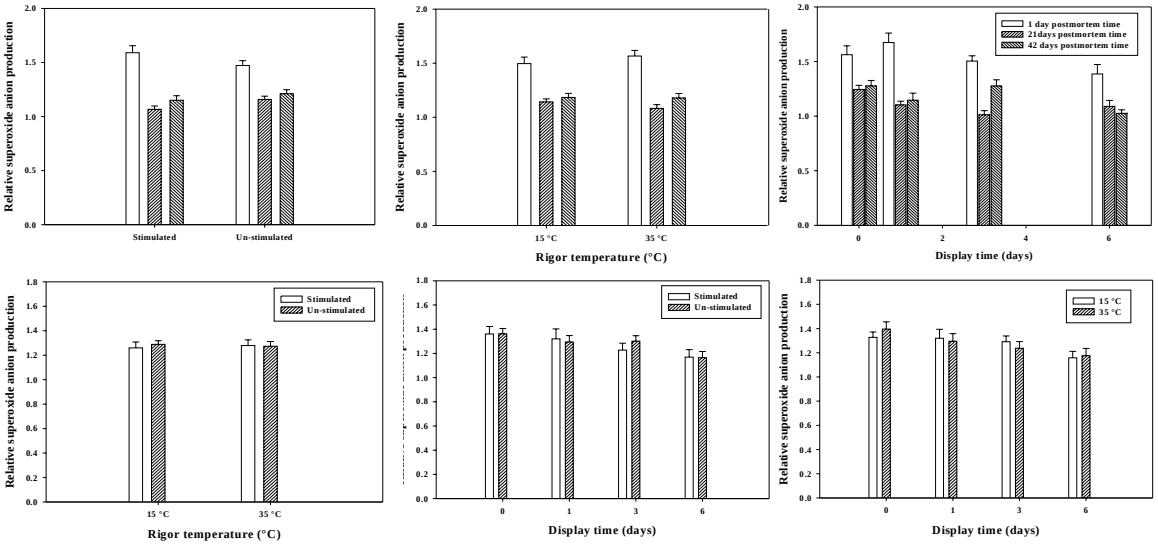
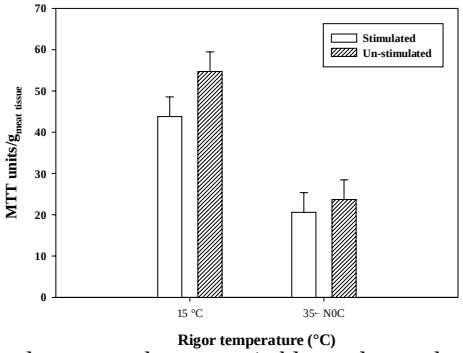


Figure 2. MTT activity in venison LD muscles from electrically stimulated and un-stimulated carcasses incubated during rigor at 15 and 35 °C at postmortem time.



Conclusions

In the present study, we streamlined systems that are suitable to detect the metabolic activity and superoxide production in meat. The reported assays could be useful as tools to investigate the oxidative mechanisms during postmortem storage and the possibility of using these assays to predict the shelf-life of red meat will be reported elsewhere.

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