Effects of Light on Non-Enzymatic Metmyoglobin Reduction *in-vitro*

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Introduction

- Consumers prefer a bright cherry-red color of meat (Carpenter et al., 2001).
- Discoloration of meat negatively impacts purchasing decisions, leading to food waste.
- Discoloration of meat is caused by the oxidation of oxymyoglobin, forming a brown pigment called metmyoglobin (metMb) (AMSA, 2012).
- Oxidation of myoglobin can be influenced by common retail settings such as light exposure and oxygen (AMSA, 2012).
- Meat has an inherent ability to reduce the brown pigment to form a bright cherry-red color through metmb reducing systems.
- It is known that meat can inherently reduce metMb via three pathways, including a non-enzymatic system (Brown and Snyder, 1969).
- Previous research has shown inherently present electron donors and carriers can contribute to non-enzymatic metmyoglobin reduction ability (NMRA) (Denzer et al., 2020), but there are limited studies concerning the effects of light on NMRA.

Hypothesis and Objective

- ***Hypothesis:*** Energy rays in the display light will increase non-enzymatic metmyoglobin reduction.
- ***Objective:*** To evaluate the effect of light and dark storage conditions on NMRA *in-vitro*.

Materials and Methods

- Equine metmyoglobin solution at pH 5.6 was combined with different electron donors and carriers in a 96-well plate (Table 1).
  - Solutions of ascorbate and NADH were used as electron donors.
  - Cytochrome *c* and methylene blue were used as electron carriers.
  - Ethylenediaminetetraacetic acid (EDTA) was used as a chelator.
- To evaluate lighting effects, the well-plate was kept under LED lighting (870-1090 lux) or in dark storage.
- Readings were taken every 5 min for 25 min using a spectrophotometer set to 582 nm (wavelength that indicates metMb reduction.
- The experiment was replicated three times.
- The data were analyzed using the Mixed Procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC).
  - Data transformed to logarithmic values of nanomoles of metMb reduced due to heterogenecity of variances.
  - Least squares mean for protected F-tests ($P < 0.05$) were separated by using the PDIFF option and were considered significant at $P < 0.05$.

Table 1: Substrate combinations and concentrations added to equine metmyoglobin (0.08 mM) and used to measure non-enzymatic metmyoglobin reduction.

1 NADH + MB[1] + EDTA
2 NADH + Cyt-c[2] +EDTA
3 Ascorbate + MB[1] + EDTA
4 Ascorbate + Cyt-c[2] +EDTA

[1]MB = Methylene blue
[2]Cyt-c = cytochrome c

Results

- NMRA was significantly higher in the presence of cytochrome *c* + ascorbate compared with cytochrome *c* + NADH for light and dark conditions.
- MRA increased with the presence of light and the combination of ascorbate + methylene blue ($P < 0.001$).
- In light conditions, NADH + methylene blue + EDTA had an increase ($P < 0.0001$) in NMRA.

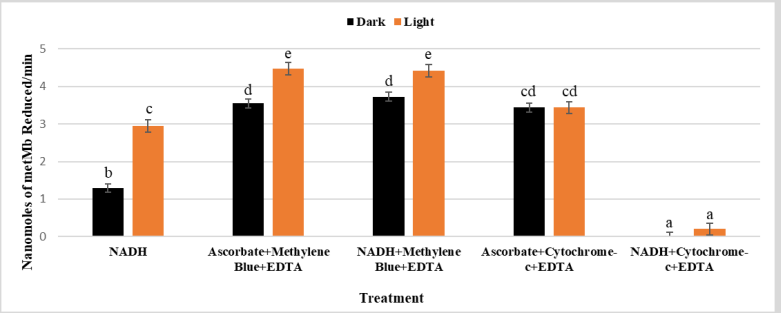
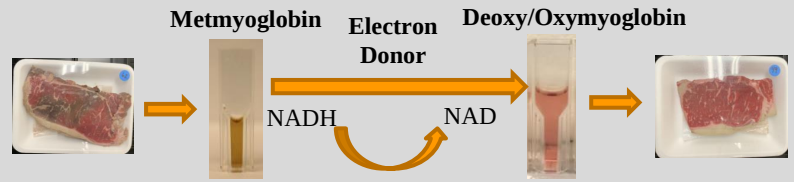


Figure 1: Least squares means for each combination evaluating the effects of lighting conditions on NMRA. Least squares means with a different letter (a-e) are significantly different ($P<0.05$). SEM = 0.17

Metmyoglobin Reduction



Metmyoglobin *in-vitro* to replicate metmyoglobin on the surface of meat.

Metmyoglobin reduced *in-vitro* to replicate any return to myoglobin (bright cherry-red).

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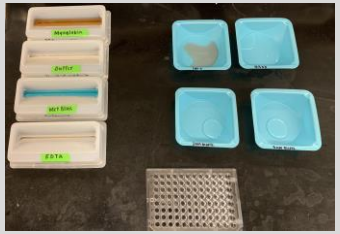
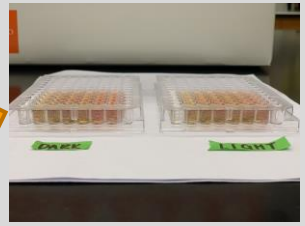
Methods and Materials

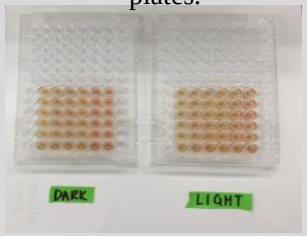
Equine myoglobin solution + phosphate buffer pH 5.6.



Solutions of NADH and ascorbate used as electron donors. Methylene Blue and Cytochrome-*c* solutions used as electron carriers.



Complete solutions pipetted into 96-well plates.



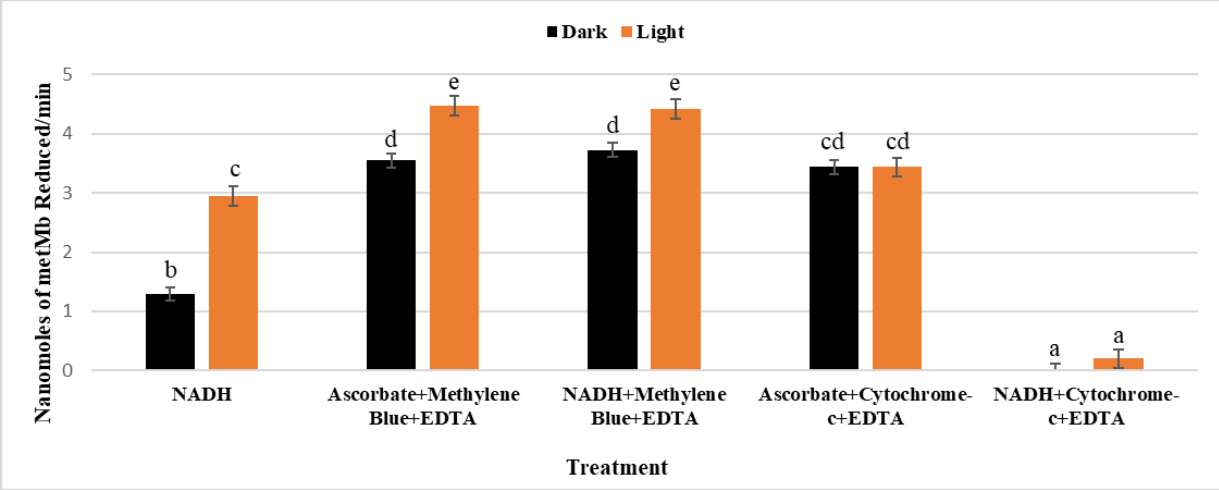
One well plate for each light condition.

Each well plate placed in its light setting; LED lamp or spectrophotometer (dark).

Molecular Devices SpectraMax M3 Multi-mode microplate reader set at 582 nm.

25 min; absorbance read every 5 min.

Results



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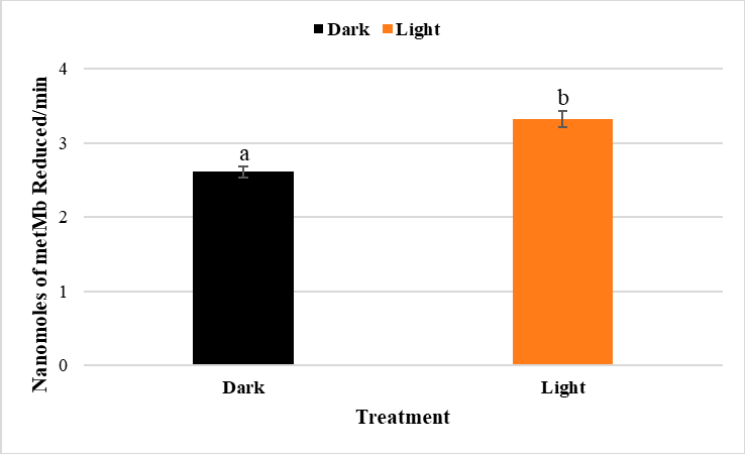

Molecular weight
NADH – 663.43
Ascorbate – 198.11

Methylene blue – 319.85
Cytochrome C – 884.9

Weights measured in g/mol.

Figure 2: Average of least squares means for both light conditions. Least squares means with a different letter (a-e) are significantly different (P<0.05).

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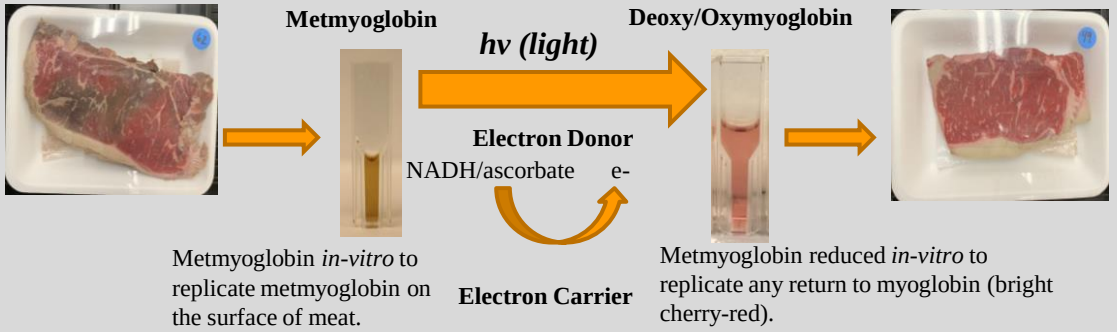
Conclusions

- NMRA was limited in the presence of cytochrome *c* in both lighting conditions.
- NMRA in the presence of methylene blue experienced a significant and consistent increase under light conditions.
- Current *in-vitro* research demonstrates inherently present electron donors and carriers can contribute to NMRA in retail light settings and meat pH.
- The study indicated the characteristics of the individual cofactors impacted the reduction under various lighting conditions.

Practical Implications

- Incorporating electron donors and carriers in post-harvest enhancement technology has the potential to extend beef color stability.
- Active packaging paired with the cofactors of NMRA have the potential to limit discoloration and increase the shelf life of meat products.

Take-away Message



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References

American Meat Science Association. 2012. AMSA Meat Color Measurement Guidelines: AMSA. American Meat Science Association.
Brown, W. D., & Snyder, H. E. (1969). Nonenzymatic reduction and oxidation of myoglobin and hemoglobin by nicotinamide adenine dinucleotides and flavins. *The Journal of biological chemistry*, *244*(24), 6702.
Carpenter, C. E., Cornforth, D. P., & Whittier, D. (2001). Consumer preferences for beef color and packaging did not affect eating satisfaction. *Meat Science*, *57*(4), 359-363. doi: https://doi.org/10.1016/S0309-1740(00)00111-X
Denzer, M., et. al. 2020. Characterization of the cofactors involved in nonenzymatic metmyoglobin/methemoglobin reduction *in-vitro*.