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 heterogeneity 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.

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.

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

<table>
<thead>
<tr>
<th>Combination</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NADH + MB&lt;sup&gt;1&lt;/sup&gt; + EDTA</td>
</tr>
<tr>
<td>2</td>
<td>NADH + Cyt&lt;sup&gt;-c&lt;/sup&gt;&lt;sup&gt;2&lt;/sup&gt; + EDTA</td>
</tr>
<tr>
<td>3</td>
<td>Ascorbate + MB&lt;sup&gt;1&lt;/sup&gt; + EDTA</td>
</tr>
<tr>
<td>4</td>
<td>Ascorbate + Cyt&lt;sup&gt;-c&lt;/sup&gt;&lt;sup&gt;2&lt;/sup&gt; + EDTA</td>
</tr>
</tbody>
</table>

<sup>1</sup>MB = Methylene blue
<sup>2</sup>Cyt-c = cytochrome c

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

Introduction

Materials and Methods

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

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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

- 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

- 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).

Molecular weight

- NADH – 663.43
- Ascorbate – 198.11
- Methylene blue – 319.85
- Cytochrome C – 884.9

Weights measured in g/mol.
Effects of Light on Non-Enzymatic Metmyoglobin Reduction in-vitro

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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

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

Deoxy/Oxy Myoglobin

Electron Donor
NADH/ascorbate

e-

Electron Carrier

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

References