EFFECTS OF LIGHT ON NONENZYMATIC METMYOGLOBIN REDUCTION IN VITRO

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I. OBJECTIVES

Predominant oxymyoglobin imparts consumer-preferred bright cherry-red meat color, but discoloration on meat negatively impacts purchasing decisions, which can lead to food waste. Discoloration occurs by the oxidation of oxymyoglobin, forming metmyoglobin, a brown pigment. Oxidation can occur more readily in common retail settings such as exposure to light and oxygen (O_2); however, meat has an inherent ability to reduce the brown pigment and form the cherry-red color through metmyoglobin reducing systems. Research has indicated that inherently present electron donors and carriers could contribute to nonenzymatic metmyoglobin reduction. However, limited knowledge is available on the impact of light on nonenzymatic reduction. The objective of this study was to evaluate the effect of light and dark storage conditions on nonenzymatic metmyoglobin reduction *in vitro*.

II. MATERIALS AND METHODS

Solutions of ascorbate and NADH were used as the electron donors and cytochrome *c* (cyt-c) and methylene blue (MB) as the electron carriers. Equine metmyoglobin solution at pH 5.6 was combined with different electron donors and carriers in a 96-well plate. There were 6 treatments used throughout the entirety of the experiment: (1) NADH, (2) NADH + MB, (3) NADH + MB + ethylenediaminetetraacetic acid (EDTA), (4) NADH + cyt-c + EDTA, (5) asborbate + MB + EDTA, and (6) ascorbate + cyt-c + EDTA. To evaluate lighting effects, the well-plate was kept under LED lighting, and readings were taken every 5 min for 25 min using a spectrophotometer set to 582 nm. The preliminary research indicated that 25-min incubation provided consistent results with less variation. The same combinations were evaluated in dark storage with the previously mentioned method. The experiment was replicated 3 times with 6 wells per treatment for each replicate. The method was never repeated on the solutions in a single 96-well plate, as each plate was only evaluated once per 25-min reading, including separate 96-well plates for each light and dark storage reading. Therefore, each treatment was evaluated 18 different times for each respective light condition and 36 times in total. The data were analyzed using the Mixed Procedure of SAS (SAS Institute Inc., Cary, NC) with a completely randomized design.

III. RESULTS

There was a significant treatment by lighting effect on the metmyoglobin reduction. The combination of NADH and MB had an increase ($P < 0.0001$) in metmyoglobin reduction in light conditions compared to dark storage. Furthermore, in the presence of light, NADH + MB + EDTA had an increase ($P < 0.0001$) in metmyoglobin reduction. The metmyoglobin reduction was limited in the presence of NADH + cyt-c in both lighting conditions. Metmyoglobin reduction was significantly higher in the presence of cyt-c and ascorbate compared with cyt-c and NADH for light and dark conditions. In addition, metmyoglobin reduction increased with the presence of light and the combination of ascorbate and MB ($P < 0.001$). The reduction of metmyoglobin in the presence of NADH alone significantly increased in light conditions.

IV. CONCLUSION

In conclusion, the current *in vitro* research demonstrates that inherently present electron donors and carriers can contribute to nonenzymatic metmyoglobin reduction in retail light settings and meat pH. Additionally, the study indicated that the characteristics of the individual cofactors impacted the reduction under various lighting conditions.

Keywords: meat color, metmyoglobin reducing ability, myoglobin