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Characterization of the Cofactors Involved in Non-Enzymatic Metmyoglobin Reduction In Vitro

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Objectives

Consumers' meat purchasing decisions are strongly influenced by color. Myoglobin is the primary meat pigment that contributes to meat color. Myoglobin consists of an iron-containing heme ring and amino acids in the form of globin chains. Both the state of the heme iron and the type of ligand affects meat color. The consumer-preferred bright cherry-red color oxymyoglobin is formed when the iron is in the ferrous state and oxygen bind to the heme. The oxidation of oxymyoglobin or deoxymyoglobin results in the formation of the brown color, ferric metmyoglobin. Predominant metmyoglobin accumulation negatively impacts consumer purchasing choices. Although muscle type and pre- and post-harvest factors can influence meat discoloration, meat has an inherent ability to reduce metmyoglobin through enzymatic pathways, mitochondria-mediated pathways, and non-enzymatic mechanisms. In the enzymatic pathway, an electron from NADH is transferred to metmyoglobin by an enzyme and an electron carrier; while in mitochondria-mediated pathway, an electron from the electron-transport chain is transferred via cytochromes. Previous research speculated the role of non-enzymatic pathway in meat color; however, limited studies have characterized the cofactors present in a meat system. The objectives of this study were to characterize cofactors in non-enzymatic metmyoglobin reduction and determine the effect of storage temperature and postmortem muscle pH *in-vitro*.

Materials and Methods

Purified equine metmyoglobin was reduced in the presence of combinations of electron carriers and donors. Methylene blue and cytochrome *c* were evaluated as the electron carriers, and NADH and ascorbate were considered as the electron donors. The cofactors were held at 4 and 25°C to determine temperature effects on the reduction of metmyoglobin, and the same cofactor combinations were evaluated at pH of 5.2, 5.6, 6.0, and 6.4 to reflect postmortem muscle pH. Spectrophotometry was utilized to monitor the rates of metmyoglobin reduction. The experiments were replicated five times, and the data were analyzed using the Mixed Procedure of SAS.

Results

The results indicated that methylene blue was a significantly more effective electron carrier than cytochrome *c* with both electron donors, ascorbate and NADH. EDTA had no impact on the non-enzymatic metmyoglobin reducing the ability of methylene blue ($P = 0.91$). Temperature and pH had cofactor specific effects on the non-enzymatic reduction of metmyoglobin. Lower temperature resulted in an increased non-enzymatic metmyoglobin reduction for methylene blue regardless of electron donor (ascorbate, $P = 0.03$, NADH, $P = 0.04$). As pH increased, the non-enzymatic metmyoglobin reducing activity reduced significantly in the presence of NADH and methylene blue.

Conclusion

In conclusion, the characteristics of the cofactors at specific temperatures and pH impacted the non-enzymatic reduction of metmyoglobin. Further, current in vitro research indicated that non-enzymatic metmyoglobin reduction is possible at lower temperature and meat pH.