Cyclic voltammetry to determine myoglobin reduction potential and oxygenation

R. Ramanathan, R. Nerimetla, S. Krishnan, D. L. VanOverbeke, G. G. Mafi

STORY IN BRIEF

Myoglobin is the protein primarily responsible for meat color and can exist in three different forms; namely deoxy-, oxy-, or metmyoglobin. Predominant metmyoglobin on the surface gives brown color; however meat has inherent capacity to delay the onset of discoloration. To date, research has been focused on the role of enzymes, mitochondria, and NADH in metmyoglobin reducing capacity. However, it is possible that reduction potential of myoglobin also can influence metmyoglobin reduction. The objectives of the current study were to investigate the usefulness of cyclic voltammetry to determine beef myoglobin reduction potential and to evaluate myoglobin oxygenation at pH 5.6 or 7.4. Myoglobin was isolated from bovine cardiac muscle by gel filtration technique. A CH electrochemical analyzer was used to perform voltammetry. Results from the current study indicate that cyclic voltammetry is a useful tool to determine myoglobin reduction potentials and to quantify myoglobin oxygenation capacity. Application of electrochemical methods will help to probe the myoglobin reduction potential changes in the microenvironment of heme cofactor. Thus, characterizing the interrelationship between myoglobin reduction potential (related to color stability) and myoglobin oxygenation (related to bloom) will increase the body of knowledge related to beef color.

Key Words: Beef color, myoglobin, reduction potential

INTRODUCTION

Meat color is an important quality attribute that influences purchasing decisions. As a result, meat discoloration has been estimated to cost the food industry \$1 billion every year (Smith et al., 2000). Myoglobin is the protein primarily responsible for meat color and can exist in three different forms; namely deoxy-, oxy-, or metmyoglobin. Oxymyoglobin provides a characteristic bright red color, while oxidation of oxy/deoxymyoglobin leads to metmyoglobin formation and meat discoloration. Although various pre- and post-harvest factors can increase myoglobin oxidation, meat has an inherent capacity to delay metmyoglobin accumulation by a process called metmyoglobin reduction. To date, research has been focused on the role of enzymes, mitochondria, and NADH levels in metmyoglobin reducing capacity. However, it is possible that reduction potential of myoglobin also can influence metmyoglobin reduction. The term reduction potential refers to the ability of iron in myoglobin to accept an electron (or to undergo reduction). Nevertheless, no reports have as yet characterized the role of myoglobin reduction potential in beef color. The objectives of the current study were to investigate the usefulness of cyclic voltammetry to determine beef myoglobin reduction potential and to evaluate myoglobin oxygenation at pH 5.6 or 7.4.

MATERIALS AND METHODS

Myoglobin was isolated from bovine cardiac muscle by a gel filtration technique. Briefly, myoglobin solution (0.15 mM) was passed through chromatography columns pre-calibrated with either pH 5.6 or 7.4 buffer to alter myoglobin pH. Metmyoglobin (10 μ L, 0.15 mM) was placed on a high purity graphite electrode, and adsorbed for 10 minutes. A CH electrochemical analyzer was used to perform voltammetry. The myoglobin reduction potential responses were recorded in millivolt (mV) units. For the second objective, a controlled amount of oxygen was allowed to bind with deoxymyoglobin at pH 5.6 or 7.4 using an oxygen mass flow controller. The experimental design was completely randomized and the data were analyzed using Proc Mixed of SAS (n = 3 replications). Least-squares means were separated with a protected pairwise t-test and were considered significant at P < 0.05.

RESULTS AND DISCUSSION

There was a significant effect of pH on myoglobin reduction potential. At pH 5.6, myoglobin reduction potential was -290 mV compared with -330 mV at pH 7.4 (P < 0.05). The reduction potential value provides information about the ability of heme within myoglobin to accept electron. A lower number indicates that the myoglobin has greater capacity for reduction. For the second objective, $0 - 1600 \mu$ M of oxygen was allowed to bind with myoglobin at pH 5.6 or 7.4. The oxygen binding to myoglobin produced electric current and this response was recorded in micro ampere. The greater the oxygen binding, the larger is the resulting current. This relationship was used to determine the myoglobin oxygen affinity. Application of electrochemistry technique, which can probe atomic level properties of myoglobin will broaden fundamental knowledge related to meat color.

LITERATURE CITED

- Faustman, C., and A. L. Phillips. 2001. Measurement of discoloration in fresh meat. Current Protocols in Food Analytical Chemistry. New York: Wiley Ch. F3 Unit F3.3.
- Ledward, D. A. 1985. Post-slaughter influences on the formation of metmyoglobin in beef muscles. Meat Sci. 15:149–171.
- Smith, G. C., Belk, K. E., Sofos, J. N., Tatum, J. D., and S. N. Williams. 2000. Economic implications of improved color stability in beef. In E. A. Decker, C. Faustman, & C. J. Lopez-Bote (Eds.), Antioxidants in muscle foods: Nutritional strategies to improve quality (pp. 397–426). New York: Wiley Interscience.

Copyright 2013 Oklahoma State University