연사 1

Metmyoglobin Reduction through Lactate-NAD-LDH System *In Vivo* and *Vitro*

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Metmyoglobin Reduction Through Lactate-NAD-LDH System *In Vivo* and *Vitro*

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Discoloration of meat surfaces due to brown metmyoglobin (MMb) formation significantly affects consumers' purchase decisions. Hood & Riordan (1973) reported that consumer discrimination against discolored meat was linearly correlated with increases in surface MMb. Oxidized myoglobin can be converted to deoxymyoglobin (DMb) via metmyoglobin reducing activity (MRA). Then, it can be oxygenated back to oxymyoglobin (OMb). It is now well established that reduction of MMb occurs through both enzymatic and non-enzymatic reducing systems and that NADH is an ultimate reducing substrate for both pathways. However, where NADH comes from has not been established. Watts, Kendrick, Zipser, Hutchins, & Saleh (1966) hypothesized that since postrigor meat contains both lactate and lactate dehydrogenase (LDH), hydrogen may be transferred from lactate to NAD by LDH. The reduction of NAD to NADH could be coupled with the reduction of MMb in the presence of intermediate electron carriers such as reductases, quinines, or methylene blue. Mancini, Kim, Hunt, & Lawrence (2004) further reported increased MRA and LDH activity of beef *longissimus* enhanced with 2% lactate.

Therefore, we hypothesize that the lactate-NAD-LDH system is partially responsible for both (1) non-enzymatic and enzymatic MMb reduction of postmortem muscle and (2) the increased color life of lactate-enhanced beef. In two experiments, this study investigated the relationship between MMb reduction and the conversion of lactate and NAD to pyruvate with subsequent production of NADH via LDH.

In experiment 1, assays of nonenzymatic MMb reduction through the lactate-NAD-LDH system were carried out in cuvettes containing: equine MMb, distilled water, FMN, NAD, L-lactic acid, methylene blue, citrate buffer, and LDH. The reaction was initiated by adding LDH to the mixture and measured at 580 nm. Oxalate or D-lactate was added to the mixture to investigate their inhibiting effects on LDH in the MMb reducing system.

In experiment 2, Twelve USDA Select strip loins were enhanced 10% with aqueous solutions containing lactate, phosphate, salt, and/or acetate. Steaks packaged in high-

oxygen MAP were stored for 2 and 9 days and then displayed for 5 days at 1ºC. Visual and instrumental color, MRA, LDH activity in both directions (Lactate ↔ Pyruvate), and NADH were measured.

In experiment 1, the nonenzymatic reduction of MMb occurred via the lactate-LDH-NAD system, but the exclusion of any one of necessary constituents resulted in no reduction reaction. Increasing the amount of NAD and L-lactic in the reaction mixture increased the reduction. Addition of oxalate or D-lactic acid reduced or eliminated MMb reduction. Reduction through the system was optimal at pH 8.0; however, same nonenzymatic reduction occurred at pH of 5.7.

In experiment 2, steaks enhanced with 2.5% lactate showed the least visual discoloration and most color stability during display. Non-enhanced controls and enhanced steaks without lactates were most discolored. Steaks containing 2.5% lactate had significantly more LDH activity in both directions, produced more NADH, and had greater MRA compared with non-enhanced controls throughout display.

These results suggest that NADH can be generated through the lactate-NAD-LDH system *in vivo* and *vitro*, and increased with the addition of lactate to the system. Lactate promotes color stability by the conversion of lactate to pyruvate via increased LDH activity and the concomitant regeneration of NADH. The NADH subsequently reduces metmyoglobin to reduced myoglobin, thus increasing color stability of lactate-enhanced beef.

References

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