INVOLVEMENT OF LACTATE DEHYDROGENASE IN METMYOGLOBIN REDUCTION AND COLOR STABILITY OF DIFFERENT BOVINE MUSCLES

Y.H. Kim*[1], J.T. Keeton[1], S.B. Smith[1]. J.W. Savell[1]

[1]*Department of Animal Science, Texas A&M University, 2471 TAMU, College Station, TX 77843-2471*

Key Words: Color, metmyoglobin reducing activity, lactate dehydrogenase, water-holding capacity, NADH

Introduction

Discoloration of raw retail meat cuts due to the formation of brown metmyoglobin on the meat surface significantly affects consumers' purchasing decisions. Hood and Riordan (1973) reported that consumers discriminate against discolored meat linearly with a corresponding increase in metmyoglobin formation. Metmyoglobin can be converted to deoxy-/oxy-myoglobin through the metmyoglobin reducing activity (MRA) of muscle. Although the general mechanism of the metmyoglobin reduction system per se is well established, the origin of the pool of NADH, an ultimate reducing substrate for the MRA, has not been clearly established. Kim et al. (2006) determined that nonenzymatic metmyoglobin reduction occurred in the lactate-LDH system with NAD^+, but that exclusion of NAD^+, L-lactic acid, or LDH eliminated the MMb reduction. Consequently, they proposed that the lactate-LDH system in post-mortem muscle can generate NADH by the reduction of NAD^+, and that a NADH-dependent reducing system, either enzymatic or non-enzymatic, can reduce metmyoglobin. Therefore, we hypothesize that the variation in color stability of different muscles could be regulated by different rates of replenishment of NADH via different LDH activities. The objectives of this study were to determine the color stability, LDH activity, NADH, and MRA of different bovine muscles, and to investigate the relationship of water-holding capacity (WHC) and pH to color stability of the muscles.

Materials and Methods

Three different bovine muscles – *Longissimus dorsi* (LD), *Semimembranosus* (SM), *Psoas major* (PM) – were fabricated 5d postmortem (n=7 for each muscle), cut into steaks (2.54 cm thick), PVC overwrapped, and then displayed for 7 days at 1°C. Instrumental color (L*, a*, and b*), surface metmyoglobin accumulation, MRA (Watts et al., 1966), LDH activity (Vassault, 1983; Wahlefeld, 1983) in both directions – LDH A and B, water-holding capacity (WHC) (Honikel, 1998), pH, and NADH (BioVision #K337 quantification kit) were measured on day 1, 3, and 7. Main effects of muscle type difference, display time, and their interaction were analyzed using the Mixed procedure of SAS for ANOVA repeated measures. LSMeans was separated ($P < 0.05$) using least significant differences generated by the PDIFF option.

Results and Discussion

Color, color stability, pH, and water-holding capacity: LD was the reddest (highest a* value) muscle, and had the least amount (%) of metmyoglobin accumulation indicating the highest color stability (Fig. 1) among muscles over the display period (7 days). SM followed the LD while PM had the lowest color stability ($P < 0.05$) and was the most discolored over the display period. Muscle pH of PM and LD was significantly higher (5.8 and 5.7, respectively) than that of SM (5.6) throughout the display time. Following the same trend of muscle pH, PM and LD had the significantly higher WHC than SM confirming that higher pH meat retains more water molecules during storage. However, regardless of high pH and WHC, PM had the lowest color stability and most rapid discoloration, which indicates that pH does not solely dictate muscle pigment change under display conditions.

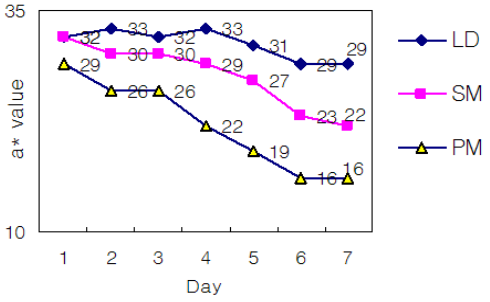

Fig. 1. Color space a* values (redness) of LD, SM, and PM over a 7 day display period.

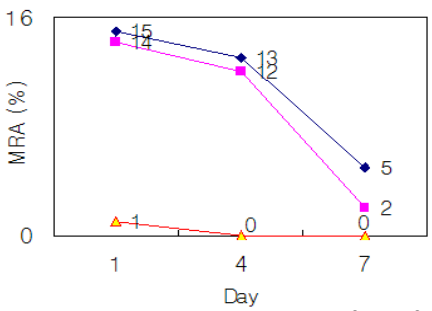

Fig. 2. Percentage MRA on the surface of LD, SM, and PM over a 7 day display period.

MRA and NADH: LD had the highest MRA (Fig. 2) followed closely by SM, while the PM had a lower MRA ($P < 0.05$) throughout the display period. These results support the observation that surface discoloration of muscle due to metmyoglobin formation varies depending upon the level of MRA in each muscle. It is now generally accepted that metmyoglobin reduction in meat occurs primarily through enzymatic pathways with NADH as a coenzyme (Renerre, 1999). NADH is the ultimate source of reducing equivalents, and increasing concentrations of NADH has been shown to result in more reducing activity (Osburn et al., 2003). We found that LD had a significantly higher NADH concentration (Fig. 3) than PM throughout the display period suggesting that NADH may be the most important substrate determining postmortem color stability of physiologically different muscles. Further, reductions in color stability during display appear primarily to be due to the depletion of NADH.

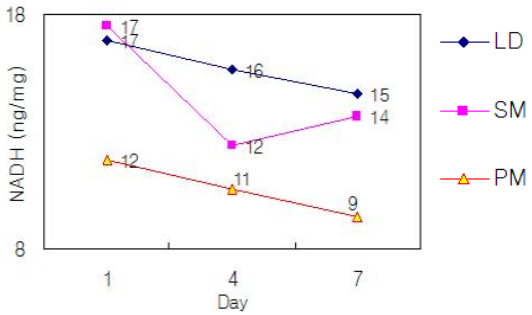

Fig. 3. NADH concentration of LD, SM, and PM over a 7 day display period.

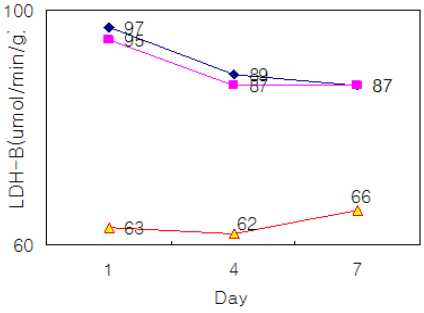

Fig. 4. LDH-B activity (reaction toward NADH production) of LD, SM, and PM during display.

LDH activity: LD had a lower LDH-A activity (Pyruvate + NADH $\longrightarrow$ Lactate + NAD^+) and higher LDH-B activity (Lactate + NAD^+ $\longrightarrow$ Pyruvate + NADH), while PM had significantly higher LDH-A, and lower LDH-B activity compared to LD and SM (Fig. 4). Thus, it can be assumed that the higher concentration of NADH from LD may be due to the higher LDH-B activity, thus favoring the reaction to replenish NADH. In other words, LDH activity of individual muscles may be directly related to the color stability of those muscles post-mortem.

Conclusions

These results suggest that the variation in color stability of physiologically different muscles could be regulated by different rates of replenishment of NADH via different LDH isozymes. LDH influences the metmyoglobin reduction system by replenishing NADH. LD maintained most stable red color, and had the highest MRA, NADH, and LDH-B activities. Although, PM had a higher pH and WHC, it showed the least color stability and lowest MRA possibly due to lower LDH-B activity and subsequently lower NADH regeneration.

References

1. Hood, D. E., and Riordan, E. B. (1973). Discolouration in pre-packaged beef: measurement by reflectance spectrophotometry and shopper discrimination. *Journal of Food Technology*, 8, 333-343.
2. Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science*, 49, 447-457.
3. Kim, Y. H., Hunt, M. C., Mancini, R. A., Seyfert, M., Loughin, T. M., Kropf, D. H., and Smith, J. S. (2006). Mechanism for lactate-color stabilization in injection enhanced beef. *Journal of Agricultural and Food Chemistry*, 54, 7856-7862.
4. Osburn, H. M., Brown, H., Adams, J. B., and Ledward, D. A. (2003). High temperature reduction of metmyoglobin in aqueous muscle extracts. *Meat Science*, 65, 631-637.
5. Renerre, M. (1999). Biochemical basis of fresh meat colour. In Proc. 45th International Congress of Meat Science and Technology. pp 344-353.
6. Vassault, A. (1983). Lactate Dehydrogenase. UV-method with pyruvate and NADH. In Methods of enzymatic analysis, 3rd ed; Bergmeyer HU., Ed.;Plenum: New York, Vol. III, p 118-125.
7. Wahlefeld, A. W. (1983). Lactate Dehydrogenase. UV-method with L-Lactate and NAD. In Methods of enzymatic analysis, 3rd ed; Bergmeyer HU., Ed. Plenum: New York, Vol. III, p 126-132.
8. Watts, B. M., Kendrick, J., Zipser, M. W., Hutchins, B. K., Saleh, B. (1966). Enzymatic reducing pathways in meat. *Journal of Food Science, 31*, 855-861.