HOW DOES LACTATE ENHANCEMENT IMPROVE BEEF COLOR STABILITY?

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Background

In both raw and cooked meat products, lactate limits discoloration by minimizing red color changes during storage and display (Brewer *et al*. 1991; Papadopoulos *et al.* 1991; Prestat *et al*. 2002; Lawrence *et al.*, 2003). However, the mechanism by which lactate improves muscle color stability is unknown.

How does lactate improve muscle color stability?
We speculate that lactate's ability to stabilize muscle color is linked to regeneration of nicotinamide adenine dinucleotide (NADH), which is necessary for metmyoglobin reduction; a reaction that promotes color stability. We believe this link between lactate and NADH regeneration is lactate dehydrogenase (LDH; Figure 1). From a meat quality standpoint, LDH may be an "overlooked" endogenous enzyme that has potential to influence color life.

Metmyoglobin reduction
Oxidation of myoglobin is inevitable; thus, maximizing metmyoglobin reduction (gain of electrons) is essential for color stability. It is widely accepted that NADH donates electrons to metmyoglobin via both enzymatic and nonenzymatic pathways (Brown and Snyder 1969; Hagler *et al*. 1979; Livingston *et al*. 1985; Renerre and Labas 1987; Stewart *et al*. 1965). However, the location of the NADH pool that provides electrons to postmortem metmyoglobin reduction is unknown (Renerre and Labas 1987).

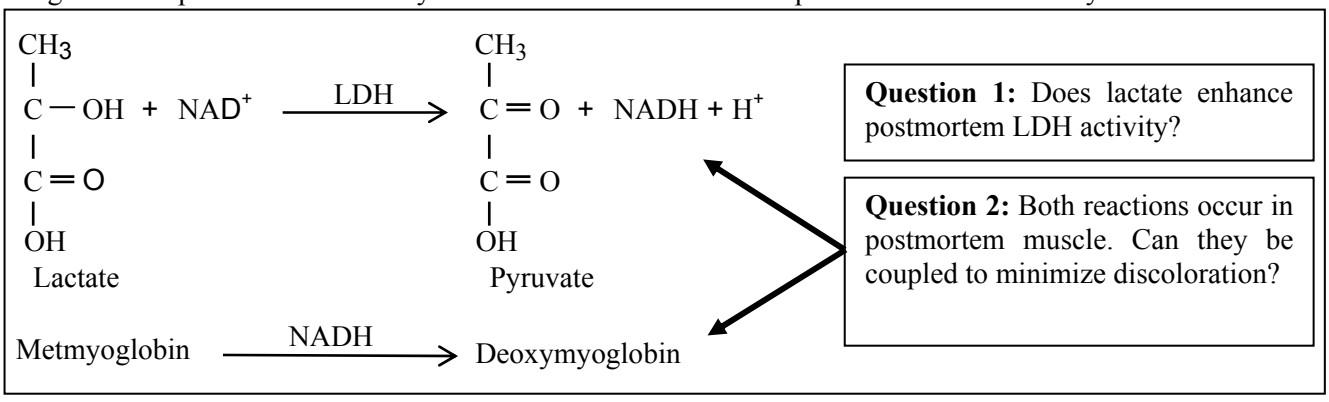
The NADH pool
Stewart *et al*. (1965) provided early speculation regarding replenishment of the NADH pool by suggesting that LDH catalyzes the production of reduced pyridine nucleotides from lactate and diphosphopyridine. These authors believed this mechanism was feasible because both lactate and LDH are abundant in postmortem muscle. However, to date, this mechanism has not been evaluated or proven.

Objectives

Hypothesis: Enhancing beef with lactate increases LDH activity, which consequently improves meat color stability by promoting metmyoglobin reduction (Figure 1).

Objective: Evaluate if enhancement of beef *longissimus* with potassium lactate increases postmortem lactate dehydrogenase and metmyoglobin reducing activity.

Figure 1: Proposed mechanism by which lactate enhancement improves beef color stability.



Materials and methods

Experiment 1: Whole loin enhancement
Five boneless beef strip loins (5 days postmortem) were divided into four equal sections. Each section within a loin was assigned randomly to one of four treatments (1 = no enhancement; 2 = enahancement with water; 3 = 1.5% potassium lactate in the finished product; 4 = 2.3% potassium lactate in the finished product). Strip loin sections were pumped at 10% of their green weight using a multineedle injector. Pumped loin sections were cut into 2.54 cm-thick steaks, overwrapped in polyvinyl chloride (PVC), and displayed for 7 days at 1ºC in open-top display cases under 1614 lux of continuous fluorescent light (3000 K). Lactate dehydrogenase activity was measured on day 1, day 3, and day 7 of display. The experimental design was a split plot where the whole plot design structure was a completely randomized block (n = 5) with a one-way treatment structure consisting of 4 treatments. Within the whole plot, loins served as blocks, and each of four loin sections were the experimental units to which enhancement treatments were applied. Steaks within a loin section (subplot experimental units) were assigned randomly to 1 of 3 display times.

Experiment 2: Model system enhancement
Eight randomly selected beef *longissimus* steaks from different animals were used in the model system. Each steak was divided into three equally sized pieces. To each piece within a steak, one of three treatments was assigned randomly (1 = nonenhanced; 2 = nonenhanced plus LDH inhibitor; and 3 = potassium lactate). Pieces assigned to treatment 3 (lactate) were injected with a syringe to a concentration of 2% lactate in the final product. Oxalate (LDH inhibitor in treatment 2) was added during quantification of LDH activity (described below). Steaks were overwrapped in PVC and stored at 4°C for 7 days. Lactate dehydrogenase activity and metmyoglobin reducing activity were measured after day 1 and day 7 of storage. The experimental design was a randomized complete block (steaks were blocks) with repeated measures. For both experiments, data were analyzed using SAS and significance is represented at $P < 0.05$.

LDH activity
Lactate dehydrogenase activity was measured using a spectrophotometric technique (Wahlefeld 1987). From each loin, a 2.0 g sample was homogenized with 0.01 M potassium buffer (pH 7.2). Homogenates were centrifuged for 30 minutes at 14,600 x *g* (4°C). In brief, β-Nicotinamide-adenine dinucleotide, Tris buffer, and L-Lactate were added to 0.1 mL of homogenized muscle supernatant. After 5 minutes of incubation at 30°C, LDH activity was measured using absorbance at 339 nm, which indicates the production of NADH via lactate oxidation (NAD reduction increases absorbance at 339 nm; L-Lactate + NAD^+ → Pyruvate + NADH + H^+). To determine activity, absorbance at 339 nm was measured at 30-second intervals for 2.5 minutes. Activity in U/L was calculated as: change in absorbance ($\Delta A/\Delta t$) x 4.21 x 10^3. For those samples assigned to treatment 2 (LDH inhibitor), oxalate was added after centrifugation to the supernatant. All assays were performed in duplicate and averaged for statistical analysis.

Metmyoglobin reducing activity
Step 1: Samples (3 x 2 x 1.27 cm^3) were submerged in 0.3% sodium nitrite. After 20 minutes, samples were blotted dry, immediately vacuum packaged, and reflectance from 400 – 700 nm was measured. Step 2: Samples were incubated at 30ºC and reflectance was measured after 2 hours. Percent metmyoglobin was determined according to AMSA (1991) and metmyoglobin reducing activity (MRA) was claculated as: (Observed decrease in metmyoglobin during incubation ÷ initial metmyoglobin concentration) x 100.

Results and discussion

Experiment 1: Whole loin enhancement
Enhancing loins with potassium lactate tended to increase LDH activity (Table 1). On day 7, both 1.5% and 2.3% lactate resulted in signifcantly more LDH activity than enhancement with only water. Using 2.3% lactate significantly increased lactate dehydrogenase activity compared to nonenhanced control loins at the end of display (day 7). Lactate dehydrogenase activity increased during display. Neither treatment influenced muscle pH (Control samples = 5.8 and lactate enhanced samples = 5.9).

Experiment 2: Model system enhancement
Compared to nonenhanced controls at the end of storage (day 7), adding 2.0% potassium lactate to beef *longissimus* significantly improved LDH and metmyoglobin reducing ability (Tables 2 and 3). However,

there was no significant difference between nonenhanced steaks and lactate-enhanced steaks early in storage (day 1). Thus, as storage time increased, the ability of potassium lactate to promote LDH and MRA activity also increased. Conversely, there was no significant change in LDH activity during storage for nonenhanced steaks. As expected, oxalate minimized LDH activity. Muscle pH was not influenced by treatment (pH = 5.9).

Lactate dehydrogenase activity in muscle has been used as an indicator of endpoint temperatures in meat products (Collins *et al*. 1991; Keeton and Morris 1996; Stadler *et al*. 1991 & 1997). However, food-safety researchers commonly measure pyruvate reduction rather than lactate oxidation, which was measured in our experiment. Nevertheless, previous work support our findings that LDH remains active postmortem.

Meat color stability is vital for maximizing consumer purchasing. Product inconsistency and central packaging have driven beef purveyors to utilize enhancement technology aimed at improving shelf life and product quality. In addition to water, salt, and phosphate, beef processors have a vested interest in color-stabilizing, antimicrobial, and palatability-enhancing ingredients such as lactate. Determining mechanisms present in postmortem muscle that support myoglobin reduction may be beneficial for developing future enhancement technologies that improve the shelf life of muscle based food products.

Conclusions

There is a lack of knowledge regarding the mechanism of lactate-induced color stability. The role of metmyoglobin reduction and NADH in meat color stability has been well documented. However, mechanisms in postmortem muscle involved in replenishing the NADH pool have received little attention. It is possible that lactate dehydrogenase is an endogenous enzyme involved in postmortem NADH production.

We conclude that enhancing beef *longissimus* with lactate can influence both postmortem LDH activity and metmyoglobin reducing ability. We speculate that LDH converted postmortem-injected lactate to pyruvate and NADH, which replenished the reducing equivalent pool of postmortem muscle and chemically reduced metmyoglobin (increased MRA). This project suggests that lactate has potential to not only benefit beef processors through improved shelf life, but also to benefit researchers by providing a better understanding of postmortem replenishment of the NADH pool.

References

Brewer M.S., McKeith F.K., Martin S.E., Dallimeir A., and Meyer J. 1991. Sodium lactate effects on shelf life, sensory and chemical characteristics of fresh pork sausage. J. Food Sci. 56:1176-1178.

Brown W.D. and Snyder H.E. 1969. Nonenzymatic reduction and oxidation of myoglobin and hemoglobin by nicotinamide adenine dinucleotides and flavins. J. Biological Chem. 244:6702-6706.

Collins S.S., Keeton J.T., and Smith S.B. 1991. Lactate dehydrogenase activity in bovine muscle as a potential heating endpoint indicator. J. Agric Food Chem 39:1291-1293.

Hagler L., Coppes R.I., and Herman R.H. 1979. Metmyoglobin reductase: Identification and purification of a reduced nicotinamide dinucleotide-dependent enzyme from bovine heart which reduces metmyoglobin. J. Biological Chem. 254:6605-6614.

Keeton J.T. and Morris C.A. 1996. Sarcoplasmic enzymes as potential indicators of heating endpoints in meat products. J. Muscle Foods 7:323-334.

Lawrence T.E., Dikeman M.E., Hunt M.C., Kastner C.L., and Johnson D.E. 2003. Effects of calcium salts on beef longissimus quality. Meat Sci. 64:299-308.

Livingston D.J., McLachlan S.J., LaMar G.N., and Brown W.D. 1985. Myoglobin: Cytochrome b5 interactions and the kinetic mechanism of metmyoglobin reductase. J. Biological Chem. 260:15699-15707.

Papadopoulos L.S., Miller R.K., Ringer L.J., and Cross H.R. 1991. Sodium lactate effect on sensory characteristics, cooked meat color and chemical composition. J. Food Sci. 56:621-626.

Prestat C., Jensen J., Robbins K., Ryan K., Zhu L., McKeith F.K., and Brewer M.S. 2002. Physical and sensory characteristics of precooked, reheated pork chops with enhancement solutions. J. Muscle Foods 13:37-51.

Renerre M. and Labas R. 1987. Biochemical factors influencing metmyoglobin formation in beef muscles. Meat Sci. 19:151-165.

Stadler J.W., Smith G.L., Keeton J.T., and Smith S.B. 1991. Lactate dehydrogenase activity in bovine muscles as a means of determining endpoint temperature. J. Food Sci. 56:895-898.

Stalder J.W., Smith G.L., Keeton J.T., and Smith S.B. 1997. Lactate dehydrogenase activity as an endpoint heating indicator in cooked beef. J. Food Sci. 62:316-320.

Stewart M.R., Hutchins B.K., Zisper M.W., and Watts B.M. 1965. Enzymatic reduction of metmyoglobin by ground beef. J. Food Sci. 30:487-491.

Wahlefeld, A.W. 1987. UV method with L-lactate and NAD. Chapter 2.2.2. In Methods of Enzymatic Analysis. Volume III. Oxidoreductases: Dehydrogenases acting on CHOH groups. p. 126. VCH Publishers, New York.

Table 1: Effects of enhancing beef strip loins with potassium lactate on lactate dehydrogenase activity during display at 1ºC.

Display time (days)	Nonenhanced control	Enhanced with water	1.5% Potassium lactate	2.3% Potassium lactate
1	342.6a	320.2a	379.4ab	407.6b
3	352.3ab	313.4a	358.9ab	386.6b
7	816.7ab	770.9a	858.3bc	916.7c
Average[d]	503.8b	468.2a	532.2bc	570.3c

[abc]Least square means (activity determined via NADH production and expressed as U/L) within a row with a different letter differ ($P < 0.05$).

[d]Least square means averaged across display times.

Table 2: Effects of potassium lactate on beef *longissimus* lactate dehydrogenase activity during storage.

Storage time at 4ºC	Nonenhanced control	2.0% Potassium lactate	Oxalate*
1 days	146.6ay	156.6ay	100.8by
7 days	190.0by	223.4az	120.0cy

[abc]Treatment effects: least square means within a row with a different letter differ ($P < 0.05$).
[yz]Storage time effects: least square means within a column with a different letter differ ($P < 0.05$).
**Longissimus* was enhanced with lactate and oxalate was added during quantification of LDH activity. Activity determined via NADH production and expressed as U/L.

Table 3: Effects of potassium lactate on beef *longissimus* metmyoglobin reducing activity* during storage.

Storage time at 4ºC	Nonenhanced control	2.0% Potassium lactate
1 days	88.5a	85.2a
7 days	51.3c	70.8b

[ab]Least square means with a different letter differ ($P < 0.05$).
*Metmyoglobin reducing activity (MRA) was calculated as: (Observed decrease in metmyoglobin during incubation ÷ initial metmyoglobin concentration) x 100.