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Effects of L- or D-lactate-enhancement on the internal cooked colour development and biochemical characteristics of beef steaks in high-oxygen modified atmosphere

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ABSTRACT

The effects of L- or D-lactate on internal cooked colour development of steaks packaged in high-oxygen (80% O_2/20% CO_2) modified atmosphere packaging (MAP) was investigated. Ten USDA Select beef strip loins were divided individually into 4 equal-width sections, and one of four treatments (control, 0.3% sodium tripolyphosphate, 2.5% L-lactate + 0.3% sodium tripolyphosphate, and 2.5% D-lactate + 0.3% sodium tripolyphosphate) was assigned randomly to the loin sections. Loin sections were injected to approximately 10% of their raw weight. Steaks packaged in high-oxygen MAP were stored in the dark at 1 °C for 10 days. Instrumental internal colour of raw and cooked steaks (70 °C), total reducing activity (TRA), NADH concentration, and percent myoglobin denaturation (PMD) were measured. Cooked steaks enhanced with 2.5% L-lactate/phosphate maintained higher a^*/b^* ratios, lower hue values, higher TRA and NADH concentration, and lower PMD than the control and D-lactate-injected steaks, whereas enhancement with 2.5% D-lactate did not affect cooked colour, TRA, NADH, or PMD. Thus, inclusion of an L-lactate/alkaline phosphate blend increased the reducing activity of muscle tissues by replenishing NADH and subsequently decreased the thermal denaturation of myoglobin by maintaining the reduced state of myoglobin in the high-oxygen package.

1. Introduction

Modified atmosphere packaging (MAP) systems with a high-oxygen (80%) level are used widely in retail meat markets because they sustain the bright-red colour of meat, which consumers find attractive. However, high oxygen levels are likely to increase the incidence of oxidative changes in meat and consequently accelerate muscle surface discolouration, leading to decrease in the desirable flavour and tenderness of meat (Grobbel, Dikeman, Hunt, & Milliken, 2008; Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007). Furthermore, meat with higher concentrations of oxymyoglobin (OMb) or metmyoglobin (MMb) can develop brown colour quicker at a relatively lower cooking temperature resulting in "premature browning" of cooked meat (Hunt, Sørheim, & Slinde, 1999; King & Whyte, 2006; Seyfert, Hunt, Mancini, Kropf, & Stroda, 2004; Warren, Hunt, & Kropf, 1996). Consequently, premature browning defects could pose significant food safety issues if consumers were to rely solely on the appearance of cooked internal meat colour as a measure of degree of doneness.

Lactate injection is used commonly in fresh and processed meat products as a microbial inhibitor (Brewer, Rostogi, Argoudelis, & Sprouls, 1995; Choi & Chin, 2003; Maca, Miller, Bigner, Lucia, & Acuff, 1999; Papadopoulos, Miller, Acuff, Vanderzant, & Cross, 1991a), and to increase juiciness, flavour, and tenderness (Lawrence, Dikeman, Hunt, Kastner, & Johnson, 2004; Papadopoulos, Miller, Ringer, & Cross, 1991b). Lactate inclusion also has been shown to improve colour stability of fresh beef by prolonging the reduced state of myoglobin through its superior antioxidant capacity (Kim et al., 2009b, 2009c; Mancini & Ramanathan, 2008) and increased reducing activity of myoglobin (Kim et al., 2006, 2009b). Kim et al. (2006) determined that lactate-enhancement promoted colour stability by the conversion of lactate to pyruvate via increased flux through lactate dehydrogenase (LDH) and the concomitant regeneration of NADH – a source of reducing power for metmyoglobin reducing activity. Thus, it can be hypothesised that lactate inclusion may reduce premature browning by increasing MMb-reducing activity through increased NADH concentration by coupling reactions with LDH in muscle tissue. Injection of muscle with D-lactate will test this proposed hypothesis, because LDH only reacts with L-lactate to regenerate NADH. The objective of this study, therefore, was to determine the effects of L- or D-lactate

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inclusion on internal cooked colour development and other biochemical characteristics of beef steaks packaged in high-oxygen MAP.

2. Materials and methods

2.1. Raw materials and processing

USDA (1997) Select (A-maturity) beef strip loins (n = 10; *M. longissimus lumborum*) were obtained from a commercial meat processing facility. At 4 d postmortem, each strip loin was cut into four equal-length sections and trimmed of all subcutaneous fat. One of four treatments, including (1) non-injected control (CON), (2) no lactate control (NLAC) – containing 0.3% sodium tripolyphosphate (Brifisol® STP; BK Giulini Corp; Simi Valley, CA), (3) 2.5% L-Lactate (L-LAC) – potassium L-lactate (PURASAL HiPure P, 60% potassium L-lactate/40% water; PURAC America, Inc., Lincolnshire, IL) + 0.3% sodium tripolyphosphate, and (4) 2.5% D-Lactate (D-LAC) – potassium D-lactate (SKr-2007-125, 60% potassium D-lactate/40% water; PURAC America, Inc., Lincolnshire, IL) + 0.3% sodium tripolyphosphate, was assigned randomly to one of four muscle sections (n = 10 strip loin sections/treatment). Injection enhancement was applied to assigned muscles (120% of raw weight) by using a multi-needle injector (model N30, Wolftec, Inc., Werther, Germany), and actual injection levels (approximately 10%) were calculated by weighing each injected muscle before and 30 min after injection. Individual injected muscle sections were sliced into 2.54-cm-thick steaks for MAP.

2.2. Packaging

Steaks were placed in 22.5 cm × 17.3 cm × 4.1 cm, preformed trays (polypropylene/ethylene vinyl alcohol; Rock-Tenn, Franklin Park, IL, USA), packaged in a high-oxygen atmosphere (80% O_2/20% CO_2, Certified Standard within ±2%, Airgas Specialty Gases; Austin, TX, USA) with a Ross Inpack Jr. (Model S3180; Ross Industries, Inc., Midland, VA, USA). A shrinkable, 1.5-mil high-barrier sealing film (MAP-Shield AF; nylon/ethylene vinyl alcohol/methallocenece polyethylene with an oxygen-transmission rate of 0.02 cc/645.16 cm^2/24 h at 10 °C and 80% relative humidity and a water-vapour transmission rate of 0.92 g/645.16 cm^2/24 h at 37.8 °C and 100% relative humidity; Honeywell, Morristown, NJ, USA) was used. Packages were stored in the dark at 2 °C for 10 d prior to determining the headspace oxygen/carbon dioxide gas composition (PBI Dansensor, Glen Rock, NJ, USA), raw and cooked colour, and other biochemical analyses.

2.3. Raw and cooked steak instrumental colour

Packages were opened after 10 d storage, and each steak was cut in half perpendicular to the meat surface. One half of the steak was divided horizontally through the middle, and instrumental colour (CIE L^*, a^*, b^* for Illuminant A) was measured immediately using a HunterLab MiniScan™XE Spectrophotometer (Model 45/0 LAV; Hunter Associates Laboratory, Inc., Reston, VA, USA) with Illuminant A, 10° standard observer, and 3.18-cm-diameter aperture. Reflectance from 400 to 700 nm at 10-nm increments and CIE L^*, a^*, and b^* values were measured and used to calculate hue angle $[(b^*/a^*)^{\tan^{-1}}]$ and a^*/b^* ratio (AMSA, 1991). The other half of the steak was cooked on an electric grill (Hamilton Beach Indoor/Outdoor Grill; Hamilton Beach/Proctor Silex, Inc., Southern Pines, NC, USA), turned at 35 °C, and cooked to 70 °C as monitored by an Omega trendicator (Omega Engineering, Inc., Stamford, CT, USA). Cooked steaks were divided horizontally through the middle, and instrumental colour analysis (same as for raw steak measurements) was performed by scanning two different locations per steak, which were averaged for statistical analyses.

2.4. Sample preparation for biochemical analysis

Raw and cooked steak samples from each treatment after storage were trimmed free of subcutaneous and seam fat and any visible connective tissue, frozen in liquid nitrogen, pulverised in a Waring® table-top blender (Dynamics Corporation of America, New Hartford, CT, USA), and stored at −80 °C until used for all biochemical analysis.

2.5. pH determination

Approximately 5 g of pulverised raw steak, which had been previously stored at −80 °C, were combined with 20 ml of distilled water, and blended for 20 s. Then, pH values were determined with a pre-calibrated (pH 4.0–7.0) combination pH electrode attached to a pH metre (Accumet 50; Fisher Scientific, Fair Lawn, NJ, USA).

2.6. NADH concentration

Concentrations of NADH in raw steak samples were determined after an alkaline extraction of NADH as described by Klingenberg (1974). Sample preparation consisted of combining 1 g of frozen sample in 8 ml of 0.5 M alcoholic KOH solution, vortexing for 30 s, agitating in a water bath for 5 min at 90 °C, and cooling rapidly to 0 °C in a −80 °C freezer. Then, 6 ml of a triethanolamine-HCl-phosphate mixture was added to the muscle mixture to neutralise (pH 7.8). After holding at room temperature for 10 min to flocculate the denatured protein, the mixture was centrifuged at 25,000× g for 10 min at 4 °C (J2-21; Beckman Instruments, Inc., Palo Alto, CA), and the supernatant filtered through Whatman # 42 filter paper (Whatman, Inc., Clifton, NJ, USA). Reduction of 2,6-dichlorophenolindophenol (DCIP) by muscle extracts followed a modified assay of McCormick and Lemuel (1971), and absorbance was measured at 600 nm (Beckman DU-7; Beckman Coulter, Inc., Fullerton, CA, USA) to determine NADH. Concentrations of NADH (nmol/g) were calculated based on the equation obtained from the standard curve using known NADH concentrations.

2.7. Total reducing activity

Total reducing activity (TRA) of raw meat samples was determined by the method of Lee, Cassens, and Fennema (1981). Briefly, 2 g of pulverised frozen muscle tissue were homogenised in 10 ml of 25 mM PIPES (piperazine-*n*,*n*-bis-2-ethane-sulfonic acid) buffer. Then, 5 ml of homogenate were mixed with 2 ml of 5 mM potassium ferricyanide, stored at 2 °C for 1 h with occasional vortexing, before 0.1 ml of 0.5% ammonium sulphamate and 0.2 ml of 0.5 M lead acetate were added. After standing 5 min at room temperature, the homogenate mixture was blended with 2.5 ml of 20% trichloroacetic acid, and the solution was filled to volume (10 ml) with distilled water. The solution was filtered through Whatman # 42 filter paper (Whatman, Inc., Clifton, NJ, USA) and absorbance of the filtrate was read at 420 nm using a Beckman DU-7 (Beckman Coulter, Inc., Fullerton, CA) spectrophotometer. A solution of 1 mM potassium ferricyanide was read as a standard, and TRA (an arbitrary value) was calculated by the following formula: [absorbance of 1 mM potassium ferricyanide – absorbance of sample filtrate].

2.8. Percent myoglobin denaturation

Undenatured (raw meat) and denatured (cooked) myoglobin were extracted from 5 g of pulverised frozen muscle tissue by blending for 1 min with 50 ml of 0.04 M potassium phosphate

buffer (pH 6.8) as described by Warriss (1979) and Hunt et al. (1999). After holding in a dark cold room (4 °C) for 1 h, the muscle extract was centrifuged 15,000× for 30 min at 4 °C (J2-21; Beckman Instruments, Inc., Palo Alto, California), and the supernatant was filtered through Whatman # 42 filter paper (Whatman, Inc., Clifton, NJ, USA). Absorbance of the filtrate was read at 525, 572, and 700 nm using a Beckman DU-7 (Beckman Coulter, Inc., Fullerton, CA) spectrophotometer. Concentration of percent myoglobin denaturation (PMD) due to heating was calculated using the formula of Krzywicki (1979):

$$\text{Myoglobin (mg/mL)} = (A_{525} - A_{700}) \times 2.303 \times \text{dilution factor}$$

$$\text{PMD} = [1 - (\text{myoglobin concentration after heating/myoglobin concentration before heating})] \times 100$$

2.9. Data analysis

Data were analysed as a split-plot design, where the 10 strip loins served as the whole-plot portion to which 1 of 4 treatments were assigned to 40 strip loin sections within a muscle [$n = 10$; (10 loins × 4 sections per loin)/4 treatments]. In the subplot, each steak from the strip loin sections was used as an experimental unit. Data were analysed using PROC MIXED of SAS (SAS, 2007), and least squares means were separated (F test, $P < 0.05$) using least significant differences.

3. Results and discussion

3.1. Gas composition and pH

The average gas composition of each package at the end of storage was 76.4 ± 1.8% oxygen; thus, a high-oxygen modified atmosphere was maintained during the storage. Injection enhancement of beef steaks increased ($P < 0.05$) pH over a range of 5.70–5.78 when compared to CON (5.47; Table 1). Increased pH of enhanced steaks was due likely to the effect of the alkaline phosphates (Trout & Schmidt, 1984). Steaks injected with L-LAC had a higher ($P < 0.05$) pH than NLAC steaks, but pH did not differ ($P > 0.05$) between L-LAC and D-LAC steaks.

3.2. Instrumental colour

Internal L^* values (lightness) for raw steaks were not ($P > 0.05$) different among treatments; however, internal a^* values (redness) and b^* values (yellowness) of the raw L-LAC steaks were lower ($P < 0.05$) than all other treatments (Table 2). The decreased redness of L-LAC steaks was probably due to the formation of deoxymyoglobin (DMb), which is a purplish-red colour. Other studies also have reported that lactate-enhanced beef muscles formed the reduced state of myoglobin, most likely DMb (Kim et al., 2006; Knock et al., 2006; Mancini et al., 2005). The D-LAC steaks had a higher ($P < 0.05$) a^* value than the L-LAC steaks, suggesting that different isoforms of lactate may affect the alteration of myoglobin redox state differently.

The cooked internal L^* and b^* values for the L-LAC steaks were lower ($P < 0.05$) than other treatments (Table 2). Although the internal cooked a^* values did not differ ($P > 0.05$) among treatments, the L-LAC steaks had higher ($P < 0.05$) a^*/b^* ratios (higher values indicate more redness; Fig. 1) and lower ($P < 0.05$) hue angles ($(b^*/a^*)^{\tan^{-1}}$, lower values indicate more redness; Fig. 2). The NLAC steaks also had higher ($P < 0.05$) a^*/b^* ratios and lower ($P < 0.05$) hue angles than either CON or D-LAC steaks, which was most likely due to higher pH of the alkaline phosphate blend in the solution. Trout (1989) reported that the increased redness of cooked beef muscles enhanced with sodium tripolyphosphate was caused by the high pH condition markedly reducing the amount of myoglobin denaturation during cooking.

Interestingly, the D-LAC steaks had lower ($P < 0.05$) a^*/b^* ratios and higher ($P < 0.05$) hue angle values than L-LAC steaks, but a^*/b^*

Table 1
Least squares means for pH, total reducing activity (TRA), and NADH concentration of raw bovine *M. longissimus lumborum* steaks, and percent myoglobin denaturation (PMD) of cooked (70 °C) bovine *M. longissimus lumborum* steaks packaged in a high-oxygen modified atmosphere and stored 10 days at 1 °C.

Trait	Treatment[d]				
	CON	NLAC	L-LAC	D-LAC	SE[e]
pH	5.47[a]	5.70[b]	5.78[c]	5.76[bc]	0.02
TRA[f]	0.26[a]	0.26[a]	0.28[b]	0.25[a]	0.008
NADH[g]	25.59[a]	31.0[ab]	34.72[b]	25.17[a]	2.24
PMD[h]	70.1[a]	56.3b[c]	51.9[c]	63.4[ab]	3.4

[abc]Within a row, least squares means lacking common letters are different ($P < 0.05$).
[d]CON = non-enhanced control; NLAC = enhanced with 0.3% sodium tripolyphosphate only; L-LAC = enhanced with 2.5% potassium L-lactate and 0.3% sodium tripolyphosphate; and D-LAC = enhanced with 2.5% potassium D-lactate and 0.3% sodium tripolyphosphate.
[e]Standard error.
[f]TRA = total reducing activity (unitless value).
[g]NADH concentration (nmol/g).
[h]PMD = percent myoglobin denaturation (%).

Table 2
Least squares means for instrumental colour values of the internal surfaces of raw and cooked (70 °C) bovine *M. longissimus lumborum* steaks packaged in a high-oxygen modified atmosphere and stored 10 days at 1 °C.

Trait	Treatment[d]				
	CON	NLAC	L-LAC	D-LAC	SE[c]
Raw					
L^*	43.5[a]	44.4[a]	42.5[a]	45.1[a]	0.9
a^*	28.67[a]	27.3[ab]	21.8[c]	26.0[b]	0.8
b^*	20.4[a]	19.1[a]	14.8[c]	17.2[b]	0.6
Cooked					
L^*	55.7[a]	55.5[a]	50.4[b]	54.7[a]	1.3
a^*	11.94[a]	14.07[a]	13.84[a]	12.83[a]	0.9
b^*	17.5[a]	17.5[a]	14.9[b]	16.5[a]	0.5

[ab]Within a row, least squares means lacking common letters are different ($P < 0.05$).
[c]Standard error.
[d]CON = non-enhanced control; NLAC = enhanced with 0.3% sodium tripolyphosphate only; L-LAC = enhanced with 2.5% potassium L-lactate and 0.3% sodium tripolyphosphate; and D-LAC = enhanced with 2.5% potassium D-lactate and 0.3% sodium tripolyphosphate.

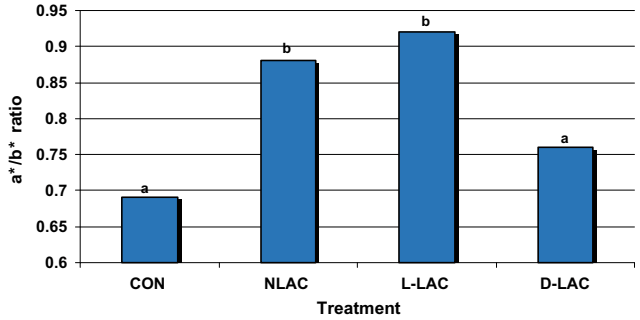

Fig. 1. Least squares means for a^*/b^* ratios of the internal surfaces of cooked (70 °C) bovine *M. longissimus lumborum* steaks packaged in a high-oxygen modified atmosphere and stored 10 days at 1 °C. [ab]Means with different letters are different ($P < 0.05$). Standard error of the mean was 0.052. CON = non-enhanced control; NLAC = enhanced with 0.3% sodium tripolyphosphate only; L-LAC = enhanced with 2.5% potassium L-lactate and 0.3% sodium tripolyphosphate; and D-LAC = enhanced with 2.5% potassium D-lactate and 0.3% sodium tripolyphosphate.

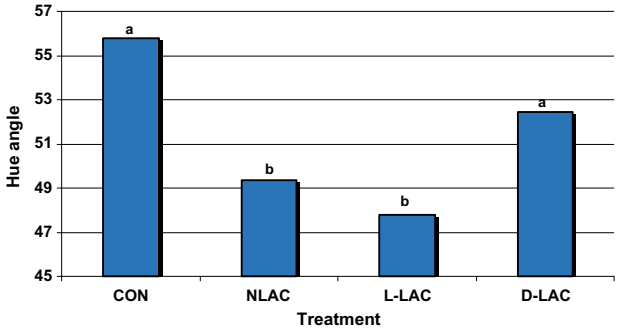

Fig. 2. Least squares means for hue angle $[(b^*/a^*)^{\tan^{-1}}]$ values of the internal surfaces of cooked (70 °C) bovine *M. longissimus lumborum* steaks packaged in a high-oxygen modified atmosphere and stored 10 days at 1 °C. [ab]Means with different letters are different ($P < 0.05$). Standard error of the mean was 1.6. CON = non-enhanced control; NLAC = enhanced with 0.3% sodium tripolyphosphate only; L-LAC = enhanced with 2.5% potassium L-lactate and 0.3% sodium tripolyphosphate; and D-LAC = enhanced with 2.5% potassium D-lactate and 0.3% sodium tripolyphosphate.

ratios and hue angles were similar ($P > 0.05$) to CON steaks, regardless of its elevated pH. This phenomenon suggests some other mechanisms besides the pH effect are involved in the thermal resistance of myoglobin denaturation in meat enhanced with lactate. The two different isomers of lactate (L- and D-lactate) are distinguished by the selective reaction of muscle LDH for the L- isomer. The oxidation of L-lactate is catalysed by LDH, in the presence of NAD^+, producing pyruvate and NADH (a reducing equivalent). In postmortem muscles, LDH-A activity (reaction toward lactate) is predominant over LDH-B activity (reaction toward pyruvate) due to its low K_m and high V_{max} (Wahlefeld, 1983). However, Kim et al. (2006) reported an increased LDH-B flux in beef muscle enhanced with lactate. These authors reported that exogenous lactate addition to postmortem muscle resulted in increased oxidation of lactate and NAD^+ to pyruvate and NADH via elevated LDH-B flux, resulting in a prolonged reduced state of myoglobin (presumably DMb). Different chemical states of myoglobin inside raw meat tissue affect the internal cooked colour development (Hunt et al., 1999; Warren et al., 1996). Hunt et al. (1999) found that ground beef containing predominantly DMb only appeared red at 55 °C, suggesting that DMb was the least sensitive to heat denaturation followed by OMb and MMb.

3.3. NADH concentration

There were differences in NADH concentrations in the raw portion of steaks among the treatments (Table 1). The L-LAC steaks had higher ($P < 0.05$) NADH concentrations than CON and D-LAC steaks, but NADH concentrations of NLAC steaks were similar ($P > 0.05$) to those of CON and D-LAC steaks. The generation of NADH through the lactate-LDH system in postmortem muscle was hypothesised initially by Watts, Kendrick, Zipser, Hutchins, and Saleh (1966), and was tested by Kim et al. (2006), both *in vitro* and in intact muscle systems. Kim et al. (2006) demonstrated increased MMb reduction in a model cuvette system containing lactate, NAD^+, and LDH with a higher concentration of L-lactate. When D-lactate replaced L-lactate in the model system, almost no reduction of MMb occurred. In agreement with the test cuvette system of Kim et al. (2006), the present study demonstrated that postmortem muscle reacts differently to the two lactate isoforms. Thus, it would be reasonable to assume that postmortem LDH distinguished the two isoforms of lactate, then coupled with exogenous L-lactate, and generated NADH. The role of NADH in the MMb-reducing system has been well studied (Arihara, Cassens, Greaser, Luchansky, & Mozdziak, 1995; Bekhit & Faustman, 2005; Giddings, 1974). Bekhit and Faustman (2005) showed that NADH can reduce MMb to the reduced state of myoglobin through either enzymatic reduction (mainly NADH-cytochrome b5 reductase) or a non-enzymatic system in the presence of electron carriers. Although the involvement of NADH in the MMb-reducing system is well known, the origin of the pool of NADH has not been clearly understood. Kim, Keeton, Smith, Berghman, and Savell (2009a) suggested that the variation in colour stability of different bovine muscles may be regulated by different NADH supply rates through different LDH flux. They found that beef *M. longissimus thoracis et lumborum* was a more colour stable muscle, having higher MMb-reducing activity, greater NADH concentrations, and higher LDH flux compared to the *M. psoas major*, concluding that LDH may be involved in the MMb-reduction system by replenishing NADH.

3.4. Total reducing activity

Following a similar trend of increasing NADH concentration with the addition of L-lactate, the TRA of L-LAC steaks were higher ($P < 0.05$) than all other treatments (Table 1). In contrast, the TRA of D-LAC steaks did not differ ($P > 0.05$) from the CON and NLAC steaks, indicating that L-lactate was metabolized differently than D-lactate within postmortem muscle in the reducing system. An increase in TRA and a decrease in the lipid oxidation of ground beef when treated with food-grade reducing agents, such as sodium erythorbate, sodium ascorbate, and ascorbyl palmitate, was reported by Sepe et al. (2005). They determined that the addition of reducing agents to ground beef can prevent premature browning of ground beef by maintaining myoglobin in the DMb state. The chemical state of myoglobin before cooking plays a very important role in the development of the internal cooked colour of meat (Hague et al., 1994; Hunt et al., 1999).

3.5. Percent myoglobin denaturation

The PMD of the L-LAC steaks was lower ($P < 0.05$) than the PMD of CON and D-LAC steaks (Table 1). Moreover, the PMD of NLAC steaks was lower ($P < 0.05$) than CON steaks, but PMD did not ($P > 0.05$) differ between NLAC and D-LAC steaks. The lower PMD of the NLAC steaks was probably in response to the elevated pH caused by phosphate addition. Trout (1989) found reduced PMD of high pH meats by the addition of sodium tripolyphosphate. Interestingly, the PMD of the D-LAC steaks was not different ($P > 0.05$) from that of the CON steaks despite a greater pH value. The trend of PMD results was in agreement with the a^*/b^* ratios and hue angle values, suggesting that, although pH plays an important role in internal cooked colour development, it is not solely responsible for the PMD and myoglobin redox stability. D-lactate addition to muscle might offset the increased pH effect in the redox state of myoglobin.

4. Conclusions

In this study, L-LAC steaks stored in a high-oxygen MAP had the lowest L^* values, as well as higher a^*/b^* ratios and lower hue angles than CON and D-LAC steaks. Moreover, L-LAC steaks packaged in the high-oxygen MAP appeared more typical of the internal colour of cooked of a medium degree of doneness temperature (70 °C). These results suggest that L-lactate/phosphate enhancement increases the reducing activity of beef steaks by replenishing NADH, and subsequently decreases the amount of myoglobin denaturation by maintaining a reduced state of myoglobin. Difference in the internal cooked colour development and biochemical charac-

teristics of steaks enhanced with either L- or D-lactate suggest that the lactate-LDH coupling reaction may be the possible mechanism for the lactate-enhanced beef colour development.

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