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Evaluation of antioxidant capacity and colour stability of calcium lactate enhancement on fresh beef under highly oxidising conditions

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ABSTRACT

Fifteen USDA Select beef strip loins were divided individually into four equal width sections, and one of six treatments containing phosphate and/or calcium lactate (CAL) enhancement solutions were assigned randomly to each loin section ($n = 10$). Steaks from each loin section were packaged with high-oxygen (80% O_2) modified-atmosphere packaging, and/or irradiated at 2.4 kGy, stored 10 days and then displayed for 5 days at 1 °C. Instrumental colour, total reducing activity (TRA), 2-thiobarbituric acid value (TBARS), and NADH concentration were measured. Loins with CAL and phosphate maintained the most stable red colour, increased NADH ($p < 0.05$), and were the least oxidised. Among irradiated steaks, CAL with phosphate treatment significantly minimised lipid oxidation, increased NADH and TRA, and consequently had a higher a^* value. These results suggest that lactate inclusion improves colour stability of fresh beef by providing superior antioxidant capacity and increased reducing activity of myoglobin by elevating NADH concentration.

1. Introduction

Injection enhancement of beef and pork muscles with brine solutions or marinades has been widely practiced in the value-added meat industry. The calcium salts of lactic acid are commonly incorporated into both raw and cooked meat products as microbial inhibitors, as well as to minimise off-odours and flavours, increase water-holding capacity and cook yield, and improve juiciness and tenderness (Devatkal & Mendiratta, 2001; Lawrence, Dikeman, Hunt, Kastner, & Johnson, 2003, 2004; Weaver & Shelef, 1993). Infusion of lactate by injection enhancement has also been recognised to stabilise the colour of both raw and cooked meat products (Kim et al., 2006; Lawrence et al., 2004; Maca, Miller, Bigner, Lucia, & Acuff, 1999; Naveena, Sen, Muthukumar, Vaithiyanathan, & Babji, 2006; Sawyer, Apple, & Johnson, 2008). However, the exact mechanism by which lactate improves meat colour stability has not been fully explained.

Meat colour is the one of the most important factors affecting consumer purchasing decisions (Hood, 1980). Meat discolouration due to formation of metmyoglobin (MMb), the oxidised form of myoglobin, is considered to indicate an inferior or old product. Hood and Riordan (1973) found a 50% reduction in retail sales of beef when MMb formation reached 20% of the total pigment present. The formation of MMb resulting from the oxidation of ferrous haem proteins is positively associated with lipid oxidation (Chan, Faustman, Yin, & Decker, 1997). It has been suggested that free radicals produced by lipid oxidation can initiate the reaction of oxymyoglobin (OMb) to MMb (Yin & Faustman, 1994). Furthermore, 4-hydroxynonenal (4-HNE), a secondary aldehyde product from lipid oxidation, accelerated MMb formation through the covalent modification of histidine residues of myoglobin (Lee, Phillips, Liebler, & Faustman, 2003).

The oxidised form of myoglobin can be converted to the reduced state through a nicotinamide adenine dinucleotide (NADH)-dependent muscle reducing system (Bekhit & Faustman, 2005). Ledward (1985) suggested that metmyoglobin reducing activity (MRA) is the most important intrinsic factor controlling the rate of MMb accumulation in beef. Reddy and Carpenter (1991) reported that the higher the MRA of muscles, the greater the colour stabilities. Because the reducing system is NADH-dependent, the depletion of NADH during storage and/or display periods results in decreasing MRA, and increased surface discolouration caused by MMb accumulation (Kim, Keeton, Smith, & Savell, 2007). However, it also has been reported that MRA can be increased with NADH regeneration by several dehydrogenases in the cytoplasm with appropriate substrates (Arihara, Itoh, & Kondo, 1996; Bodwell, Pearson, & Fennell, 1965; Pong, Chiou, Ho, & Jiang, 2000; Watts, Kendrick, Zipser, Hutchins, & Saleh, 1966). Kim et al. (2006) determined increased colour stability of beef *longissimus*

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steaks injection-enhanced with 2.5% potassium lactate solution. They concluded that lactate enhancement improved colour stability, by replenishing NADH through the increased LDH flux (reaction toward pyruvate), and consequently increasing MRA.

We hypothesise that calcium lactate improves colour stability of fresh beef by hindering oxidation of myoglobin *via* increased reducing capacity and enhancing antioxidant properties. Thus, the objectives of this study in two experiments were: (1) to evaluate MMb reduction through the generated NADH *via* the reaction between calcium lactate and muscle LDH *in vitro*, and (2) to determine the influence of calcium lactate enhancement on colour stability and lipid oxidation of bovine muscles under highly oxidising conditions *in situ*.

2. Materials and methods

2.1. Experiment 1: equine MMb reduction by calcium lactate–NAD–LDH system

2.1.1. Reagents

Horse heart metmyoglobin, NAD, calcium L-lactate, 2,6-dichlorophenolindophenol (DCIP), and phenazine methosulphate (PMS) were obtained from Sigma (St. Louis, MO).

2.1.2. Reduction of equine MMb

The reduction of equine MMb through calcium lactate (CaLac) with muscle extract was tested by following the modification method of Kim et al. (2006). Reactants in various combinations were added to 10 mm path length polystyrene cuvettes with 1.0 ml final reaction volume. The standard reaction mixtures at pH 5.7 contained one or more of the following (Table 1): 0.3 ml of 0.5 mM equine MMb in 30 mM phosphate buffer pH 7.0, 0.1 ml of citrate buffer (50 mM), 0.175 ml of distilled water, 0.025 ml of 30 mM PMS, 0.1 ml of bovine *longissimus* muscle extract, 0.1 ml of 6.5 mM NAD, 0.1 ml of 200 mM calcium lactate, and 0.1 ml of 0.6 mM DCIP.

The effects of different CaLac concentrations (50, 100, 150, and 200 mM) and different assay pH on the rate of MMb reduction were determined. The final assay pH (4.9, 5.4, 5.7, and 6.7) was varied by altering the concentration of the citrate (100, 80, or 50 mM, respectively) or by adding sodium phosphate buffer (pH 8.3, 30 mM). The bovine muscle extract was prepared previously; in brief, powdered frozen muscle tissue (2.0 g) was homogenised (Polytron Model PT 10/35, Kinematica, Luzernerstrasses, Switzerland) in 8 ml of a 0.01 M sodium phosphate buffer (pH 7.5) for 30 s on ice. The homogenate was centrifuged at 13,823*g* for 30 min at 4 °C, and then was filtered through Whatman No. 42 filter paper (Whatman, Inc., Clifton, NJ). The reaction was initiated by adding 0.1 ml of the filtered supernatant. Absorbance at 580 nm was recorded every 2 s for 120 s. Nonenzymatic reducing activity was calculated as μmol MMb reduced (equal to nmol OMb formed) per min per g of muscle during the initial linear phase of the assay, using a difference in molar absorption of 12,000 l mol^{-1} cm^{-1} at 580 nm (the wavelength at which the difference in absorption between MMb and OMb is maximal). Activity was expressed as the mean of triplicate samples.

2.2. Experiment 2: effects of calcium lactate enhancement on colour stability, reducing activity, and antioxidant capacity of injection-enhanced beef under highly oxidised conditions

2.2.1. Raw materials and processing

Fifteen USDA (1997) Select beef strip loins *M. longissimus lumborum* were obtained from a commercial meat facility, and were transferred to the Rosenthal Meat Science and Technology Center at Texas A&M University. At 4 days *post mortem*, each muscle was divided into four equal-length sections (60 total sections), and one of six treatment combinations (control, CON; 0.3% phosphate, PHS; 0.2% calcium lactate with 0.3% phosphate, CLPS; irradiated (2.4 kGy) non-enhanced control, I-CON; irradiated with 0.2% calcium lactate, I-CAL; and irradiated with 0.2% calcium lactate plus 0.3% phosphate, I-CLPS) were assigned randomly to each loin section, resulting in 10 replications per treatment. Enhancement solutions contained one or more of the following (Table 2): distilled water, calcium L-lactate (PURACAL; PURAC America, Inc., Lincolnshire, IL), and/or sodium tripolyphosphate (Brifisol® 512; BK Giulini Corp; Simi Valley, CA). Sequential injections of calcium lactate followed by phosphate were applied at 12% of raw weight because phosphates chelate calcium in solution if mixed together. Loin sections were weighed individually before and after injection with a multineedle injector (model N30, Wolftec, Inc., Werther, Germany) to calculate actual injection levels (12%). Each enhanced product was sliced (2.54 cm thick steaks) into steaks for packaging.

2.2.2. Packaging

Each steak was placed in preformed trays (polypropylene/ethylene vinyl alcohol, 22.5 cm × 17.3 cm × 4.1 cm; Rock-Tenn., Franklin Park, IL). Trays were flushed with a high-oxygen atmosphere (80% O_2/20% CO_2, Certified Standard, Airgas Specialty Gases; Austin, TX) and sealed by a ROSS INPACK JR (Model S3180; Ross Industries, Inc.; Midland, VA) and a shrinkable barrier sealing film (MAP-Shield AF; 1.5 mil high-barrier nylon/ethylene vinyl alcohol/metallocene polyethylene with an oxygen-transmission rate of 0.02 cm^3/645.16 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) was used for modified-atmosphere packaging (MAP).

2.2.3. Irradiation

After packaging, samples assigned for the irradiation treatment (I-CON, I-CAL, and I-CLPS) were transported to the Electron Beam Food Research Facility located at Texas A&M University's Research

Table 1
Reduction of horse MMb through calcium lactate + NAD + muscle extract system at 22 °C and pH 5.7 (n = 3).

Solution components[a]							
MMb (0.5 mM)	DCIP (0.6 mM)	Citrate (50 mM)	NAD (6.5 mM)	CaLac (200 mM)	Extract	PMS (30 mM)	Activity (μmol/min/g)
+	+	+	+	+	+	+	0.30 ± 0.006
+	–	+	+	+	+	+	0.27 ± 0.003
+	+	+	–	+	+	+	0
+	+	+	+	–	+	+	0
+	+	+	+	+	–	+	0
+	+	+	+	+	+	–	0
–	+	+	+	+	+	+	0

[a] Substances present (+) or absent (–) in mixtures. MMb, equine metmyoglobin; DCIP, 2, 6-dichlorophenolindophenol; NAD, nicotinamide dinucleotide; CaLac, calcium L-lactate; PMS, phenazine methosulphate. Assays were conducted with 0.5 mM equine MMb in 30 mM phosphate buffer pH 7.0.

Table 2
Composition of experimental injection-enhancement solutions for beef muscles.

	Treatment[a]					
	Non-irradiated			Irradiated		
	CON	PHS	CLPS	I-CON	I-CAL	I-CLPS
Sodium tripoly phosphate	0	0.3	0.3	0	0	0.3
Calcium lactate	0	0	0.2	0	0.2	0.2

[a] Non-enhanced control (CON); no lactate control (PHS); calcium L-lactate/phosphate (CLPS); non-enhanced irradiated control (I-CON); calcium lactate only with irradiation (I-CAL); calcium L-lactate/phosphate with irradiation (I-CLPS).

Park. The high-oxygen MAP packaged samples were irradiated to doses of 2.5 kGy using a 10 MeV, 15 KW electron beam linear accelerator (L-3 Communications, Pulse Sciences, San Leandro, CA). After irradiation, the samples were placed in cardboard boxes and were transferred immediately to the Rosenthal Meat Science and Technology Center at Texas A&M University. Packages were stored in the dark at 2 °C for 10 days before display for 5 days at 1 °C under 2150 lux of fluorescent light.

2.2.4. pH

A sample from each muscle on day 14 was frozen in liquid nitrogen and pulverised in a Waring® table-top blender (Dynamics Corp. of America, New Hartford, CT). About 5 g of muscle tissue was mixed with 20 ml of distilled water for 20 s, and pH values were measured with a standardised combination pH electrode attached to a pH meter (Accumet 50; Fischer Scientific, Fair Lawn, NJ).

2.2.5. Instrumental colour

Instrumental colour (CIE $L^*a^*b^*$ for Illuminant A) was evaluated during storage and display periods using a HunterLab MiniScan™XE Spectrophotometer (Model 45/0 LAV, Illuminant A, 3.18 cm diameter aperture, 10° standard observer; Hunter Associates Laboratory, Inc., Reston, VA). Reflectance from 400 to 700 nm with 10 nm increment readings and CIE $L^*a^*b^*$ values (Illuminant A) were measured and used to calculate saturation index $[(a^{*2}+b^{*2})^{1/2}]$ and hue angle $[(b^*/a^*)^{\tan^{-1}}]$ (AMSA, 1991). Three different locations per steak for day 3, 9, and 14 were scanned and averaged for statistical analyses.

2.2.6. NADH concentration

Alkaline extraction of NADH as described by Klingenberg (1974) was used to measure NADH concentration of muscle tissue from day 14 steaks. In brief, 1 g of frozen pulverised muscle tissue in 8 ml of 0.5 M alcoholic potassium hydroxide solution was vortexed for 30 s, placed in an agitating water bath for 5 min at 90 °C, and rapidly cooled down to 0 °C in an −80 °C ultra freezer. The muscle mixture was neutralised (pH 7.8) by adding 6 ml of triethanolamine–HCl–phosphate mixture. After holding at room temperature for 10 min to flocculate the denatured protein, the mixture was centrifuged at 25,000g for 10 min at 4 °C, and the supernatant filtered through Whatman No. 42 filter paper. Then, a modified assay of McCormick and Lemuel (1971) was used to determine NADH by measuring the reduction of DCPIP by muscle extracts using absorbance at 600 nm (UV-2010; Hitachi Instruments, Inc., San Jose, CA). The NADH concentration (nmol/g) was calculated using the equation obtained from the standard curve using known NADH concentration.

2.2.7. Total reducing activity

The method of Lee, Cassens, and Fennema (1981) was used to determine total reducing activity (TRA) of muscle tissue from day 14 steaks. In brief, powdered frozen muscle tissue (2.0 g) was homogenised in 10 ml of 25 mM PIPES (piperazine-N,N-*bis*-2-ethanesulfonic acid) buffer. Five millilitres of homogenate were mixed with 2 ml of 5 mM potassium ferricyanide, chilled at 2 °C for 1 h with occasional stirring, and 0.1 ml of 0.5% ammonium sulphamate and 0.2 ml of 0.5 M lead acetate were added. After holding at room temperature for 5 min, 2.5 ml of 20% trichloroacetic acid were added to the mixture, and the solution was filled to volume (10 ml) with distilled water. After 5 min, the solution was filtered through Whatman No. 42 filter paper. A solution of 1 mM potassium ferricyanide was used as a standard. The absorbance of filtrate was read at 420 nm using a Beckman DU-7 spectrophotometer (Beckman Coulter, Inc., Fullerton, CA). TRA, an arbitrary value, was expressed as absorbance of 1 mM potassium ferricyanide minus absorbance of sample filtrate.

2.2.8. Lipid oxidation

Lipid oxidation of steaks from day 14 was measured using the 2-thiobarbituric acid method described by Yin, Faustman, Riesen, and Williams (1993). In brief, powdered frozen muscle tissues (5.0 g) were mixed with trichloroacetic acid solution (11%), homogenised for 30 s, and filtered through Whatman No. 42 filter paper. The filtrate (1 ml) was mixed with 1 ml of thiobarbituric acid (20 mM) and incubated at 25 °C for 20 h. The absorbance of samples was measured spectrophotometrically at 520 nm and reported as thiobarbituric acid-reactive substances (TBARS).

2.2.9. Data analysis

For Experiment 1, tests were conducted in triplicate under a completely randomised design. Least squares means and standard errors were used to determine the effects of lactate and LDH on *in vitro* metmyoglobin reduction. The design for Experiment 2 was a split plot. For the whole plot portion, 15 muscles served as blocks to which 1 of 6 treatments were assigned to sections within a muscle as incomplete randomised block design [$n = 10$; (15 muscle × 4 sections per muscle)/6 treatments]. For the subplot, each of the steaks from a loin section was considered as an experimental unit. Type-3 tests of fixed effects for injection treatment, display time, and their interaction (2-way) were analysed by analysis of variance using the SAS (1996) PROC MIXED. Least squares means were separated (*F*-test, $p < 0.05$) by using least significant differences generated by the PDIFF option.

3. Results and discussion

3.1. Experiment 1: equine MMb reduction by calcium lactate–NAD–LDH system

The equine MMb was effectively reduced by the calcium lactate–NAD–muscle extract system. Exclusion of any substrate (NAD, calcium lactate, and muscle extract) for NADH generation resulted in no MMb reduction activity (Table 1). Omission of PMS, an electron transfer catalyst, caused no MMb reduction through the system. PMS is often used to mediate electron transfer between NADH and the oxidising cofactor (Halaka, Babcock, & Dye, 1982). Our data suggest that the substrate mixture of calcium lactate and NAD with muscle extract containing LDH can generate NADH, resulting in concomitant reduction of MMb to OMb through its electron donation. Increasing the concentration of calcium lactate resulted in increasing the equine MMb reduction (Fig. 1). Kim et al. (2006) found identical results for non-enzymatic MMb reduction, which increased with greater amounts of either NAD or lactate. The reduction of equine MMb was positively affected by pH condition (Fig. 2). At pH 4.9, there was no reduction through the lactate–NAD–LDH system, but at a normal pH range of *post mortem* muscle (5.3–5.7), the MMb reduction increased as well.

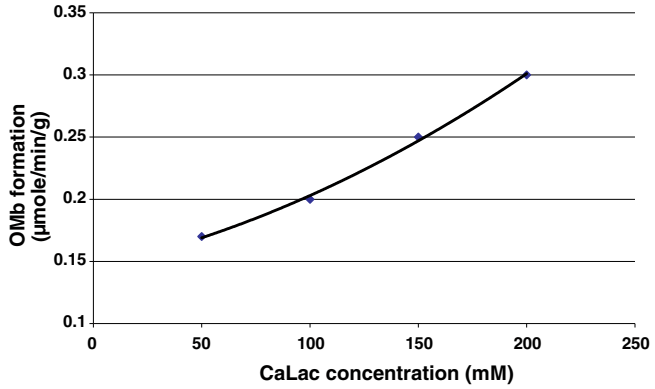

Fig. 1. Least squares means for equine MMb reduction (equal to OMb formation, μmol/min/g) through lactate–NAD–muscle extract system. Assays were conducted with 0.5 mM equine MMb in 30 mM phosphate buffer pH 7.0 giving final pH 5.7 for a test cuvette.

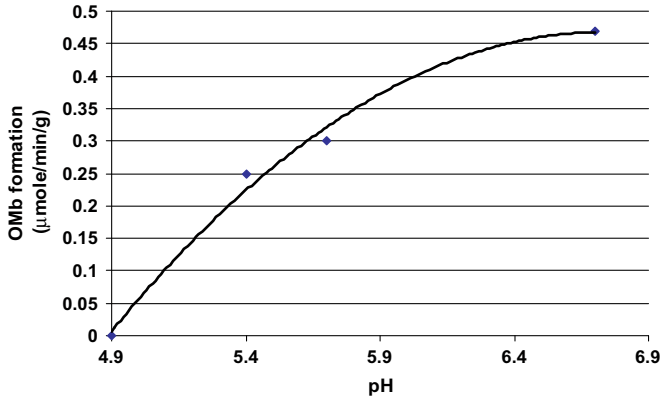

Fig. 2. Least squares means for equine MMb reduction (equal to OMb formation, μmol/min/g) through lactate–NAD–muscle extract system within different assay pH by altering the concentration of the citrate (100, 80, or 50 mM, respectively) or adding sodium phosphate buffer (pH 8.3, 30 mM). Assays were conducted with 0.5 mM equine MMb in 30 mM phosphate buffer pH 7.0, NAD (6.5 mM), and calcium lactate (200 mM) mixture in test cuvettes.

The higher the pH, the more MMb was reduced by NADH generation, *via* the lactate–LDH system. This is mostly due to the higher LDH-B activity (reaction toward pyruvate + NADH) under alkaline conditions (Wahlefeld, 1983).

3.2. Experiment 2: effects of calcium lactate enhancement on colour stability, reducing activity, and antioxidant capacity of injection-enhanced beef under highly oxidised conditions

3.2.1. pH

The pH of the enhancement solutions ranged from 6.5 to 8.2 (Table 3). All enhanced steaks containing phosphate had higher pH values compared to the non-enhanced control (CON). Irradiated steaks with only calcium lactate enhancement (I-CAL) had lower pH compared to the steaks enhanced with a combination of calcium lactate and phosphate, due to the lower pH (6.3) of calcium lactate injection solution.

3.2.2. Instrumental colour and colour stability

CIE L^* (lightness) values for the non-irradiated treatments (Table 4) were not different ($p > 0.05$). Calcium lactate enhancement did not cause any surface darkening, which was reported in other lactate enhancement studies (Kim et al., 2006; Knock et al., 2006; Mancini et al., 2005; Prestat et al., 2002). Irradiated steaks (I-CON, I-CLPS) had lower L^* values ($p < 0.05$) compared to non-irradiated steaks at the initial storage day. However, as storage and display time increased, lightness values for all irradiated steaks increased ($p < 0.05$). Lightness values for non-irradiated steaks did not significantly change during storage and display periods.

Table 3
Least squares means for pH of treatment solution and *M. longissimus lumborum* (LD) steaks at day 14 (end of display) at 1 °C.

	Treatment[A]						
	Non-irradiated			Irradiated			
	CON	PHS	CLPS	I-CON	I-CAL	I-CLPS	SE[B]
Solution		8.2	8.0		6.5	8.0	
LD	5.48a	5.69c	5.73cd	5.42b	5.39b	5.74d	0.03

[abcd]Means within a row with different letters are different ($p < 0.05$).
[A] Standard errors.
[B] Non-enhanced control (CON); no lactate control (PHS); calcium L-lactate/phosphate (CLPS); non-enhanced irradiated control (I-CON); calcium lactate only with irradiation (I-CAL); calcium L-lactate/phosphate with irradiation (I-CLPS).

There were significant day × treatment interactions in redness values (a^*). Initial redness values for non-irradiated steaks were not different. However, during display periods, a^* values for all steaks decreased significantly except the calcium/phosphate (CLPS) enhanced steaks. The CLPS-treated steaks maintained higher redness values ($p < 0.05$) among all other irradiated/non-irradiated steaks at the end of the storage and display period (day 14). Irradiation significantly decreased redness values for all enhanced- and non-enhanced steaks, compared to the non-irradiated steaks throughout time. There were no differences in a^* values among different treatments within the irradiated steaks at day 3. However, at the end of storage and display periods, the calcium/phosphate enhanced steaks (I-CLPS) had the highest redness value among other irradiated steaks.

Chroma values (indication of colour intensity) for the treatments presented similar trends to a^* values. The CLPS-enhanced steaks maintained higher colour intensity ($p < 0.05$) compared to the CON and PHS treated steaks at day 14. Although irradiation significantly decreased colour intensity of steaks for all treatments, the I-CLPS steaks had higher ($p < 0.05$) chroma values among the other irradiated steaks at day 14. The hue angle values (indicative of surface discolouration) for both the calcium lactate/phosphate enhanced steaks (CLPS, I-CLPS) were significantly lower than those of both non-irradiated/irradiated controls (CON, I-CON). The CLPS and I-CLPS were not significantly different in hue angle throughout storage and display periods. The I-CAL steaks had the highest hue angle value among all other treatments.

Decreases in redness and colour intensity of muscles due to irradiation were reported previously (Nam & Ahn, 2003; Nanke, Sebranek, & Olson, 1999). Free radicals generated by ionising irradiation can oxidise myoglobin directly or they can oxidise lipids, producing lipid radicals, which subsequently oxidise myoglobin, resulting in changing from a bright cherry-red to a dull red colour (Brewer, 2004; Nanke et al., 1999). Irradiation of oxygen permeable packaged beef decreases a^* values and increases visual and brown colours inducing a metmyoglobin-like pigment (Nanke et al., 1999). Although the steaks were exposed to severe oxidising conditions by applying irradiation to high-oxygen (80%) MAP, the calcium lactate/phosphate enhanced steaks maintained stable red colour and resulted in less surface discolouration during display periods. Enhancement by lactate of fresh beef improves colour stability during retail display (Kim et al., 2006; Lawrence et al., 2003; Mancini, Kim, Hunt, & Lawrence, 2004). Our data suggest that lactate enhancement protects the meat pigment from myoglobin oxidation under strongly oxidising conditions.

Table 4
Least squares means for instrumental colour scores of *M. longissimus lumborum* steaks stored for 9 days and displayed up to 14 days at 1 °C.

Trait		Treatment[A]						
		Non-irradiated			Irradiated			
	Day	CON	PHS	CLPS	I-CON	I-CAL	I-CLPS	SE[B]
L^*	3	43.1ax	44.1ax	43.9ax	39.8bx	41.4abx	39.9bx	0.82
	9	43.8ax	43.8ax	44.8ax	43.1ay	44.9ay	42.5ay	0.82
	14	42.8bcx	44.1abx	42.9bcx	41.7cz	45.6ay	41.4by	0.82
a^*	3	32.1ax	33.7ax	33.2ax	15.8bxy	16.4bx	17.6bx	0.77
	9	27.9ay	30.4by	30.3by	16.6cy	15.4cx	18.9dx	0.77
	14	23.1az	26.1bz	29.3cy	14.9dx	12.5ey	18.6fx	0.77
b^*	3	21.8ax	24.1bx	23.6bx	12.1cx	12.7cx	12.7cx	0.46
	9	18.6ay	21.7by	21.4by	13.1cxy	13.4cxy	13.5cx	0.46
	14	16.4az	19.0bz	20.8by	13.4cy	13.9cy	13.7cx	0.46
Hue[C]	3	34.1ax	35.6ax	35.4ax	37.5ax	37.6ax	35.8ax	1.69
	9	34.1ax	35.6ax	35.2ax	39.1bxy	41.7by	35.6ax	1.69
	14	40.7acy	36.0bx	35.2bx	42.9cy	48.9dz	36.5abx	1.69
Chroma[D]	3	38.8ax	41.4ax	40.7ax	20.0bx	20.8bx	21.7bx	1.01
	9	33.5ay	37.3ay	37.1by	21.3cx	20.5cx	23.2cx	1.01
	14	25.4az	32.3bz	35.9by	20.2cx	18.8cx	23.1dx	1.01

[abcd]Means in a row within day having different letters are different ($p < 0.05$). [xyz]Means in a column of treatment with different letters are different ($p < 0.05$).
[A] Non-enhanced control (CON); no lactate control (PHS); calcium L-lactate/phosphate (CLPS); non-enhanced irradiated control (I-CON); calcium lactate only with irradiation (I-CAL); calcium L-lactate/phosphate with irradiation (I-CLPS).
[B] Standard errors.
[C] Hue angle = $(b^*/a^*)^{\tan^{-1}}$.
[D] Saturation index = $(a^{*2} + b^{*2})^{1/2}$.

3.2.3. NADH concentration

The calcium lactate/phosphate enhancement increased ($p < 0.05$) NADH concentration (Fig. 3) for both non-irradiated/irradiated steaks, compared to the controls (CON, I-CON, respectively). The phosphate enhancement (PHS) and calcium-only injected steaks (I-CAL) did not affect NADH concentration. Irradiation decreased NADH concentration ($p < 0.05$) of beef steaks, probably due to oxidation. Kim et al. (2006) reported increased NADH concentration of beef strip steaks injection-enhanced with 2.5% potassium lactate/phosphate solution. They determined that endogenous NADH can be regenerated by exogenous substrate (lactate) addition *via* increasing LDH-B flux (reaction toward pyruvate and NADH). The replenished NADH was utilised for transferring electrons to the ferric state of haem maintaining the reduced state of myoglobin (Kim et al., 2006, 2007).

3.2.4. Total reducing activity

Total reducing activity (TRA) of non-irradiated steaks was not significantly different (Fig. 4). Irradiation significantly decreased TRA for the control (I-CON) and calcium-only injected (I-CAL) steaks. However, the I-CLPS enhanced steaks maintained higher TRA ($p < 0.05$) than other irradiated steaks (I-CON, I-CAL), and were similar to other non-irradiated steaks ($p > 0.05$) suggesting that increasing muscle pH by adding a combination of phosphate and lactate provided higher reducing capacity of myoglobin under severe oxidising conditions. Nam, Ahn, Du, and Jo (2001) found that irradiation of high-pH (>6.2) pork meat caused less colour change and lower lipid oxidation than low-pH (<5.4) pork. Sammel, Hunt,

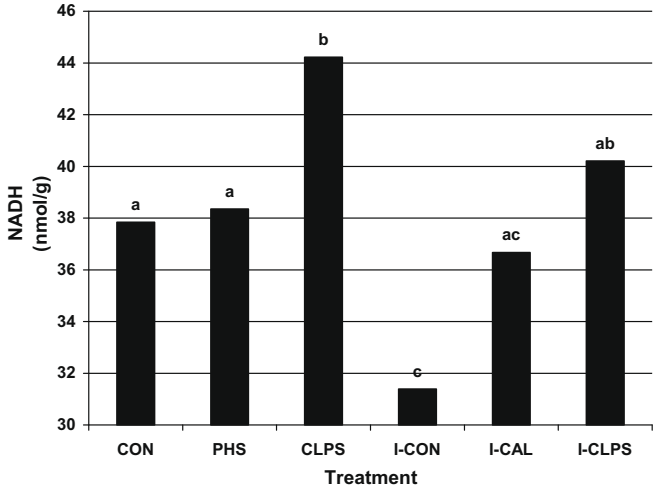

Fig. 3. Least squares means for NADH concentration (nmol/g) of bovine *M. longissimus lumborum* steaks for each treatment at day 14 (end of storage and display) at 1 °C. [abc]Means with different letters are different ($p < 0.05$). Non-enhanced control (CON); no lactate control (PHS); calcium L-lactate/phosphate (CLPS); non-enhanced irradiated control (I-CON); calcium lactate only with irradiation (I-CAL); calcium L-lactate/phosphate with irradiation (I-CLPS). Standard error of the mean: 2.05.

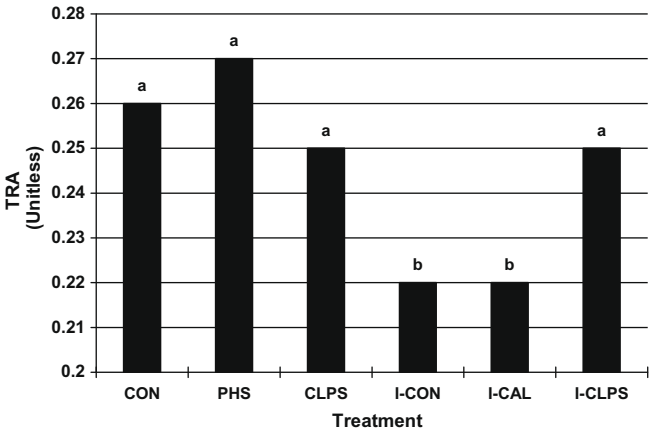

Fig. 4. Least squares means for TRA values (unitless) of bovine *M. longissimus lumborum* steaks for each treatment at day 14 (end of storage and display) at 1 °C. [abc]Means with different letters are different ($p < 0.05$). Non-enhanced control (CON); no lactate control (PHS); calcium L-lactate/phosphate (CLPS); non-enhanced irradiated control (I-CON); calcium lactate only with irradiation (I-CAL); calcium L-lactate/phosphate with irradiation (I-CLPS). Standard error of the mean: 0.007.

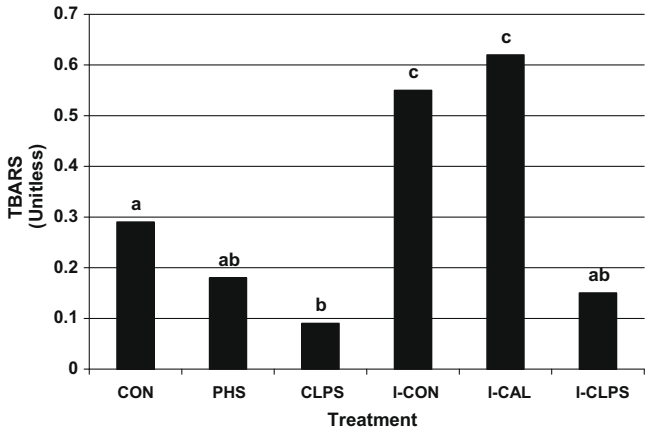

Fig. 5. Least squares means for TBARS values (unitless) of bovine *M. longissimus lumborum* steaks for each treatment at day 14 (end of storage and display) at 1 °C. [abc]Means with different letters are different ($p < 0.05$). Non-enhanced control (CON); no lactate control (PHS); calcium L-lactate/phosphate (CLPS); non-enhanced irradiated control (I-CON); calcium lactate only with irradiation (I-CAL); calcium L-lactate/phosphate with irradiation (I-CLPS). Standard error of the mean: 0.075.

Kropf, Hachmeister, and Johnson (2002) reported that TRA was correlated to NADH and visual colour of beef *semimembranosus* muscle. Sepe et al. (2005) determined that the addition of reducing agents (sodium erythorbate, sodium ascorbate, ascorbic acid, and ascorbyl palmitate) increased surface a^* values, TRA, and decreased lipid oxidation of ground beef. Nam and Ahn (2003) found that incorporation of ascorbic acid (0.1%) to ground beef before irradiation prevented colour changes in irradiated beef. They concluded that the addition of ascorbic acid accelerated MMb reduction by donating electrons to the ferric state of haem and subsequently resulted in the conversion of ferri-myoglobin to ferrous-myoglobin. It is not clear why the CLPS-enhanced steaks were not higher than other non-irradiated steaks in TRA. However, under severe oxidising conditions, the CLPS-enhanced steaks maintained higher TRA, which was mostly due to the increased NADH concentration through calcium lactate enhancement with a higher pH.

3.2.5. TBARS value

The CLPS-enhanced steaks underwent significantly lower lipid oxidation (Fig. 5) than non-irradiated control (CON). Irradiation significantly increased the TBARS values of the I-CON and I-CAL steaks, whereas the TBARS values of the I-CLPS-enhanced steaks were not influenced by the irradiation treatment. The difference in TBARS values for the I-CAL (pH 5.39) and I-CLPS (pH 5.74) enhanced steaks suggests that the lower pH meat is more susceptible to lipid oxidation. The antioxidant property of lactate has been reported in several studies (Choi & Chin, 2003; Wang & Brewer, 1999). Mancini and Ramanathan (2008) found less MMb formation of equine oxymyoglobin (OMb) incubated with sodium lactate, and they concluded that lactate itself may increase colour stability through a non-enzymatic interaction with myoglobin. However, our data indicated that calcium lactate-only enhanced steaks (I-CAL) did not protect myoglobin oxidation under severe oxidising condition. Seyfert, Hunt, Lundesjö Ahnström, and Johnson (2007) also reported adverse effects of using calcium lactate only on colour stability of ground beef. They did not incorporate lactate with phosphate for their ground beef, and consequently it did not increase pH (5.4–5.5). In this study, the PHS-only enhanced steaks were not significantly different from the CON in TBARS values. However, when the combination of phosphate and calcium lactate was applied to steaks (CLPS), it decreased lipid oxidation, whether irradiated or non-irradiated, suggesting that the combination of phosphate and lactate has a synergistic effect on reducing oxidation (Fig. 6).

4. Conclusions

These data suggest that calcium lactate/phosphate enhancement actively minimises lipid oxidation and subsequently protects myoglobin oxidation from either direct oxidation by hydroxyl radicals or indirect oxidation thorough lipid radicals (Brewer, 2004; Chan et al., 1997; Lee et al., 2003). Further, the calcium lactate/phosphate combination induces an increase in NADH concentration, and concomitant increase in reducing capacity of muscle tissue, resulting in maintenance of the ferrous state of haem for a longer period of time. This novel research determined improved colour stability of fresh beef through lactate/phosphate inclusion by providing superior antioxidant capacity and increased reducing activity of myoglobin by elevating NADH concentration. Future research should focus on the addition of lipid oxidation catalase into the oxymyoglobin–lactate–NAD–LDH system and combination of other reducing agents on myoglobin oxidation.

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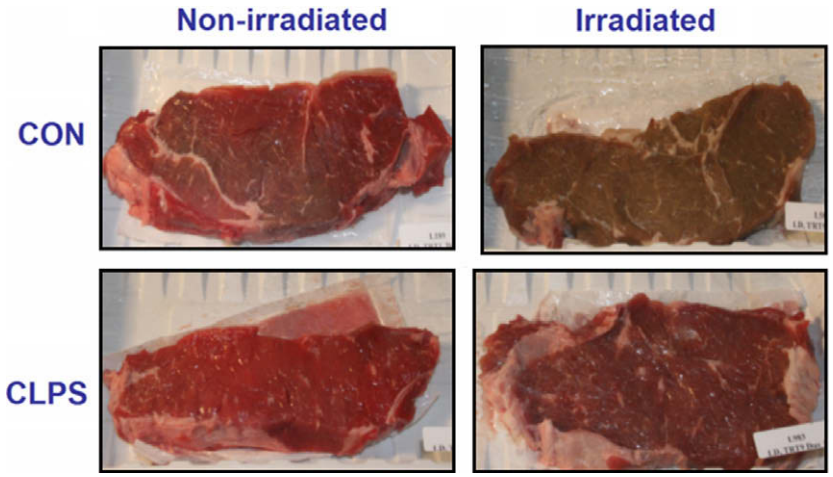

Fig. 6. Pictures of bovine *M. longissimus lumborum* steaks for each treatment at day 14 (end of storage and display) at 1 °C. Non-enhanced control (CON); calcium L-lactate/phosphate (CLPS).

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