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Nitrite Embedded Vacuum Packaging Improves Retail Color and Oxidative Stability of Bison Steaks and Patties

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Abstract: Bison meat is prone to rapid discoloration under traditional aerobic retail packaging conditions. The aim of this study was to determine if the color stability of bison steaks and burger patties could be improved through packaging meat with a vacuum-sealed film containing embedded sodium nitrite. Bison bulls ($n = 40$) were slaughtered and the *longissimus lumborum* (LL) and *rhomboideus* (RH) were removed. Following a postmortem aging period of 6, 13, or 20 d steaks were obtained from the LL. RH muscles aged 6 d were ground (85:15 lean to fat) and formed into 140 g patties. One steak and two burger patties from each carcass side were placed into either a polystyrene tray overwrapped with oxygen permeable polyvinyl chloride film (CONT) or a polyethylene tray vacuum sealed with film coated in sodium nitrite (113 mg × m^{-2}; NIT); meat was placed under simulated retail conditions for 4 d. A 3-way interaction was observed between packaging type, whole muscle aging and time in retail display for objective (L*, Chroma, and Hue) and subjective (lean color score and proportion of surface discoloration) color measures from steaks ($P < 0.0001$). The CONT packaged meat showed an increased area of discoloration and in metmyoglobin after 4 d in retail display ($P < 0.0001$); NIT meats did not show a higher area of discoloration or metmyoglobin after retail display. Additionally, NIT packaged steaks and burger patties lightened (higher L*) and became redder over the course of the retail display period. Thiobarbituric acid reactive substances (products of lipid peroxidation) did not increase in NIT packaged burger patties after 4 d under retail conditions, however, there was a significant increase observed for CONT packaged burger patties ($P < 0.0001$). NIT packaging appears to effectively improve the color stability of bison meat under retail conditions, making this packaging strategy well suited to address the issue of rapid discoloration.

Keywords: bison, meat color, muscle pigment, packaging systems, TBARS
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Introduction

Bison (*Bison bison*) herds are kept commercially in North America for meat production. Their meat is valued both domestically and internationally as an alternative high protein, game meat (Marchello and Driskell, 2001; Galbraith et al., 2006). When available, bison can be sold fresh in steaks and burger patties at grocery stores. However, fresh bison meat discolors faster than beef (Janz et al., 2000; Dhanda et al., 2002; Pietrasik et al., 2006). This may be due to the higher pigment concentration and polyunsaturated fat content promoting

oxidative instability in the meat (Galbraith et al., 2016). As consumers use meat color as a sign of freshness and wholesomeness (Mancini and Hunt, 2005), a reduction in color stability in bison may result in costly losses to retail revenue due to discounts or discarded product.

Recently developed technology (Vacuum Skin Package, FreshCase; Curwood Inc., Oshkosh, WI) incorporates small quantities (25 to 113 mg × m^{-2}) of sodium nitrite into the film during manufacturing. Normally, deoxymyoglobin (Mb) and oxymyoglobin (OMb) are oxidized to metmyoglobin (MMb) under partial pressures of oxygen which would occur in polyvinyl chloride film (PVC) overwrapped packaging environments sometime after the initial bloom. MMb is a major contributor to discoloration of meat (Mancini and Hunt, 2005). The nitrite embedded in the film can, however, cause nitrosylation of the Mb to form nitrosomyoglobin (NOMb), which is bright red in color. Consequently, sodium nitrite coated films when vacuum sealed have been shown to improve color stability in beef (Claus and Du, 2013). The aim of the present study was to determine if the problem of early retail browning in bison can be mitigated using FreshCase packaging film.

Materials and Methods

Sample collection

Commercially finished bison bulls (n = 40; 481 ± 60 kg live weight; 26 to 30 mo of age) were slaughtered at the Lacombe Research and Development Center abattoir following the humane practices described in the Canadian Council of Animal Care (2009); the study was approved by the Lacombe Research and Development Center Animal Care Committee (2012F029R). Further details on carcass evaluation and procurement of samples were reported by Ding et al. (2016). Following carcass splitting, within 45 min of exsanguination, high voltage electrical stimulus (HVES; 400 V peak, 5 ms pulses at 15 pulses s^{-1} for 30 s) was applied to each right carcass side. *Longissimus lumborum* (LL) from the left and right sides from bison carcasses (n = 40) were extracted 48 h postmortem. The muscles were squared off and extraneous fat was trimmed from muscles exposing the overlying connective tissue. The trimmed LL was cut into 3 equal portions (approximately 10 cm). Individual portions (cranial, mid and caudal) balanced between muscle locations were assigned to an aging period of 6, 13, or 20 d postmortem. Muscle portions were weighed, vacuum packaged and kept in a cooler (2°C with wind speeds of 0.5 m × sec^{-1}) for the designated aging period. Remaining end portions of the right and left LL were pooled and finely comminuted (Robot Coupe Blixir BX3, Robot Coupe USA Inc., Ridgeland, MS) and frozen (–80°C) prior to analysis. Ground meat was analyzed for protein, moisture and fat (AOAC, 1995a, 1995b) with CEM rapid analyzer systems (Sprint Protein Analyzer Model 558000, Smart Turbo Moisture Analyzer Model 907990, and Smart Trac Fat Analyzer Model 907955; CEM Corporation, Matthews, NC). A sample of ground meat was analyzed for pigment concentration as described by Lindahl (2011). Briefly, samples were prepared by thoroughly homogenizing 2.5 g of sample in 25 mL of phosphate buffer (0.04 M) and filtering through Whatman 1 filter paper. A 0.5-mL aliquot of filtrate was added to 3.5 mL of phosphate buffer (0.04 M), 1.4 mL of Triton X-100 and 0.1 mL Sodium Nitrite (0.065 M). Spectrophotometer readings were then collected from prepared samples using the wavelengths 730 nm and 409 nm. Additionally, tocopherol determinations were completed on ground meat using fluorescence HPLC (Waters, Separation Module e2695 equipped with a Photodiode Array Detector 2998 and Multi-wavelength Fluorescence Detector 2475; Waters Corporation, Milford, MA) according to Hewavitharana et al. (2004); this method determines the concentration for all tocopherol and tocotrienol homologs in meat. Finally, fatty acid composition was determined from ground meat following extraction, methylation (Kramer et al., 1997), and gas chromatography [GC analyses (Cruz-Hernandez et al., 2004; Cruz-Hernandez et al., 2007; Kramer et al., 2008)]. For fatty acid analysis, intramuscular lipids were extracted from LL ground meat using a chloroform–methanol mixture (2:1, v/v) according to Folch et al. (1957). Aliquots of LL lipids (10 mg) were methylated separately using acid (5% methanolic HCl) and base (0.5 N sodium methoxide) reagents (Kramer et al., 2008). Internal standard, 1 mL of 1 mg c10–17:1 methyl ester/ml toluene (standard no. U-42M form Nu-Check Prep Inc., Elysian, MN), was added before the addition of methylating reagents. Fatty acid methyl esters (FAME) were analyzed by GC using the temperature programs described by Kramer et al. (2008). The FAME (1 µL) were injected at 0.25 mg/ml in hexane using a 20:1 split onto a CP-Sil88 column (100 m, 25 µm ID, 0.2 µm film thickness) in a CP-3800 gas chromatograph equipped with an 8600-series autosampler (Varian Inc., Walnut Creek, CA). Hydrogen was used as the carrier gas under constant pressure (25 psi, initial flow rate of 1.9 mL/min). Injector and flame ionization detector temperatures were held at 250°C.

For the identification of FAME by GC, reference standard no. 601 (Nu-Check Prep Inc, Elysian, MN) was used. Branched-chain FAME were identified us-

ing a GC reference standard BC-Mix1 acquired from Applied Science (State College, PA). For conjugated linoleic acid (CLA) isomers, the UC-59M standard from Nu-Chek Prep Inc. was used which contains all four positional CLA isomers. *Trans*-18:1, CLA and other biohydrogenation intermediates not included in the standard mixtures were identified by their retention times and elution orders as reported in literature (Cruz-Hernandez et al., 2004; Kramer et al., 2008; Gómez-Cortés et al., 2009; Vahmani et al., 2016). The FAME were quantified using chromatographic peak area and internal standard based calculations. Fatty acids were expressed as a percentage of total fatty acids.

At 48 h postmortem, 150 g of subcutaneous (SQ) fat and 850 g of *rhomboideus* (RH) muscle from both carcass sides were collected and individually vacuum packaged in a nitrite-free film. The RH muscle was placed into a cooler while SQ fat removed from over the LL was frozen at –35°C until 13 d postmortem. These samples were used for manufacturing burger patties, as described below.

Packaging

Following 6, 13, and 20 d aging, two 2.5 cm LL steaks were cut using aseptic technique to minimize contact with the steak surface. One steak was weighed and placed onto a polystyrene tray with a dri-loc pad (UZ Soaker Ultra Zap Pads, Paper Pak Industries Washington, GA), over-wrapped with a polyvinyl chloride oxygen permeable film (8,000 mL × m^{-2} 24 h^{-1} vitafilm choice wrap; Goodyear Canada Inc.; CONT); the second steak was pre-weighed onto a polyethylene tray with a dric-loc pad and vacuum skin packaged with 113 mg × m^{-2} nitrite embedded vacuum skin oxygen impermeable film (NIT; Vacuum Skin Package, FreshCase; Curwood Inc., Oshkosh, WI; 0.1 mm thickness, water vapor transmission rate < 0.5 g/645 cm^2/24 h) using a Multivac Tray Sealer T200 (Multivac Inc.; Wolfertschwenden, Germany). The final pressure in the packaging environment was set to 1 kPa.

At 13 d two 140-g patties (11.5 cm diameter × 0.64 cm thick) were formed from each carcass side with ground lean of the RH muscle and SQ fat (lean 85%, subcutaneous fat 15%) using a single hamburger press (Cabelas, Sydney, NE). Two patties from each carcass were placed into either the CONT or NIT packaging in the same manner described above.

Retail evaluation

Following packaging, steaks were placed into a fan assisted, horizontal (chest type) retail display case (Hill Refrigeration of Canada Ltd., Barrie, ON) under fluorescent room lighting (GE deluxe cool white F40CWC41/ECO; GE, Bucyrus, OH) and incandescent lighting directly above the display case [Philips Halogen Par 38 lamp (90 W, 120 V) spaced 91.5 cm apart; Philips Lighting Company, Somerset, NJ]. The lighting had an intensity of 1020 lux at the meat surface for 14 h × d^{-1} (Jeremiah and Gibson, 2001). For the remaining 10 h d^{-1} s, the direct display case lights were shut off and the fluorescent room lights remained on. Prior to collecting objective color measures from one packaging type, the spectrophotometer (Minolta CM-700D with SpectraMagic NX Lite Color Data Software CM-S100w Version 2, illuminant C and 2° observer, 3 mm aperture; Minolta Canada Inc., Mississauga, ON) was calibrated to each packaging type. The calibration block was wrapped in the packaging material (CONT or NIT) and calibrations were performed according to manufacturer directions. At 0, 2, and 4 d of retail display, steak subjective and objective color [CIE L*- brightness; a*- red-green axis; b*- yellow-blue axis; (CIE, 1978)] were assessed through the packaging material. Hue (Hab = arctan [b*/a*]) and Chroma (Cab = $[a^{*2} + b^{*2}]^{0.5}$) were calculated from collected measures. Approximately 1 h after placing samples into retail display (0 d) objective color was measured in triplicate. To approximate the myoglobin oxidative states, spectral reflectance readings were collected along with objective color measures and converted to a reflex attenuance (Shibata, 1966). Interpolation of isobestic points for 473, 525, 572, and 730nm from the reflex attenuance were calculated and converted to absorption values (the logarithm of the reciprocal for these reflectance values). These absorption values were used to calculate a_1 = (A572 – A700) / (A525 – A700) and a_2 = (A473 – A700) / (A525 – A700). Finally, a_1 and a_2 were used to calculate relative contents of metmyoglobin (MMb = 1.395 – a_1), deoxymyoglobin (Mb = 2.375 × [1– a_2]) and oxymyoglobin (OMb = 1 – [MMb + Mb]; Krzywicki, 1979). In addition to the proportions of myoglobin forms, the ratio of reflectance values obtained at 650 nm and 570 nm were calculated for NIT packaged meats. The 650 nm / 570 nm ratio gives an indication of the amount of NOMb in cured meat products (American Meat Science Association, 2012); it was expected this value would increase after exposure to the NIT film. After these measures were obtained, steaks under the different packaging conditions were subjectively evaluated by an established 5-member meat sensory panel for lean color using an 8-point scale (1 = light pink and 8 = extremely dark red, with 5 = bright cherry red) and percent surface discoloration using a 7-point scale (1 = no surface discoloration and 7 = complete surface

discoloration; Basarab et al., 2007). Lean color score assignments were based on standards using Munsell color tiles developed at the Lacombe RDC originally used for beef. The panelists selected for this study had been screened and trained according to American Meat Science Association (2012), with additional training for evaluation of discoloration. Prior to the present study the panelists had participated in multiple studies and refreshing training sessions for visually assessing beef under retail conditions. For the present study panelists received two 1-h training sessions specifically on bison retail samples at various stages of discoloration (8 to 10 bison steak samples each time). At 0, 2, and 4 d under retail conditions panelists scored the appearance of each steak and burger patty; for these evaluations meat was kept in packaging. Panelists scored all packages in the retail display case each day (20 to 40 steaks and 40 burger patties were scored for each day of evaluation) and were allowed to break as needed to prevent fatigue. Objective and subjective evaluations were repeated at 2 and 4 d of retail display. Following color measurements obtained at 4 d retail display, a final weight of the steak was recorded to determine steak drip-loss. Steaks were cooked to an internal end-point temperature of 71°C and peak shear force was determined on a 19 mm core perpendicular to the fiber grain (TA-XT Plus Texture Analyzer equipped with a Warner-Bratzler shear head at a crosshead speed of 200 mm min^{-1} and a 30-kg load cell using Texture Exponent 32 Software; Texture Technologies Corp., Hamilton, MA; Ding et al., 2016).

Following packaging, all burger patties were put in retail display cases for objective and subjective color measurements at 0, 2, and 4 d as described above. In addition to measures of color, at 0 and 4 d in retail display, one patty from each carcass and packaging treatment was removed from the packaging, thoroughly homogenized (Robot Coupe Blixir BX3, Robot Coupe USA Inc., Ridgeland, MS) and thiobarbituric acid reactive substances (TBARS) were determined from 10-g aliquots of ground mixture as a measure of lipid oxidation (Nielsen et al., 1997). Briefly, the samples were added to 30 mL of 7.5% TCA, homogenized, and filtered through Whatman 5 filter paper; 2.5 mL of filtrate were then collected and incubated at 94°C for 40 min. Spectrophotometer readings at 531 nm were obtained from incubated samples. The TBARS concentrations for samples were then calculated from a standard curve.

Statistical analysis

The means and standard deviation of measures obtained from ground meat (proximate, tocopherol, pigment concentration, and fatty acid composition) were calculated using SAS (SAS Inst. Inc., Cary, NC). All other data were analyzed using a Mixed Effects Model, with the packaging treatment, and for the measures from steaks, whole muscle aging duration as main effects; time in retail was included in all models as a repeated measure with an auto regressive covariance structure. Individual carcasses and side × carcass were included as random factors. For all models the denominator degrees of freedom were adjusted using Kenward-Roger procedure and least square means were separated (F test, $P < 0.05$) by using least significant differences generated by the PDIFF option. The HVES and interactions to the treatments were included as a fixed effect in initial models, however, these factors did not have a significant effect on any characteristics measured here. Hence, the HVES was removed as a fixed effect and carcass side was included as a random effect.

Results and Discussion

Fatty acid and proximate composition of bison loin and burgers

Overall composition and fatty acid profile of the bison loins are presented in Table 1. Paralleling the visual perception of marbling (Ding et al., 2016), the total proportion of chemically determined fat was relatively low compared to beef (Galbraith et al., 2016), with a mean overall fat content in bison loin of 18.9 mg × g^{-1} (range 7.5 – 44.1 mg × g^{-1}). Pigment concentration averaged 10.02 mg × g^{-1} (range: 7.71 – 12.28 mg × g^{-1}). Galbraith (2011) reported pigment levels in bison averaged 8.45 mg × g^{-1} from the LL, which were higher than those found in beef 6.87 mg × g^{-1}. The primary structure of bison and beef myoglobin have 100% similarity in amino acid sequence (Joseph et al., 2010). Thus, the pigment structure does not likely contribute to poor color stability in fresh bison meat. Rather, poor color stability in bison meat is likely the result of the higher pigment content, difference in postmortem oxygen consumption and metmyoglobin reducing ability (Suman and Joseph, 2013). In addition, oxidative stability at retail can be affected by the fatty acid composition of the meat (Aalhus and Dugan, 2014). Although total saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) in the present study were similar to those reported for a different bison population by Galbraith (2011), the total polyunsaturated fatty acid (PUFA) content was lower (9.32%) in the current study using feedlot finished bison versus those reported

Table 1. Overall compositional analyses with standard deviation (SD) of bison loin (n = 40)

Trait	Mean	SD	Minimum	Maximum
Composition, mg × g^{-1}				
Moisture	738.0	11.9	699.5	760.1
Fat	18.9	7.9	7.5	44.1
Protein	201.4	7.9	185.8	217.0
Pigment, mg × g^{-1}	10.02	1.15	7.71	12.28
Tocopherol, mg × g^{-1}	1.13	0.31	0.66	2.47
Fatty acid, %				
SFA[1]	42.76	1.95	38.93	46.97
Total MUFA[2]	47.24	3.88	36.34	54.26
cis-MUFA	44.53	3.42	34.40	50.70
trans-MUFA	2.71	0.46	1.94	3.56
Total PUFA[3]	9.32	3.46	4.42	22.36
n-6	8.42	3.03	4.17	20.50
n-3	0.90	0.43	0.25	1.86
SFA:PUFA	4.59	0.56	2.10	8.81
BCFA[4]	1.49	0.15	1.21	1.93
AD[5]	0.41	0.11	0.19	0.66
CLA[6]	0.20	0.05	0.09	0.34
CLnA[7]	0.07	0.03	0.03	0.17

[1]Saturated fatty acids (SFA) include C14:0, C15:0, C16:0, C17:0, C18:0, C19:0, C20:0, C22:0, C23:0, C24:0.

[2]Monounsaturated fatty acids (MUFA) include the cis-MUFA (c9–14:1, c9–15:1, c7–16:1, c9–16:1, c11–16:1, c9–17:1, c9–18:1, c11–18:1, c12–18:1, c13–18:1, c14–18:1, c15–18:1, c16–18:1, c9–20:1, c11–20:1) and the trans-MUFA (t6-t8–18:1, t9–18:1, t10–18:1, t11–18:1, t12–18:1, t13-t14–18:1, t15–18:1, t16–18:1).

[3]Polyunsaturated fatty acids (PUFA) include the n-6 (C18:2n-6, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:4n-6) and n-3 (C18:3n-3, C22:3n-3, C20:5n-3, C22:5n-3, C22:6n-3) fatty acids.

[4]Branched chain fatty acids (BCFA) include C15:0iso, C15:0anti-iso,C16:0iso, C17:0iso, C17:0anti-iso, and 18:0iso.

[5]Atypical dienes (AD) include c9t13–18:2, t8c12–18:2, t8c13–18:2, c9t12–18:2 and c9,c15–18:2.

[6]Conjugated linoleic acid (CLA) includes isomers of 18:2 (t12,t14; t11,t13; t10,t12; t9,t11; t8,t10; t11,c13; t10,c12; c9,t11; t8,c10; and t7,c9).

[7]Conjugated linolenic acid (CLnA) includes c9,t11,c15–18:3.

by Galbraith (2011) for grass finished animals (21.11%). The PUFA content in the present study was closer to levels reported for typical feedlot finished beef (6.43%) in Galbraith (2011). Therefore it appears the fatty acid composition of bison meat is influenced by diet, as it is with beef. Animal diet needs to be considered when generalizing about the healthfulness of bison meat. It is also worth noting the fatty acid profile of bison in the present study had a higher n-6: n-3 than that observed previously in grain finished beef (Galbraith et al., 2016). In the context of meat color, PUFA are generally unstable in the postmortem environment and can contribute to oxidative instability (Aalhus and Dugan, 2014). Oxidative instability in fatty acids may also have an effect on the oxidative state of myoglobin leading to a more rapid deterioration of meat color (Wood et al., 2004; Faustman et al., 2010). The total tocopherol concentration from the meat averaged 1.13 μg × g^{-1} (range: 0.66 to 2.47 μg × g^{-1}). This is low relative to the concentrations found in meat from grass fed bison (3.47 μg × g^{-1}) and grain finished beef (2.20 μg × g^{-1}; Galbraith et al., 2016). In lamb, concentrations of vitamin E in muscle below 3 μ × g^{-1} has resulted in a higher rates of lipid oxidation (Ponnampalam et al., 2014). Proximate analysis of the ground lean and fat used to manufacture burger patties had an average fat content of 114.6 ± 22.2 mg × g^{-1} (range: 70.2 to 169.5 mg × g^{-1}).

Effect of NIT packaging on steak shear force, cook time, drip, and cooking losses

The shear force measured from NIT packaged steaks, after retail display, was not significantly different than CONT steaks (CONT 7.74, NIT 7.79 ± 0.33 kg; P = 0.708); cook time was also similar between the packaging treatments (CONT 6.4 s × g^{-1}, NIT 6.7 s × g^{-1} ± 0.2 s × g^{-1}; P = 0.102). However, there was a higher retail drip loss associated with CONT packaging compared to NIT (CONT 40.1, NIT 37.5 mg × g^{-1} ± 1.0, P = 0.001); this appeared to be offset by slightly higher cooking losses (CONT 209, NIT 215 ± 1 mg × g^{-1}, P = 0.022). NIT packaging does not appear to have any detrimental effect on shear force, cooking time, drip loss and cooking loss.

Effect of NIT on burger patty lipid oxidation

There was a significant interaction between packaging and retail display period for measures of TBARS (P < 0.001; Table 2). TBARS, were unchanged in NIT packaged burger patties after 4 d in retail, while in CONT packaging, TBARS increased significantly. Galbraith et al. (2016) observed a similar increase in TBARS from bison steaks when aged for 3 d under retail conditions in aerobic packaging conditions. The TBARS were measured as an indicator of lipid oxidation; the present results indicate the use of NIT was effective in reducing lipid oxidation occurring in bison burger patties. It is highly likely the lower lipid oxidation in NIT packaging resulted from the oxygen impermeability of the film. Pietrasik et al. (2006) observed that beef stored in vacuum packaging without embedded nitrite did not have a significant increase in TBARS over 3 wk of storage. While sensory characteristics were not measured here, the present result may also have implications for the eating quality of bison patties as higher levels of lipid oxidation in beef has been associated with higher perception of rancid flavors (Resconi et al., 2012).

Table 2. Thiobarbituric acid reactive substances (TBARS), subjective color measures, objective color measures and proportion of pigments from bison burgers under retail display conditions in polyvinyl chloride film overwrapped packaging (CONT) or vacuum skin packaged with 113 mg × m^{-2} nitrite embedded vacuum skin oxygen impermeable film (NIT). Burgers were exposed to 14h d^{-1} of direct overhead incandescent lighting (1020 lux)

	CONT			NIT				
Retail display period, d	0	2	4	0	2	4	SEM	*P*-value
TBARS, μmol × L^{-1}	0.22^{b}		1.24^{a}	0.22^{b}		0.20^{b}	0.05	< 0.0001
Mb[1]	–0.01^{d}	0.00^{cd}	0.02^{c}	0.29^{b}	0.29^{b}	0.36^{a}	0.02	< 0.0001
OMb[1]	0.74^{a}	0.62^{b}	0.53^{c}	0.34^{f}	0.45^{d}	0.39^{e}	0.02	< 0.0001
MMb[1]	0.27^{c}	0.37^{b}	0.459^{a}	0.37^{b}	0.26^{cd}	0.25^{d}	0.01	< 0.0001
650nm/570nm[2]	–	–	–	1.59^{b}	1.96^{a}	1.96^{a}	0.02	< 0.0001
Lean[3]	5.42^{e}	6.38^{d}	7.01^{b}	7.16^{a}	6.48^{cd}	6.52^{c}	0.06	< 0.0001
Discoloration[4]	1.02^{c}	1.66^{b}	3.73^{a}	1.00^{c}	1.10^{c}	1.05^{c}	0.07	< 0.0001
L*	52.92^{a}	51.68^{b}	50.43^{c}	51.88^{b}	52.09^{b}	51.98^{b}	0.22	< 0.0001
Chroma, %	13.33^{a}	10.49^{b}	8.83^{d}	8.26^{e}	10.40^{b}	10.00^{c}	0.19	< 0.0001
Hue, °	39.67^{d}	49.03^{b}	53.92^{a}	47.24^{c}	38.98^{d}	38.66^{d}	0.51	< 0.0001

$^{a–f}$Means within the same measurement variable not sharing a common letter were significantly different ($P < 0.05$).

[1]Mb, OMb, and MMb proportions were calculated according to reflex attenuation at 730nm (Krzywicki, 1979).

[2]The ratio of reflectance values is indicative of the amount of NOMb formed on the burger patty surface (American Meat Science Association, 2012).

[3]Eight point descriptive scale indicating the subjective score of lean color (1 = light pink and 8 = extremely dark red, with 5 = bright cherry red).

[4]Seven point descriptive indicating the approximate percent of meat surface which was discolored (1 = 0%; 2 = 1 to 10%; 3 = 11 to 25%; 4 = 26 to 50%; 5 = 51 to 75%; 6 = 76 to 99% and 7 = 100% discoloration) following 3 d of retail display.

Effect of NIT on myoglobin state for steaks and burger patties

In the present study there was a significant 3-way interaction between the packaging type, whole muscle aging duration and retail display period for Mb, OMb, and MMb calculated from measurements collected from steaks ($P < 0.0001$). A significant interaction between packaging type and retail display period was also found for Mb, OMb, and MMb measured on the surface of the burger patties ($P < 0.0001$). Mb, when exposed to a sufficient partial pressure of oxygen forms OMb; meat with high proportions of OMb has a bright cherry-red color. Oxidation of OMb or Mb produces MMb, resulting in a change from bright red to brown (Mancini and Hunt, 2005).

Proportions of Mb were negligible in the CONT packages with all whole muscle aging durations and retail display periods (Fig. 1a). This is likely due to rapid oxygenation of Mb with aerobic packaging. In the NIT packaging, the proportion of Mb increased through the retail display period in steaks aged 6 and 13 d. Since the film used for NIT steaks was oxygen impermeable, it is perhaps unsurprising to see a larger proportion of myoglobin in the Mb state. NIT steaks aged 20 d had a lower proportion of Mb at 0 d retail display than NIT steaks aged 6 or 13 d. After 4 d under retail conditions, the proportion of Mb had increased in NIT steaks aged 20 d, however, the proportion of Mb was still lower than steaks aged for 6 and 13 d after the same retail aging. In burger patties, similar to steaks, the Mb proportions from CONT burger patties were negligible through the retail display, while in NIT packaging Mb proportions were higher and there was a significant increase in Mb after 4 d under retail conditions. The change in Mb in NIT packaged steaks over the retail display contrasts with results observed by Li et al. (2012) where LL steaks from beef in vacuum packaging and vacuum skin packaging, with no embedded nitrite in the films, showed a decline in Mb over retail display. It is unclear if the embedded sodium nitrite present in the NIT packaged steaks of the present study contributed to this increase, as the sodium nitrite free-vacuum packaging systems were not evaluated in the present study.

The proportion of OMb decreased in CONT packaged steaks over the retail display period; this decrease was greater in magnitude for steaks aged for 13 and 20 d prior to retail display (Fig. 1b). Again, initially high OMb proportions were likely due to rapid oxygenation of Mb to OMb under aerobic conditions. The decline in the proportion of OMb in CONT packaging at 2 and 4 d under retail conditions was likely caused by the oxidizing environment under oxygen permeable packaging conditions (Faustman et al., 2010). As the calculations for the proportion of OMb were determined from the difference between all myoglobin forms and Mb + MMb, the OMb proportions calculated for the NIT packaged meat were not negligible as may be expected from a vacuum sealed

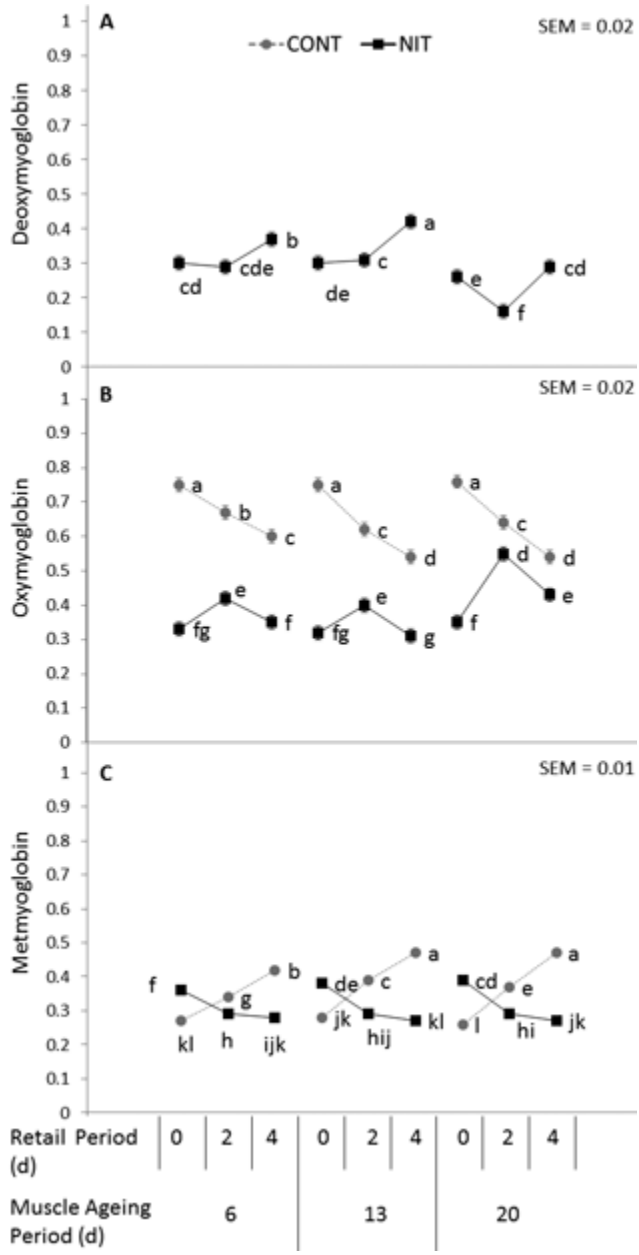

Figure 1. Proportions of myoglobin in the a) deoxymyoglobin, b) oxymyoglobin, and c) metmyoglobin oxidation states through a 4 d retail display from steaks packaged in vacuum-sealed film containing embedded sodium nitrite (NIT) or oxygen permeable polyvinyl chloride film (CONT); steaks were obtained from bison *longissimus lumborum* aged 6, 13, or 20 d. $^{a–k}$Means within the same measurement variable not sharing a common letter were significantly different ($P < 0.05$). The proportions of deoxymyoglobin calculated for CONT steaks was ≤ 0 for all whole muscle aging durations and as such, values are not shown a). Deoxymyoglobin, oxymyoglobin and metmyoglobin proportions were calculated according to reflex attenuation at 730 nm (Krzywicki, 1979). Steaks were exposed to 14h d^{-1} of direct overhead incandescent lighting (1,020 lux).

packaging. The proportion of OMb calculated for NIT steaks were lower than in CONT steaks, and showed the highest proportions at 2 d under retail display. The peak proportion of OMb for NIT steaks at 2 d was greatest for steaks aged 20 d prior to retail display. The same pattern was observed with the burger patties, where there was a decline in the initially high OMb of CONT patties and NIT patties showed a peak in proportion occurring at 2 d under retail conditions. As discussed in the introduction, the nitrite embedded in the NIT film dissolves as nitric oxide at the surface of the meat and binds to the myoglobin, resulting in a fixed red color from the NOMb (Siegel, 2011). The NOMb has been shown to have absorption maxima close to those for OMb (Millar et al., 1996). The absorption maxima for NOMb have been measured at 421 nm, 548 nm, and 579 nm; the maxima for OMb have been observed at 418 nm, 544 nm, and 582 nm (Millar et al., 1996). Given that the NIT packaging is oxygen impermeable, it is likely a large portion of the OMb calculated in the NIT steaks was actually NOMb.

Using the ratio of reflectance values collected at 650 nm / 570 nm as an indication of NOMb, there was a significant interaction between whole muscle aging period and retail display ($P < 0.0001$). The largest increase in this ratio (indicating a higher proportion of NOMb) occurred between 0 and 2 d under retail display for all aging durations. Steaks aged for 13 or 20 d had a slightly higher 650 nm / 570 nm ratio by 2 d in retail conditions than steaks aged 6 d (6 d: 1.48 – 1.67 ± 0.01, 13 d: 1.46 – 1.70 ± 0.01, 20 d: 1.46 – 1.70 ± 0.01). Between 2 and 4 d retail display, the NOMb did not increase further in the steaks aged 13 and 20 d (13 d: 1.70 – 1.69, 20 d: 1.75 – 1.73; $P > 0.05$), while there was a slight increase in the NOMb of steaks aged 6 d (1.67 – 1.70 $P < 0.05$). A very similar pattern was observed for NIT packaged burger patties (Table 2). These results suggest the nitrosylation of myoglobin at the meat surface largely occurs within 2 d. This is consistent with the product indications which suggest allowing NIT packaged meat to cure for 12 to 60 h prior to display.

The MMb increased through the retail display period for CONT packaged steaks, to a greater extent in steaks aged 13 and 20 d prior to packaging. The MMb in NIT packaged steaks, however, declined over the retail display period; with the largest decline in MMb occurring between 0 and 2 d retail display for all whole muscle aging periods. A very similar pattern was observed between packaging treatments and the retail display period with the burger patties. The oxidation of OMb to MMb is thought to be influenced by several factors for meat, including the partial pressure of oxygen and the degree of lipid oxidation (Faustman et al., 2010). Lipid oxidation increases with aging duration for beef stored in vacuum sealed conditions prior to retail display (Vitale et al., 2014); therefore, the greater MMb present after 4 d under retail conditions for CONT packaged steaks aged 13 and 20 d could be the result of greater lipid oxidation driving the oxidation of OMb to MMb.

The MMb present at 0 d under retail conditions in the NIT packaged steaks and burger patties for all aging durations was higher than the CONT packaged meat. As measurements were obtained after only 1 h in packaging, the higher MMb concentration in NIT packaging may be the result of residual oxygen in the packaging; the very low, but not absent levels of oxygen in packaging (i.e., ~1%) favors oxidation to MMb. However, as residual oxygen is thought to be rapidly consumed in vacuum packaging, this could explain why MMb declined by 2 d under retail conditions. The oxidation to MMb may also be promoted by the higher concentration of myoglobin in bison meat (Johnson, 1991). In the presence of NO, MMb can also change state to nitrosometmyoglobin, which has a red-brown color (Schwartz et al., 2009); this could also potentially explain the observed decline in MMb of NIT packaged steaks and burger patties from 0 d to 2 d under retail conditions.

Effect of NIT on retail characteristics for steaks and burger patties

For subjective retail evaluations of lean steak color, a significant 3-way interaction was found between packaging × whole muscle aging × retail display period ($P < 0.0001$; Fig. 2). For each whole muscle aging period, at

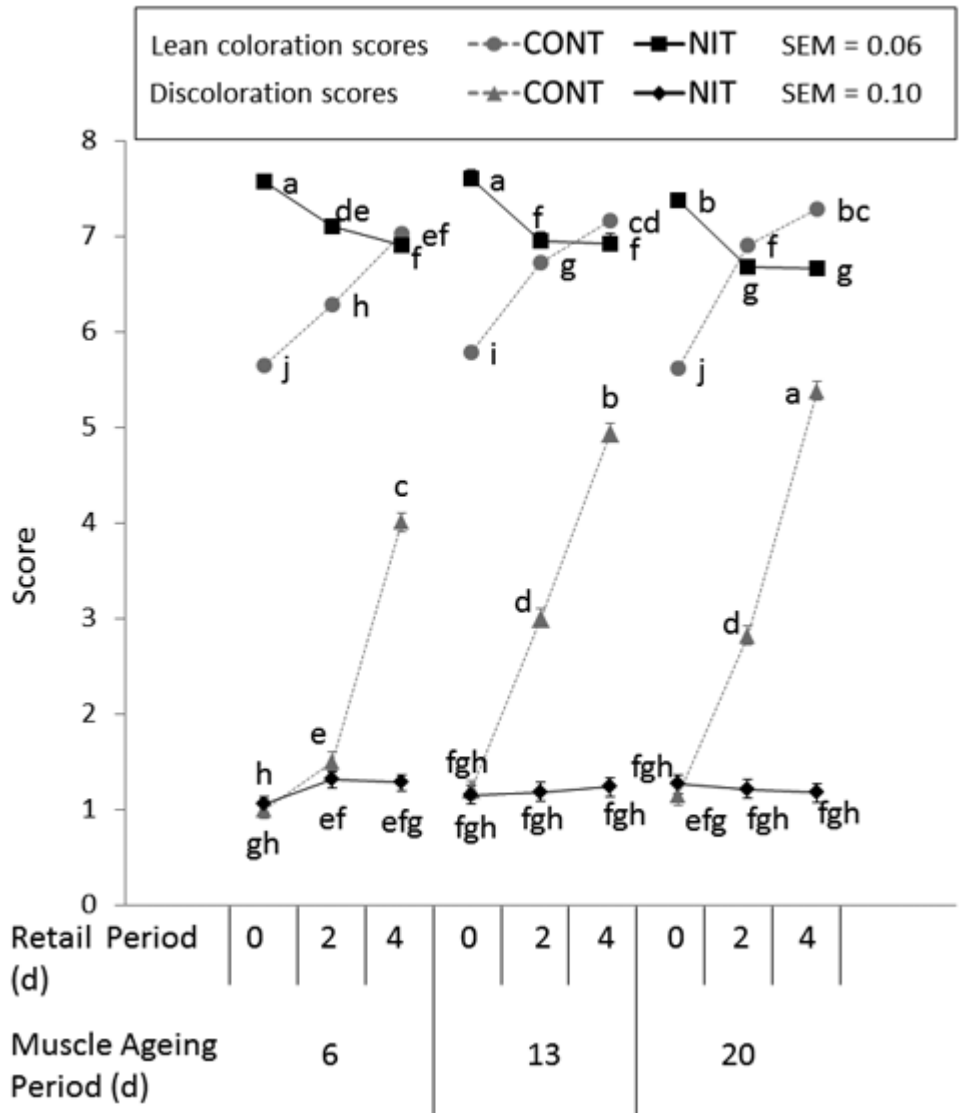

Figure 2. Color measures obtained from bison steaks packaged in vacuum-sealed film containing embedded sodium nitrite (NIT) or oxygen permeable polyvinyl chloride film (CONT) through a 4 d retail display; steaks were obtained from bison *longissimus lumborum* aged 6, 13 or 20 d. Steaks were assigned subjective scores for lean color using an eight point descriptive scale (1 = white and 8 = extremely dark red, with 5 = bright cherry red) and for approximate percent of meat surface which was discolored using a 7 point descriptive scale (1 = 0%; 2 = 1 to 10%; 3 = 11 to 25%; 4 = 26 to 50%; 5 = 51 to 75%; 6 = 76 to 99% and 7 = 100% discoloration). [a–h]Means within the same measurement variable not sharing a common letter were significantly different ($P < 0.05$). Steaks were exposed to 14h d^{-1} of direct overhead incandescent lighting (1020 lux).

0 d retail display, the NIT packaged steaks were rated as having a darker red color than CONT packaged meat. In general, NIT packaged steaks shifted from darker to lighter red, while CONT steaks shifted from lighter to darker red during retail display, but these changes were attenuated between 2 and 4 d of retail display when whole muscle cuts were aged for a longer period (i.e., 20 versus 6 d). Scores of lean coloration for CONT steaks aged 20 d, decreased to a greater extent than the steaks aged for 6 or 13 d. At 0 d under retail conditions NIT steaks aged 20 d had a darker red color than steaks aged 6 and 13 d. However, lean color was similar for steaks aged 20 d between CONT at 0 d and NIT after 4 d under retail conditions. After 4 d in NIT packaging for steaks aged 6 and 13 d, the ratings of lean color did not reach the lighter red values assigned to CONT steaks at 0 d under retail conditions (i.e., shortly after blooming). Similar to steaks, burger patty lean color scores were lower for NIT versus CONT packaged burger patties at 0 d of retail display. After 2 d in retail display NIT lean color scores increased and CONT scores decreased, but at 4 d the NIT color score continued to increase, while the CONT scores remained stable.

The subjective discoloration scores for steaks showed a significant 3-way interaction between packaging × whole muscle aging × retail display period ($P < 0.0001$; Fig. 2). The NIT steaks largely did not show an increase in discoloration through 4 d of retail display, while surface discoloration values for CONT steaks significantly increased during retail display and the extent of discoloration was greater with longer whole muscle aging (Fig. 2). For the burger patties, there was a significant interaction between packaging and retail display period where discoloration increased in CONT packaged patties over the 4 d retail aging period while discoloration scores did not increase significantly in the NIT packaged patties (Table 2). After 4 d under retail conditions, CONT steaks and patties had scores corresponding to more than 50% surface discoloration during retail display. The discoloration scores appear to align well with the increase in proportion of MMb in CONT but not NIT steaks through the retail display period. The MMb concentrations are perceptible in beef steaks when regional MMb proportions are above 60% (Seideman et al., 1984). This may indicate the higher MMb observed during 0 d retail display of the NIT packaged steaks was below a visibly perceptible threshold at the locations where the color measures were obtained.

Regarding objective color evaluation, L*, Chroma and Hue measures obtained from the steaks showed an interaction effect between packaging × whole muscle aging × retail display period ($P < 0.001$). For all aging periods at 0 d under retail conditions, the CONT packaged steaks presented higher values of L* and Chroma but lower Hue values, indicating a more vivid red color than NIT (Fig. 3). However, after 4 d under display conditions, the NIT steaks had significantly higher L* but lower Hue values (more bright red color) than CONT; particularly at 13 d and 20 d of muscle aging. The patterns of change to Hue for both NIT and CONT steaks appears to correspond well with the measures of MMb obtained through

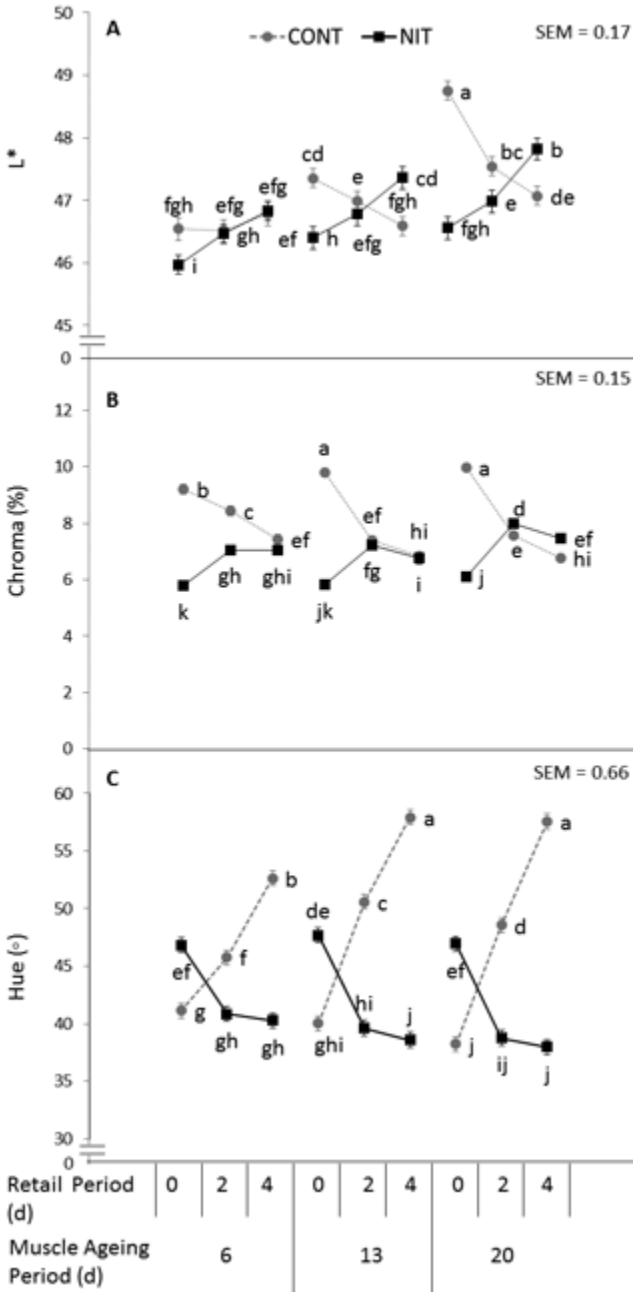

Figure 3. Color measures obtained from bison steaks packaged in vacuum-sealed film containing embedded sodium nitrite (NIT) or oxygen permeable polyvinyl chloride film (CONT) through a 4 d retail display, steaks were obtained from bison *longissimus lumborum* aged 6, 13, or 20 d. a) L*, b) Chroma (%), c) Hue (°). $^{a–k}$Means within the same measurement variable not sharing a common letter were significantly different ($P < 0.05$). Steaks were exposed to 14h d^{-1} of direct overhead incandescent lighting (1,020 lux).

the retail display. Additionally, the Hue of NIT packaged steaks appears to match expectations based on the 650 nm / 570 nm ratio. The Hue of NIT became redder by 2 d under retail conditions, but did not increase further by 4 d. Chroma was higher for CONT steaks aged 6 d after 4 d under retail display; however, for steaks aged 13 d, the Chroma at 4 d was similar to NIT. In contrast for 20 d aged steaks, the Chroma of NIT packaged steaks was higher than CONT after 4 d under retail display.

Similar changes to objective color measures were observed in the burger patties (Table 2). There was an interaction effect between packaging and retail display period for L*, Chroma and Hue ($P < 0.001$). The CONT packaged burger patties at 0 d under retail display conditions had higher L* and Chroma but lower Hue values (bright vivid red color) than NIT packaged burger patties. However, after 4 d under retail display conditions, the CONT packaged burger patties showed lower L* and Chroma but higher Hue values than NIT burger patties, likely due to browning caused by the increased proportion of MMb. NIT packaged burger patties showed no significant change in L* over the entire retail display duration. As discussed above, differences in the lightness of meat between packaging types can be attributed to differences in the packaging environments affecting myoglobin state. In addition to effects of the packaging partial pressure of oxygen and the embedded nitrite on color, the ground mixture used to form the patties, had further oxygen exposure from the grinding process and a higher level of surface area exposed. A bloom Mb to OMb prior to packaging followed by subsequent nitrosylation to NOMb after packaging could explain no change to L* was observed in NIT packaged burger patties.

The effects of packaging strategy on the color measures obtained in the present study for both burger patties and steaks across the objective and subjective evaluations of meat are generally consistent and appear to correspond to the changes in myoglobin oxidative state, at least in the CONT packaged meats. The CONT packaging, due to the high partial pressure of oxygen, bloomed rapidly resulting in lighter and redder meat at 0 d under retail conditions. As mentioned above, all burger patties were exposed to oxygen during the grinding process, as such, the L* of the NIT packaged patties was relatively high, only slightly lower than CONT at 0 d of display. However, after 4 d under retail conditions, CONT packaged meat darkened and showed higher levels of discoloration; additionally, there were higher levels of MMb observed in these steaks. The NIT packaged meat appeared to improve in color over the retail display period with meat becoming lighter and redder. It appears NIT packaged steaks did not reach lightness (from both subjective and L* measures) observed in CONT steaks shortly after bloom (0 d under retail conditions). Also, there were no increases in discoloration of NIT packaged meat over the retail display period. The present results are largely in agreement with the improved color stability in beef treated with nitrite in vacuum packaged systems for beef (Claus and Du, 2013; Song et al., 2015).

Narváez-Bravo et al., (2016) also demonstrated bison meat in NIT packaging has lower bacterial growth relative to PVC oxygen permeable overwrap, but not vacuum packaging film without embedded nitrite; therefore this effect was attributed to the anaerobic packaging conditions not the sodium nitrite present in the film. In addition to improved color stability in bison meat NIT can likely be utilized to increase the shelf life of bison meat relative to PVC oxygen permeable overwrap. Extended shelf-life of NIT packaged beef and pork, relative to PVC oxygen permeable overwrap packaging has been demonstrated (Yang et al., 2016a; Yang et al., 2016b). However, as the NIT packaged meat becomes redder due to NOMb, there is a risk for spoilage masking as the discoloration associated with spoilage will not occur as rapidly. As such, it highly is important to provide best before dates on any NIT packaged meats.

The time required for bison meat to lighten relative to CONT suggests utilizing this packaging strategy might be well suited for centralized packaging prior to shipment to retail. Utilizing a centralized packaging of bison meat with NIT may allow sufficient time for the meat to lighten and allow retailers to display meat at or near optimal appearance. The nitrite embedded packaging material has been deemed generally regarded as safe (GRAS) status by the FDA and GRAS application is currently under review for Canada. The application to the FDA claimed residual unbound nitrite had a concentration of < 0.7 mg/kg in meat (U.S. Food and Drug Administration, 2010). The concentrations of sodium nitrite embedded in the film can be adjusted according to the concentration of myoglobin in the meat. In the present study, the films used for the NIT treatment were designed for use with beef (113 mg × m^{-2}), despite the higher pigment concentration generally observed in bison (Galbraith, 2011). However, the present results suggest this concentration was sufficient for the bison steaks to reach a lighter color within 4 d, with the largest color changes appearing to occur within the first 2 d under retail conditions. Future research could explore if increasing sodium nitrite concentrations in packaging film would cause a more extensive and rapid change in surface color in bison. Additionally, as there were no signs of increased discoloration through 4 d of aging for NIT packaged meat, it would likely be appropriate to display NIT packaged bison meat for a

longer duration than tested here (Narváez-Bravo et al., 2017). Evaluations of this packaging system for beef have shown the color remains stable 9 d after packaging (Claus and Du, 2013). Further research needs to be conducted to determine how long bison meat retains its acceptable appearance after NIT packaging.

Conclusions

The use of NIT packaging appeared to be an effective means of reducing discoloration in bison steaks and burger patties relative to PVC overwrap packaging. The NIT packaging also did not appear to have a detrimental effect on cooking losses or drip loss when both measures were considered together. Packaging type also did not have an effect on shear force. The initial appearance (0 d retail display) of CONT packaged meat was lighter and redder than NIT packaging. This was due to rapid blooming occurring in CONT packaged meat with oxygen permeable film. In key measures of color (discoloration scores, lean color scores, lightness and hue) by 4 d of retail display the appearance of CONT packaged meat had clearly deteriorated, whereas meat in NIT packaging had a largely stable color. Lower lipid oxidation was also observed through the lower TBARS of NIT packaged burger patties relative to CONT. While bison meat in general may have lower color stability than beef, use of NIT in packaging appeared to improve its color stability under retail conditions. Additional research to determine if higher concentrations of nitrite embedded in the packaging film could further improve the color of high pigment bison meat may be warranted.

Literature Cited

Aalhus, J. L., and M. E. Dugan. 2014. Spoilage, factors affecting (b) oxidative and enzymatic. In: W. K. Jensen, C. E. Devine, and M. E. Dikeman, editors, Encyclopedia of meat sciences. 2nd ed. Elsevier, Oxford. p. 394–400. doi:10.1016/B978-0-12-384731-7.00091-X

American Meat Science Association. 2012. Meat color measurement guidelines. Am. Meat. Sci. Assoc., Champaign, Il.

AOAC. 1995a. Method 960.39. Fat (crude) or ether extract in meat final action. AOAC, Gaithersburg, MD.

AOAC. 1995b. Method 992.15. Crude protein in meat and meat products including pets. Combustion method. Final action 1992. Or ether extract in meat final action. AOAC, Gaithersburg, MD.

Basarab, J. A., J. L. Aalhus, M. A. Shah, P. S. Mir, V. S. Baron, M. Dugan, E. K. Okine, and W. M. Robertson. 2007. Effect of feeding sunflower seeds on the performance, carcass characteristics, meat quality, retail stability and sensory characteristics of pasture-fed and feedlot finished beef. Can. J. Anim. Sci. 87(1):15–27. doi:10.4141/A06-047

Canadian Council of Animal Care. 2009. Guidelines on: The care and use of farm animals in research, teaching and testing. Canadian Council on Animal Care, Ottawa, Canada.

CIE. 1978. Recommendations on uniform colorspaces-color equations, psychometric color terms. Commission Internationale de l'Eclairage (CIE). Paris, France.

Claus, J. R., and C. Du. 2013. Nitrite-embedded packaging film effects on fresh and frozen beef color development and stability as influenced by meat age and muscle type. Meat Sci. 95(3):526–535. doi:10.1016/j.meatsci.2013.05.029

Cruz-Hernandez, C., Z. Deng, J. Zhou, A. R. Hill, M. P. Yurawecz, P. Delmonte, M. M. Mossoba, M. E. R. Dugan, and J. K. G. Kramer. 2004. Methods for analysis of conjugated linoleic acids and trans-18:1 isomers in dairy fats by using a combination of gas chromatography, silver-ion thin-layer chromatography/gas chromatography, and silver-ion liquid chromatography. J. AOAC Int. 87(2):545–562.

Cruz-Hernandez, C., J. K. G. Kramer, J. J. Kennelly, D. R. Glimm, B. M. Sorensen, E. K. Okine, L. A. Goonewardene, and R. J. Weselake. 2007. Evaluating the conjugated linoleic acid and trans 18:1 isomers in milk fat of dairy cows fed increasing amounts of sunflower oil and a constant level of fish oil. J. Dairy Sci. 90(8):3786–3801. doi:10.3168/jds.2006-698

Dhanda, J. S., R. B. Pegg, J. A. M. Janz, J. L. Aalhus, and P. J. Shand. 2002. Palatability of bison semimembranosus and effects of marination. Meat Sci. 62(1):19–26. doi:10.1016/S0309-1740(01)00222-4

Ding, C., A. Rodas-González, Ó. López-Campos, J. Galbraith, M. Juárez, I. Larsen, Y. Jin, J. Aalhus, and J. Plaizier. 2016. Effects of electrical stimulation on meat quality of bison striploin steaks and ground patties. Can. J. Anim. Sci. 96(1):79–89. doi:10.1139/cjas-2015-0001

Faustman, C., Q. Sun, R. Mancini, and S. P. Suman. 2010. Myoglobin and lipid oxidation interactions: Mechanistic bases and control. Meat Sci. 86(1):86–94. doi:10.1016/j.meatsci.2010.04.025

Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226(1):497–509.

Galbraith, J. K. 2011. Meat characteristics and stress of bison slaughtered in a mobile or stationary abattoir. PhD. University of Alberta. Edmonton, Canada.

Galbraith, J. K., J. L. Aalhus, M. Juárez, M. E. R. Dugan, I. L. Larsen, N. Aldai, L. A. Goonewardene, and E. K. Okine. 2016. Meat colour stability and fatty acid profile in commercial bison and beef. J. Food Res. 5(3). 10.5539/jfr.v5n3p92

Galbraith, J. K., G. Hauer, L. Helbig, Z. Wang, M. J. Marchello, and L. A. Goonewardene. 2006. Nutrient profiles in retail cuts of bison meat. Meat Sci. 74(4):648–654. doi:10.1016/j.meatsci.2006.05.015

Gómez-Cortés, P., A. Bach, P. Luna, M. Juárez, and M. A. de la Fuente. 2009. Effects of extruded linseed supplementation on n-3 fatty acids and conjugated linoleic acid in milk and cheese from ewes. J. Dairy Sci. 92(9):4122–4134. doi:10.3168/jds.2008-1909

Hewavitharana, A. K., M. C. Lanari, and C. Becu. 2004. Simultaneous determination of vitamin e homologs in chicken meat by liquid chromatography with fluorescence detection. J. Chromatogr. A 1025(2):313–317. doi:10.1016/j.chroma.2003.10.052

Janz, J. A. M., J. L. Aalhus, M. A. Price, and A. L. Schaefer. 2000. The influence of elevated temperature conditioning on bison (bison bison bison) meat quality. Meat Sci. 56(3):279–284. doi:10.1016/S0309-1740(00)00054-1

Jeremiah, L. E., and L. L. Gibson. 2001. The influence of packaging and storage time on the retail properties and case-life of retail-ready beef. Food Res. Int. 34:621–631. doi:10.1016/S0963-9969(01)00080-1

Johnson, B. 1991. Csiro workshop-production of chilled meat for export: Colour of chilled meat. http://www.meatupdate.csiro.au/data/Chilled_meat_for_export_03-91.pdf. (Accessed 9 March 2017).

Joseph, P., S. Suman, S. Li, C. Beach, L. Steinke, and M. Fontaine. 2010. Characterization of bison (bison bison) myoglobin. Meat Sci. 84(1):71–78. doi:10.1016/j.meatsci.2009.08.014

Kramer, J. K. G., V. Fellner, M. E. R. Dugan, F. D. Sauer, M. M. Mossoba, and M. P. Yurawecz. 1997. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. Lipids 32(11):1219–1228. doi:10.1007/s11745-997-0156-3

Kramer, J. K. G., M. Hernandez, C. Cruz-Hernandez, J. Kraft, and M. E. R. Dugan. 2008. Combining results of two gc separations partly achieves determination of all cis and trans 16:1, 18:1, 18:2 and 18:3 except cla isomers of milk fat as demonstrated using ag-ion spe fractionation. Lipids 43(3):259–273. doi:10.1007/s11745-007-3143-4

Krzywicki, K. 1979. Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. Meat Sci. 3(1):1–10. doi:10.1016/0309-1740(79)90019-6

Li, X., G. Lindahl, G. Zamaratskaia, and K. Lundström. 2012. Influence of vacuum skin packaging on color stability of beef longissimus lumborum compared with vacuum and high-oxygen modified atmosphere packaging. Meat Sci. 92(4):604–609. doi:10.1016/j.meatsci.2012.06.006

Lindahl, G. 2011. Colour stablility of steaks from large beef cuts aged under vacuum or high oxygen modified atmosphere. Meat Sci. 87(4):428–435. doi:10.1016/j.meatsci.2010.10.023

Mancini, R. A., and M. C. Hunt. 2005. Current research in meat color. Meat Sci. 71(1):100–121. doi:10.1016/j.meatsci.2005.03.003

Marchello, M. J., and J. A. Driskell. 2001. Nutrient composition of grass- and grain-finished bison. Great Plains Res. 11(1):65–82.

Millar, S., B. Moss, and M. Stevenson. 1996. Some observations on the absorption spectra of various myoglobin derivatives found in meat. Meat Sci. 42(3):277–288. doi:10.1016/0309-1740(94)00045-X

Narváez-Bravo, C., A. Rodas-Gonzalez, C. Ding, J. Ye, O. López-Campos, J. Galbraith, D. Siegel, and J. Aalhus. 2016. Effects of novel nitrite packaging film on the microbiological flora of bison strip-loin steaks. Meat Sci. 112:122. doi:10.1016/j.meatsci.2015.08.053

Narváez-Bravo, C., A. Rodas-González, C. Ding, O. López-Campos, J. Galbraith, I. L. Larsen, J. Ye, D. Siegel, and J. L. Aalhus. 2017. Effects of novel nitrite packaging film on the bacterial growth of bison strip-loin steaks. J. Food Process. Preserv. 2017:e13311. doi:10.1111/jfpp.13311

Nielsen, J. H., B. Sørensen, L. H. Skibsted, and G. Bertelsen. 1997. Oxidation in pre-cooked minced pork as influenced by chill storage of raw muscle. Meat Sci. 46(2):191–197. doi:10.1016/S0309-1740(97)00016-8

Pietrasik, Z., J. S. Dhanda, P. J. Shand, and R. B. Pegg. 2006. Influence of injection, packaging, and storage conditions on the quality of beef and bison steaks. J. Food Sci. 71(2):S110–S118. doi:10.1111/j.1365-2621.2006.tb08913.x

Ponnampalam, E. N., S. Norng, V. F. Burnett, F. R. Dunshea, J. L. Jacobs, and D. L. Hopkins. 2014. The synergism of biochemical components controlling lipid oxidation in lamb muscle. Lipids 49(8):757–766. doi:10.1007/s11745-014-3916-5

Resconi, V. C., A. Escudero, J. A. Beltrán, J. L. Olleta, C. Sañudo, and M. D. Mar Campo. 2012. Color, lipid oxidation, sensory quality, and aroma compounds of beef steaks displayed under different levels of oxygen in a modified atmosphere package. J. Food Sci. 77(1):S10–S18. doi:10.1111/j.1750-3841.2011.02506.x

Schwartz, S. J., von Elbe, J. H., and M.M., G. 2009. Colorants. In: Damodaranm, S., K. L. Parkin, and O. R. Fennema, editors, Food chem. 4th ed. CRC Press, Boca Raton, FL. p. 571–638.

Seideman, S. C., H. R. Cross, G. C. Smith, and P. R. Durland. 1984. Factors associated with fresh meat color: A review. J. Food Qual. 6(3):211–237. doi:10.1111/j.1745-4557.1984.tb00826.x

Shibata, K. 1966. Spectrophotometry of translucent biological materials—opal glass transmission method. In: D. Glick, editor, Methods biochem. Anal. Interscience, New York, NY. p. 217.

Siegel, D. G. 2011. An update on packaging fresh meat with nitrite containing film. 64th Reciprocal Meat Conference. Kansas State University

Song, X., D. Cornforth, D. Whittier, and X. Luo. 2015. Nitrite spray treatment to promote red color stability of vacuum packaged beef. Meat Sci. 99:8–17. doi:10.1016/j.meatsci.2014.08.003

Suman, S. P., and P. Joseph. 2013. Myoglobin chemistry and meat color. Annu. Rev. Food Sci. Technol. 4(1):79–99. doi:10.1146/annurev-food-030212-182623

U.S. Food and Drug Administration. 2010. Grn no. 228 sodium nitrite embedded on one side of a food packaging film at a maximum level of 113 milligrams per square meter https://www.accessdata.fda.gov/scripts/fdcc/?set = GRASNotices&id = 228. (Accessed: 22 February 2017).

Vahmani, P., D. C. Rolland, K. E. Gzyl, and M. E. R. Dugan. 2016. Non-conjugated cis/trans 18:2 in beef fat are mainly δ-9 desaturation products of trans-18:1 isomers. Lipids (in press). doi:10.1007/s11745-016-4207-0

Vitale, M., M. Pérez-Juan, E. Lloret, J. Arnau, and C. E. Realini. 2014. Effect of aging time in vacuum on tenderness, and color and lipid stability of beef from mature cows during display in high oxygen atmosphere package. Meat Sci. 96(1):270–277. doi:10.1016/j.meatsci.2013.07.027

Wood, J. D., R. I. Richardson, G. R. Nute, A. V. Fisher, M. M. Campo, E. Kasapidou, P. R. Sheard, and M. Enser. 2004. Effects of fatty acids on meat quality: A review. Meat Sci. 66(1):21–32. doi:10.1016/S0309-1740(03)00022-6

Yang, X., D. R. Woerner, J. D. Hasty, K. R. McCullough, I. Geornaras, J. N. Sofos, and K. E. Belk. 2016a. An evaluation of the effectiveness of freshcase technology to extend the storage life of whole muscle beef and ground beef. J. Anim. Sci. 94:4911–4920. doi:10.2527/jas.2016-0508

Yang, X., D. R. Woerner, K. R. McCullough, J. D. Hasty, I. Geornaras, G. C. Smith, J. N. Sofos, and K. E. Belk. 2016b. An evaluation of the effectiveness of freshcase technology to extend the storage life of whole-muscle pork and ground pork sausage. J. Anim. Sci. 94(11):4921–4929. doi:10.2527/jas.2016-0509