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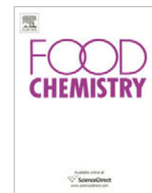

Effects of L- or D-lactate-enhancement on the internal cooked colour development and biochemical characteristics of beef steaks in high-oxygen modified atmosphere

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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.

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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

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