Meat and Muscle Biology™

Impact of Spoilage Bacterial Populations on Discoloration of Beef Steaks

Colton L. Smith[1], Ifigenia Geornaras[1], and Mahesh N. Nair[1]*

[1]Department of Animal Sciences, Colorado State University, Fort Collins, CO 80523, USA
*Corresponding author. Email: mnair@colostate.edu (Mahesh N. Nair)

Abstract: There is limited understanding of the relationship between the growth of spoilage microflora and fresh meat color. Therefore, the objective of this study was to investigate the impact of the growth of common spoilage bacteria on the color stability of color-stable (*longissimus lumborum*; LL) and color-labile (*psoas major*; PM) beef muscles. Beef striploins (LL) and tenderloins (PM) (USDA Choice, $n = 8$) were wet aged (14 d), after which they were decontaminated and fabricated into 1.27-cm thick steaks. Steaks were randomly assigned as decontaminated (DCON) or inoculated (INOC). The surface of INOC steaks was inoculated (ca. 4 log CFU/cm^2) with a mixture of spoilage bacteria, while an equivalent volume of phosphate-buffered saline was applied to the surface of DCON steaks. Steaks were aerobically retail displayed for up to 9 d. Each day, objective and subjective color evaluation and microbiological analyses were conducted. Aerobic plate counts on INOC steaks were 8.9 (LL) and 9.3 (PM) log CFU/cm^2 at the end of retail display. Corresponding counts on DCON steaks were <2.7 (LL) and <3.4 (PM) log CFU/cm^2. For LL steaks, there was a treatment × display day interaction ($P < 0.05$) for lightness (L^*), redness (a^*), yellowness (b^*), lean color scores, surface discoloration, and bacterial levels. On days 6–8, redness was lower ($P < 0.05$) for INOC compared to DCON LL steaks, while lean color scores and surface discoloration were lower ($P < 0.05$) for DCON compared to INOC LL steaks. For PM, there was a treatment × display day interaction ($P < 0.05$) for a^* values, surface discoloration, and bacterial levels. Surface discoloration was greater ($P < 0.05$) for INOC steaks compared with DCON steaks on days 4 and 5. The results indicate a connection between surface discoloration and microbial growth on beef LL and PM steaks, and differences in bacterial growth kinetics could explain some of the differential color stabilities between these muscles.

Key words: fresh beef, meat color, meat spoilage, microbial growth, shelf-life
Meat and Muscle Biology 8(1): 17796, 1–13 (2024) **doi:10.22175/mmb.17796**
Submitted 29 April 2024 Accepted 5 July 2024

Introduction

The demand for fresh meat is expected to increase drastically over the next 3 decades with global economic growth, leading to increased demands on the natural resources needed to produce it (Godfray et al., 2018). At the same time, food wastage, especially wastage of perishable animal proteins, is increasing. In high-income countries, animal protein availability exceeds population needs (Ederer et al., 2023) and results in food waste. For example, approximately 26% of fresh meat produced in the U.S. is wasted annually at the retail and consumer levels (Gunders, 2012). The U.S. produced an estimated retail equivalent of 8.94 billion kg of beef in 2022 (USDA ERS, 2023), and approximately 194.7 million kg of that is wasted annually (Ramanathan et al., 2022), with one primary cause of fresh beef wastage being surface discoloration. Moreover, recent studies have indicated that 2.55% of beef produced in the U.S. is discarded due to discoloration, leading to an economic loss of $3.7 billion/year to the beef industry (Ramanathan et al., 2022). The wastage is partially attributed to consumers' preference for fresh beef that is bright cherry-red colored and their reluctance to purchase meat products that do not meet these color expectations (Viana et al., 2005; Grebitus et al., 2013a; Feuz et al., 2020). The importance of fresh

© 2024 Smith, et al.
This is an open access article distributed under the CC BY license (https://creativecommons.org/licenses/by/4.0/)

beef color on consumer perceptions of quality has been reported previously (Glitsch, 2000; Carpenter et al., 2001; Robbins et al., 2003; Killinger et al., 2004; Thies et al., 2024) as consumers commonly associate beef color with freshness and product safety (Grebitus et al., 2013b).

King et al. (2011) reported that muscle variation within carcass explained more differences in observed *a** (redness) values than animal variation, suggesting that muscle-specific factors could be driving the meat color stability differences. For example, tenderloin (*psoas major*; PM) steaks are considered color-labile, as they discolor rapidly during retail display (within 2–3 d), whereas striploin (*longissimus lumborum*; LL) steaks are considered color-stable, as they can retain a bright cherry-red color for longer than 5 d (McKenna et al., 2005; Najar-Villarreal et al., 2021). Factors such as muscle fiber type differences, myoglobin concentrations, oxygen consumption rates, proteome differences, and microbiome diversities have been examined to explain the muscle specificity in discoloration (Atkinson and Follett, 1973; Hood, 1980; Klont et al., 1998; McKenna et al., 2005; Mancini et al., 2018; Smith et al., 2024); however, less is known about the effects of bacterial growth on discoloration of beef muscles.

The initial bacterial load on fresh meat depends on a multitude of factors, such as the use of antimicrobial interventions during harvest and processing, harvest facility hygiene, fabrication facility cleanliness, temperature during transport and display, as well as retail store sanitation standard operating procedures (De Filippis et al., 2013). Preventing bacterial growth on fresh meat during retail display is nearly impossible as meat provides a nutrient-dense medium for microbial growth (Labadie, 1999). The changes in meat associated with bacterial growth during storage can lead to spoilage (Nychas et al., 2008; Argyri et al., 2015). Fresh beef is typically considered microbially spoiled when it reaches 7–8 log CFU of bacteria and is often characterized by slime formation, surface greening, and off-odor from malodorous volatile compounds (Nychas et al., 2008). The microbial types that dominate during chilled storage of fresh meat depend mostly on the storage temperature and packaging condition of the product. In general, *Pseudomonas* spp. (including *P. fragi*, *P. fluorescens*, and *P. lundensis*) are responsible for spoilage of meat stored under aerobic conditions, while lactic acid bacteria (including species of *Carnobacterium*, *Leuconostoc*, and *Lactobacillus*) are responsible for spoilage of meat stored under oxygen-restricted packaging conditions, such as vacuum and modified atmosphere packaging (Laursen et al., 2005; Leisner et al., 2007; Nychas et al., 2008; Jääskeläinen et al., 2013; Wickramasinghe et al., 2019; Zhang et al., 2019; Pellissery et al., 2020). However, studies evaluating the impact of the growth of spoilage bacteria on the surface discoloration of fresh meat are limited. Previously, Robach and Costilow (1961) reported that *Pseudomonas* can contribute to metmyoglobin formation in beef but failed to observe similar results with lactic acid bacteria. Additionally, it has been reported that *P. fluorescens* can cause brown discoloration in meat (Chan et al., 1998), whereas *P. fragi* may contribute to surface discoloration by increasing lipid and protein oxidation, thus instigating metmyoglobin formation (Bala et al., 1977). The impacts of the spoilage organisms on meat color have been primarily studied in isolation; however, bacteria are rarely present in fresh beef as a monoculture—they exist as a community (Nychas et al., 2008; Hwang et al., 2020). To the authors' knowledge, the community effects of spoilage bacteria on fresh beef steak color stability during retail display have not been examined previously. Therefore, the objective of this study was to examine the effect of a mixture of common meat spoilage microorganisms on beef discoloration during aerobic retail display using color-stable (LL) and color-labile (PM) beef muscles.

Materials and Methods

Experimental design

Beef striploins and tenderloins obtained from a commercial processing facility were wet aged and then subjected to a surface decontamination process. The goal of the decontamination process was to reduce the existing microbial load on the surface of the loins, which, based on various beef slaughter, processing, and storage practices, can be highly variable. Standardization of the initial microbial contamination level amongst the LL and PM loins and subsequently fabricated steaks allowed us to have 2 treatment groups, namely, (1) steaks with a low initial contamination level of the natural microflora (decontaminated control [DCON] treatment) and (2) steaks that were surface inoculated to a target initial concentration of ca. 4 log CFU/cm^2 with a 5-isolate mixture of common spoilage bacteria (inoculated [INOC] treatment). Steaks from LL and PM of both treatments were overwrapped and placed in a retail display case for up to 9 d. To determine the effects of microbial growth during retail display on surface discoloration,

steaks were analyzed daily for microbial counts, instrumental color, and visual color. The experimental details are provided in the sections below.

Meat collection and processing

Eight ($n = 8$) USDA Choice striploins (LL) and tenderloins (PM) were collected from a commercial beef processing facility 24 h postmortem and wet aged in individual vacuum bags (2°C) until 14 d postmortem. Muscles were selected from carcasses of similar age and background. After aging, the muscles were cut into halves (for ease of handling) and decontaminated by submerging them into a pot of boiling water for 2 min. Due to differences in size and fat coverage between LL and PM, the decontamination process was modified for PM to ensure internal temperatures did not exceed 4°C. For PM, the muscle was placed into boiling water for 1 min, removed for 30 s, and then submerged into the boiling water for another 1 min, while LL was submerged in boiling water for 2 min continuously. The internal temperature of the muscles was monitored by inserting a thermometer (ThermaPen One; ThermoWorks, American Fork, UT, USA) into the center and slightly beneath the surface to ensure that temperatures did not exceed 4°C during the decontamination process. The decontaminated muscles were aseptically trimmed, removing the entire outer heat-exposed surface of each piece. The trimmed muscles were then aseptically cut into 0.5 in (1.27 cm) thick steaks, randomly assigned as DCON or INOC, and placed onto soaker pad-lined foam trays.

Bacterial strains and inoculum preparation

The inoculum included 3 isolates of *Pseudomonas* spp. and 2 of lactic acid bacteria from our laboratory's culture collection. The isolates, all of which had been recovered from spoiled beef steaks, included *P. fragi* (CMSQ-SB3), *P. fluorescens* (CMSQ-SB4), *P. lundensis* (CMSQ-SB5), *Carnobacterium divergens* (CMSQ-SB1), and *Leuconostoc gelidum* (CMSQ-SB2). These particular bacterial species were included in the inoculum, as they are commonly associated with spoilage in aerobically packaged beef steaks (Nychas et al., 2008; Wickramasinghe et al., 2019). The isolates, which were maintained as 15% glycerol stocks at −80°C, were individually revived prior to the start of the experiment by transferring a loopful of frozen culture into 10 mL of tryptic soy broth (TSB; Difco™; Becton, Dickinson, and Company, Sparks, MD, USA). Following 24 h of incubation at 25°C, the TSB cultures were streak-plated onto tryptic soy agar (TSA; Neogen Culture Media, Lansing, MI, USA) and incubated at 25°C for 72 h. A subsequent streak plate from a single colony from each original TSA plate was used as the working culture for the experiment.

For the purpose of inoculum preparation, a single colony from the TSA plate of each isolate was separately inoculated into 10 mL TSB and incubated at 25°C (24 h). Each strain was then subcultured (25°C, 24 h) by transferring a 0.1 mL aliquot of the broth culture to 10 mL of fresh TSB (designated as TSB2). Based on preliminary work conducted to determine the cell concentration of each strain following the above culturing protocol, all strains except *P. fragi* reached a concentration of ca. 9 log CFU/mL in the TSB2 culture. The concentration of *P. fragi* was ca. 8 log CFU/mL. To ensure a similar representation of all 5 strains in the inoculum mixture used to inoculate the LL and PM samples, TSB2 cultures of *P. fluorescens*, *P. lundensis*, *C. divergens*, and *L. gelidum* were diluted tenfold in phosphate-buffered saline (PBS; pH 7.4, Sigma-Aldrich, St. Louis, MO, USA) to a ca. 8 log CFU/mL concentration. This dilution, as well as the undiluted TSB2 culture of *P. fragi*, were then combined and centrifuged (Sorvall Legend X1R; Thermo Fisher Scientific, Germany) at $6{,}000 \times g$ for 15 min at 4°C. The resulting bacterial cell pellet was washed with 10 mL PBS and centrifuged as described above. This washing step was repeated one additional time, and after the second wash, the cell pellet was resuspended in 45 mL of PBS. This cell suspension (ca. 8 log CFU/mL) was then diluted tenfold in PBS, and the resulting cell suspension (ca. 7 log CFU/mL) was used to inoculate the meat samples. To determine the actual concentration of the individual strains before centrifugation and of the mixed cell suspension used to inoculate the meat samples, cell suspensions were tenfold diluted in maximum recovery diluent (MRD; Neogen Culture Media) and were plated, in duplicate, onto TSA.

Inoculation, packaging, and retail display

The upper surface of INOC steaks was inoculated with 0.15 mL of the spoilage bacteria inoculum. The cell suspension was deposited on the meat surface with a micropipette and then spread over the entire surface with a sterile disposable spreader. The inoculated samples were left undisturbed for 15 min to allow for bacterial cell attachment. For the DCON steaks, a 0.15 mL volume of PBS was deposited, spread over the surface, and left for 15 min before packaging. Steaks were overwrapped (O_2 transmission = 23,250 mL × m^2 × d^{-1}, 72 gauge, Resinite Packaging Films, Borden, Inc., North Andover, MA, USA) and placed into a multideck retail display case with continuous lighting at 3 ± 1°C

(2800 lx, 1810LX4000 LED fixture, Kason, Newnan, GA, USA; color rendering index = 84, color temperature = 4,500 K) for up to 9 d. Steaks were allowed to bloom for 2 h after wrapping before day 0 color assessment and microbial analysis. Additionally, steaks were rotated in the retail display case daily to ensure minimal impacts from temperature and lighting variance. In a predetermined random order, one steak per loin per treatment (i.e., $n = 8$ each for LL INOC, LL DCON, PM INOC, and PM DCON), at 24 ± 1 h intervals were used to assess instrumental color, panelist visual color evaluation, and microbial population levels.

Instrumental color evaluation

Instrumental and visual color evaluations were performed on packaged steaks prior to sampling for microbiological analysis. Instrumental lightness (L^*), redness (a^*), and yellowness (b^*) were measured using a HunterLab MiniScan LabScan EZ4500 colorimeter (Hunter Associates Laboratory, Reston, VA, USA), with a 2.54-cm diameter aperture with a 12.5-mm measurement port, illuminant A, and 10° standard observer (King et al., 2023). An average was taken of 3 random locations on the light-exposed surface of INOC or DCON steaks. The colorimeter was calibrated with the black and white tiles prior to use daily.

Visual color evaluation

Using a randomized survey tool (Qualtrics, Provo, UT, USA), 5 to 8 panelists assessed lean redness and percent surface discoloration. Samples were only identified by a random four-digit code, and panelists were unaware of the treatments. This work was approved by the Colorado State University Institutional Review Board (IRB#2929). A continuous lean redness color lexicon was adapted from King et al. (2023) with values ranging from 1 to 8 (e.g., 1 = extremely bright cherry-red, 8 = extremely dark red). Panelists were trained to evaluate the lean color of non-discolored portions of the lean surface and to quantify the overall percentage discoloration. Percent discoloration was evaluated using a continuous scale from 0% to 100%. Results for both were reported as estimated marginal means and standard error on a per treatment by day basis.

Microbial analyses and bacterial growth kinetics

For LL, a 4 cm × 4 cm (16 cm^2) surface section of steak, approximately 1 mm thick, was aseptically excised using a sterile template and scalpel, with care to ensure only the lean surface was removed. The sample was placed into a filter-separated sterile sample bag (710 mL; Whirl-Pak, Pleasant Prairie, WI, USA), and 25 mL of MRD was added. For the PM samples, a 3 cm × 4 cm (12 cm^2) section of the surface (to adjust for the smaller muscle dimensions of PM as compared to those of LL) was excised (approximately 1 mm thick), and 19 mL of MRD was added to the sample bag. Other than this difference in dimensions of the excised sample, PM and LL samples were further processed and analyzed for microbial counts the same. Bags containing excised meat samples were mechanically pummeled (Masticator; IUL Instruments, Barcelona, Spain) for 2 min, for bacterial cell detachment. The resulting homogenate was tenfold serially diluted, and appropriate dilutions were surface-plated, in duplicate, on TSA for aerobic plate counts (APC), and *Pseudomonas* agar base (Oxoid Ltd., Basingstoke, Hants, UK) supplemented with *Pseudomonas* CFC supplement (comprised of cetrimide, fucidin, and cephalosporin; Oxoid Ltd.) for *Pseudomonas* spp. counts. Additionally, a pour plate overlay method was used with Lactobacilli MRS agar (MRS, Difco™, Becton, Dickinson, and Company) to obtain lactic acid bacteria counts (LABC). Plates of all 3 culture media were incubated at 25°C for 72 h, followed by manual counting of colonies. Colony counts were converted to log CFU/cm^2. The detection limit of the microbiological analysis was 0.2 log CFU/cm^2.

Estimated growth kinetic parameters of the bacterial populations recovered from LL and PM INOC samples were determined. The Baranyi and Roberts (1994) growth kinetics model was used to model the bacterial population data (log CFU/cm^2) as a function of time, with the Microsoft Excel predictor plug-in DMFit (version 3.5), made publicly available from ComBase (https://www.combase.cc). This primary model characterizes growth kinetics based on 4 parameters: (1) lag phase duration, (2) maximum specific growth rate (μ_{max}), (3) the lower asymptote corresponding to initial population counts (y_0; log CFU/cm^2), and (4) the upper asymptote corresponding to maximum population counts (y_{end}; log CFU/cm^2).

Statistical analysis

Statistical analysis for instrumental color, microbial population enumerations, and visual color data was performed in R (version 4.2.2) using the lme4 (version 1.1.33) package for mixed models. The statistical analysis was performed within muscle only because it is known that beef LL and PM differ in their biochemical properties (McKenna et al., 2005). Therefore, separate but similar factorial models were created

for LL and PM. Display day and treatment type (DCON and INOC) were considered the fixed effects for both LL and PM, and the interaction between display day and treatment was analyzed. Loin was used as a random variable. The lmerTest package (version 3.1.3) in R was used, and an analysis of variance (ANOVA) with a Kenward-Roger degrees of freedom adjustment. Estimated marginal means were calculated with the emmeans package (version 1.8.5) and used to make means comparisons for both interactions and main effects where applicable. Tukey's multiple testing correction was applied, and the significance was set at $\alpha = 0.05$.

Results

Instrumental color evaluation

There was a treatment × display day interaction ($P < 0.05$) for L^* (lightness) values of LL steaks (Table 1). The L^* values were similar ($P \geq 0.05$) for DCON and INOC until day 7, after which DCON had greater ($P < 0.05$) lightness values compared to INOC. Additionally, there was a treatment × display day interaction ($P < 0.05$) for a^* and b^* values of LL. Initially, both DCON and INOC had similar ($P \geq 0.05$) redness (a^* values) until day 5, but on days 6 through 8, the a^* values for INOC were lower ($P < 0.05$) than those of DCON (Table 1).

There was no interaction ($P \geq 0.05$) between display day and treatment for PM for L^* values. However, there was a display day main effect ($P < 0.05$) for L^* values in PM, but there was no treatment effect ($P \geq 0.05$) between DCON and INOC (Figure 1). A treatment × display day interaction ($P < 0.05$) was observed for a^* values of PM steaks (Table 2). It is noteworthy that the a^* values began to increase unexpectedly for INOC steaks during the later days of retail display. There was a treatment effect ($P < 0.05$) and display day effect ($P < 0.05$) for the b^* values of PM, but there was no interaction ($P \geq 0.05$) between them (Table 3). The DCON steaks had higher ($P < 0.05$) b^* values on day 0 compared to those of INOC, but on days 1 through 6, the b^* values of both treatments were similar ($P \geq 0.05$). On the last day, day 7, PM INOC samples had a lower ($P < 0.05$) b^* value than that of DCON (Table 3).

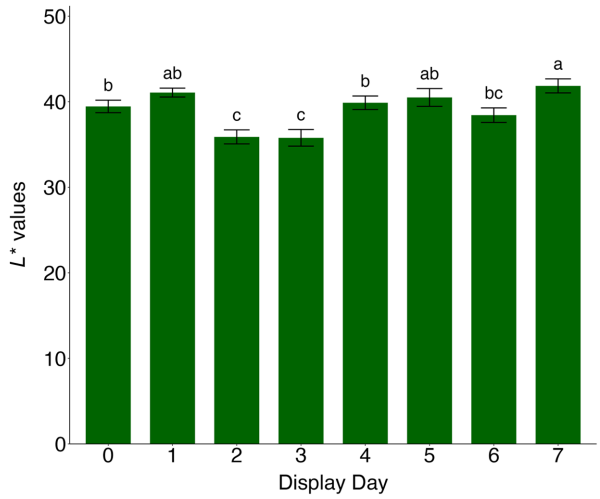

Figure 1. Marginal means ± standard error of CIE L^* (lightness) of beef *psoas major* ($n = 8$) steaks from both decontaminated and inoculated treatments averaged by display day during simulated retail display (3°C). [a–c]Bars without a common letter are different ($P < 0.05$).

Visual color assessment

A treatment × display day interaction ($P < 0.05$) was observed for LL steaks for both lean color and surface discoloration (Table 4). The lean color was

Table 1. Marginal means ± standard error of CIE L^* (lightness), a^* (redness), and b^* (yellowness) of decontaminated (DCON) and inoculated (INOC) treatment groups of beef *longissimus lumborum* ($n = 8$) steaks during simulated retail display (3°C).

	L^* value		a^* value		b^* value	
Day	DCON	INOC	DCON	INOC	DCON	INOC
0	39.61 ± 0.59^{a}	39.45 ± 0.60^{a}	28.09 ± 0.61^{abcde}	27.78 ± 0.47^{bcde}	21.90 ± 0.54^{cd}	21.52 ± 0.44^{cd}
1	38.75 ± 0.68^{abc}	38.80 ± 1.31^{abc}	28.97 ± 0.50^{abcd}	29.50 ± 0.57^{abc}	23.87 ± 0.57^{cd}	24.45 ± 0.54^{bc}
2	38.08 ± 1.04^{abc}	39.05 ± 0.90^{ab}	28.53 ± 0.86^{abcde}	28.18 ± 0.52^{abcde}	24.04 ± 0.18^{cd}	22.93 ± 0.65^{cd}
3	35.27 ± 1.05^{bcd}	35.22 ± 1.03^{bcde}	32.26 ± 1.19^{a}	31.07 ± 1.05^{ab}	28.82 ± 1.27^{a}	27.63 ± 1.05^{ab}
4	38.45 ± 0.70^{abc}	39.14 ± 1.01^{a}	28.14 ± 0.52^{abcde}	26.74 ± 0.64^{cde}	22.91 ± 0.49^{cd}	22.09 ± 0.68^{cd}
5	36.62 ± 0.62^{abcd}	35.89 ± 0.73^{abcd}	26.63 ± 0.69^{cde}	24.80 ± 0.94^{de}	22.25 ± 0.72^{cd}	21.17 ± 0.85^{d}
6	37.70 ± 0.90^{abc}	38.12 ± 0.94^{abc}	25.71 ± 0.91^{cde}	15.50 ± 1.10^{fg}	21.24 ± 0.73^{d}	16.08 ± 0.83^{e}
7	33.55 ± 0.76^{de}	31.40 ± 1.53^{e}	31.37 ± 0.99^{ab}	18.04 ± 2.16^{f}	28.94 ± 0.83^{a}	22.77 ± 1.12^{cd}
8	39.44 ± 0.97^{a}	35.11 ± 1.49^{cde}	24.66 ± 0.75^{e}	12.38 ± 0.63^{g}	21.00 ± 0.75^{d}	14.77 ± 0.53^{e}

[a–g]Marginal means within the same measurement without a common superscript letter are different ($P < 0.05$).

Table 2. Marginal means ± standard error of CIE *a** (redness) of decontaminated (DCON; $n = 8$) and inoculated (INOC; $n = 8$) treatment groups of beef *psoas major* steaks during simulated retail display (3°C).

Day	DCON	INOC
0	27.98 ± 1.21^{a}	28.45 ± 0.17^{a}
1	24.63 ± 0.65^{a}	24.50 ± 0.36^{ab}
2	20.41 ± 0.61^{bc}	20.00 ± 0.53^{c}
3	17.65 ± 0.78^{cde}	17.16 ± 0.78^{cde}
4	15.67 ± 1.01^{def}	11.68 ± 0.46^{f}
5	15.37 ± 0.81^{def}	12.28 ± 0.48^{f}
6	14.09 ± 1.52^{ef}	14.45 ± 0.93^{ef}
7	14.80 ± 1.55^{ef}	18.97 ± 1.35^{cd}

[a–f]Marginal means without a common superscript letter are different ($P < 0.05$).

Table 3. Marginal means ± standard error of CIE *b** (yellowness) of decontaminated (DCON; $n = 8$) and inoculated (INOC; $n = 8$) treatment groups of beef *psoas major* steaks during simulated retail display (3°C).

Day	DCON	INOC
0	24.77 ± 1.08^{aw}	22.68 ± 0.25^{bv}
1	21.30 ± 0.36^{ax}	21.18 ± 0.23^{awv}
2	18.88 ± 0.65^{ayx}	18.87 ± 0.55^{axw}
3	18.46 ± 0.59^{azy}	17.76 ± 0.56^{ayx}
4	16.30 ± 0.66^{az}	15.59 ± 0.38^{azy}
5	16.23 ± 0.68^{az}	15.6 ± 0.83^{azy}
6	16.22 ± 0.64^{az}	15.12 ± 0.70^{az}
7	19.05 ± 0.49^{ayx}	17.01 ± 0.44^{bzyx}

[a,b]Within each row, means without a common superscript letter are different ($P < 0.05$).

[v–z]Marginal means in the same column without a common superscript letter are different ($P < 0.05$).

similar ($P \geq 0.05$) between the treatments until day 5, after which the INOC steaks were darker ($P < 0.05$) than DCON steaks. Percentage surface discoloration was similar ($P \geq 0.05$) between the treatments, with less than 5% for both DCON and INOC on days 0 through 4 (Table 4; Figure 2). On day 5, surface discoloration increased to 13.7% for the INOC steaks and steadily increased each day, reaching nearly 80% discoloration by the end of the study. On the other hand, the discoloration score did not increase ($P \geq 0.05$) for the LL DCON steaks during the display period (Table 4).

Like LL, there was a treatment × display day interaction ($P < 0.05$) for lean color scores and surface discoloration for PM (Table 5). The color of the lean became darker ($P < 0.05$) throughout the display for both treatments. Initially, treatments had similar

Table 4. Marginal means ± standard error of panelist lean color and percentage discoloration of decontaminated (DCON; $n = 8$) and inoculated (INOC; $n = 8$) treatment groups of beef *longissimus lumborum* steaks during simulated retail display (3°C).

	Lean color[1]		% Discoloration	
Day	DCON	INOC	DCON	INOC
0	1.67 ± 0.05^{j}	1.67 ± 0.03^{j}	0.00 ± 0.00^{c}	0.02 ± 0.02^{c}
1	1.77 ± 0.07^{ij}	1.75 ± 0.08^{ij}	0.00 ± 0.00^{c}	0.16 ± 0.16^{c}
2	2.34 ± 0.17^{hij}	2.40 ± 0.13^{hi}	1.39 ± 0.24^{c}	1.29 ± 0.10^{c}
3	2.95 ± 0.17^{gh}	3.22 ± 0.18^{fg}	0.51 ± 0.27^{c}	1.04 ± 0.74^{c}
4	3.72 ± 0.25^{ef}	3.86 ± 0.21^{def}	1.81 ± 1.54^{c}	3.53 ± 2.30^{c}
5	3.72 ± 0.28^{ef}	4.16 ± 0.21^{de}	1.76 ± 0.85^{c}	13.70 ± 2.49^{c}
6	3.88 ± 0.23^{def}	4.92 ± 0.12^{c}	1.28 ± 0.82^{c}	38.03 ± 3.61^{b}
7	4.31 ± 0.22^{cde}	5.64 ± 0.16^{b}	9.59 ± 6.22^{c}	69.25 ± 7.77^{a}
8	4.55 ± 0.21^{cd}	6.60 ± 0.26^{a}	6.11 ± 2.69^{c}	79.74 ± 3.60^{a}

[a–j]Marginal means within the same measurement without a common superscript letter are different ($P < 0.05$).

[1]Panelists scored each steak to assess lean color using a continuous 8-point scale (1 = extremely bright cherry-red, 2 = bright cherry-red, 3 = moderately bright cherry-red, 4 = slightly bright cherry-red, 5 = slightly dark cherry-red, 6 = moderately dark red, 7 = dark red, and 8 = extremely dark red).

($P \geq 0.05$) surface discoloration; however, surface discoloration substantially increased ($P < 0.05$) for INOC steaks from 24.16% on day 3 to over 70% on days 4 and 5, while DCON did not display as sharp an increase (Table 5; Figure 3). By days 6 and 7, surface discoloration was again similar ($P \geq 0.05$) for both treatments on PM steaks.

Microbial populations

For all the bacterial count types assessed (APC, LABC, and *Pseudomonas* spp. counts), there was a treatment × display day interaction ($P < 0.05$) for LL steaks (Table 6). Initial (day 0) levels of APC, LABC, and *Pseudomonas* spp. counts on the INOC steaks were 3.9, 3.6, and 3.5 log CFU/cm^2, respectively, while initial bacterial levels on DCON steaks were below the analysis detection limit (<0.2 log CFU/cm^2) regardless of bacterial count type. Bacterial counts of DCON steaks remained lower ($P < 0.05$) than those of INOC samples throughout the display period. By the end of display, APC, LABC, and *Pseudomonas* spp. counts on INOC LL steaks were 8.9, 6.7, and 8.9 log CFU/cm^2, respectively, whereas those on DCON samples were <2.7, <1.7, and <2.3 log CFU/cm^2, respectively (Table 6).

A treatment × display day interaction ($P < 0.05$) for all bacterial count types was also obtained for PM steaks, and as observed for LL samples, bacterial levels on

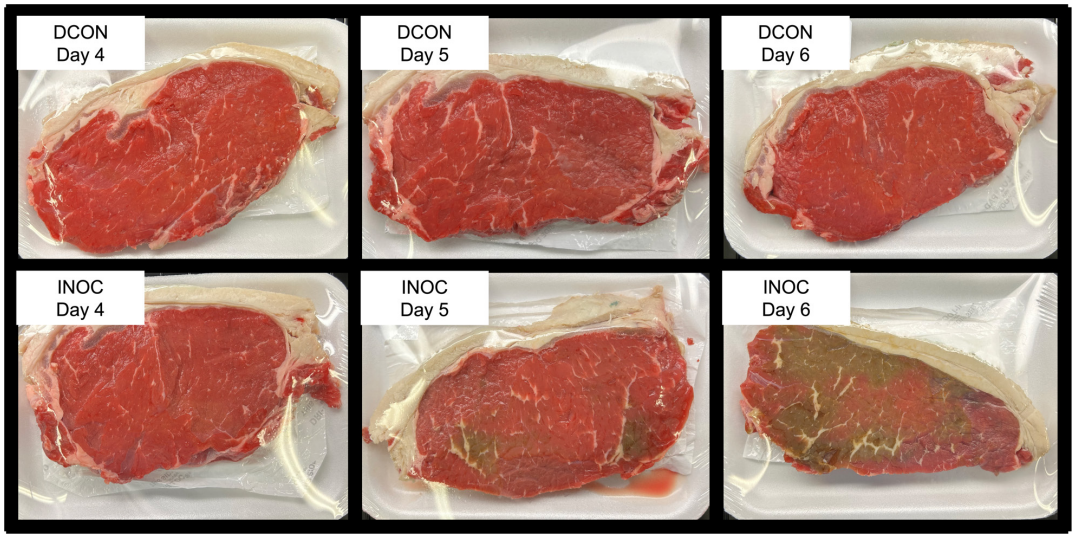

Figure 2. Representative images of beef *longissimus lumborum* steaks fabricated from the same striploin over a 3 d retail display period (days 4 to 6) showing the impact of microbial growth on color stability. Decontaminated controls (DCON; top row) and steaks inoculated with a 5-isolate spoilage bacteria mixture at ca. 4 log CFU/cm^2 (INOC; bottom row) are from the same time points during the simulated retail display at 3°C.

Table 5. Marginal means ± standard error of panelist lean color and percentage discoloration of decontaminated (DCON; $n = 8$) and inoculated (INOC; $n = 8$) treatment groups of beef *psoas major* steaks during retail display (3°C).

	Lean color[1]		% Discoloration	
Day	DCON	INOC	DCON	INOC
0	4.17 ± 0.22^{g}	4.07 ± 0.26^{g}	0.00 ± 0.00^{e}	0.10 ± 0.07^{e}
1	4.88 ± 0.29^{fg}	4.91 ± 0.17^{fg}	9.67 ± 5.41^{de}	1.09 ± 0.51^{e}
2	5.53 ± 0.15^{ef}	5.64 ± 0.25^{def}	11.82 ± 2.39^{de}	16.86 ± 4.33^{de}
3	5.99 ± 0.23^{bcde}	5.67 ± 0.19^{def}	13.96 ± 3.60^{de}	24.16 ± 7.73^{de}
4	5.99 ± 0.18^{bcde}	5.70 ± 0.30^{def}	26.19 ± 7.07^{cde}	70.40 ± 11.22^{ab}
5	5.88 ± 0.23^{cde}	6.79 ± 0.10^{abc}	39.50 ± 7.69^{bcd}	82.38 ± 6.68^{a}
6	6.53 ± 0.22^{abcd}	6.90 ± 0.17^{ab}	58.78 ± 11.03^{abc}	68.10 ± 8.25^{ab}
7	7.10 ± 0.21^{a}	7.11 ± 0.08^{a}	74.89 ± 12.00^{a}	69.06 ± 5.65^{ab}

[a–g]Marginal means within the same measurement without a common superscript letter are different ($P < 0.05$).

[1]Panelists scored each steak to assess lean color using a continuous 8-point scale (1 = extremely bright cherry-red, 2 = bright cherry-red, 3 = moderately bright cherry-red, 4 = slightly bright cherry-red, 5 = slightly dark cherry-red, 6 = moderately dark red, 7 = dark red, and 8 = extremely dark).

INOC samples were greater ($P < 0.05$) than those of DCON samples throughout retail display (Table 7). On day 0, APC, LABC, and *Pseudomonas* spp. counts were <0.3, 0.2, and 0.2 log CFU/cm^2, respectively, on DCON PM steaks and 4.0, 3.7, and 3.7 log CFU/cm^2, respectively, on INOC PM steaks. At the end of the display period, DCON steak bacterial levels were <3.4, 1.0, and 2.8 log CFU/cm^2 for APC, LABC, and *Pseudomonas* spp., respectively (Table 7). In comparison, bacterial levels of 9.3, 8.1, and 9.2 log CFU/cm^2 for APC, LABC, and *Pseudomonas* spp. counts, respectively, were obtained on INOC PM samples.

Bacterial growth kinetics for inoculated steaks

Bacterial growth kinetics parameters (Baranyi and Roberts, 1994) were only estimated for the bacterial populations recovered from the INOC treatment of LL and PM steaks. This analysis was not performed on the DCON microbial data because of the inconsistent occurrence of detectable increases in microbial counts on these steaks during the display period. As such, an accurate estimation of lag phase duration and maximum specific growth rates, when growth did occur, could not be calculated. Starting with LL, estimated lag phase durations for APC, *Pseudomonas* spp., and LABC populations were 2.2, 1.5, and 3.3 d, respectively (Table 8). Conversely, there was no (i.e., <1 d) observed lag phase for APC, LABC, and *Pseudomonas* spp. populations for INOC PM steaks. Growth parameters for populations of all count types recovered from LL INOC steaks did show a slightly higher μ_{max} compared with the populations recovered from PM INOC steaks (Table 8).

Discussion

Microbial growth and color stability of beef longissimus lumborum steaks

The color stability of beef LL during retail display has been examined previously (Joseph et al., 2012;

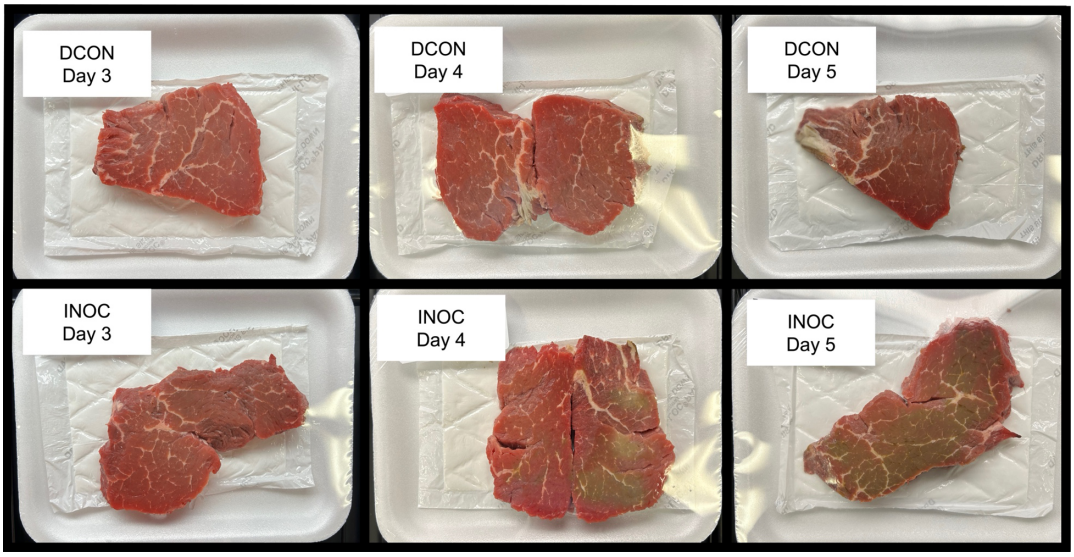

Figure 3. Representative images of beef *psoas major* steaks fabricated from the same tenderloin over a 3 d retail display period (days 3 to 5) showing the impact of microbial growth on color stability. Decontaminated controls (DCON; top row) and steaks inoculated with a 5-isolate spoilage bacteria mixture at ca. 4 log CFU/cm^2 (INOC; bottom row) are from the same time points during the simulated retail display at 3°C.

Table 6. Marginal means ± standard deviation (log CFU/cm^2) of aerobic plate counts (APC), lactic acid bacteria counts (LABC), and *Pseudomonas* spp. counts recovered from decontaminated (DCON; $n = 8$) and inoculated (INOC; $n = 8$) treatment groups of beef *longissimus lumborum* steaks during simulated retail display (3°C).

	APC		LABC		*Pseudomonas* spp.	
Day	DCON	INOC	DCON	INOC	DCON	INOC
0	$< 0.2 \pm 0.1^{g*}$	3.9 ± 0.1^{cd}	$< 0.2 \pm 0.0^{e}$	3.6 ± 0.2^{c}	$< 0.2 \pm 0.0^{f}$	3.5 ± 0.1^{d}
1	$< 0.2 \pm 0.0^{g}$	3.9 ± 0.1^{cd}	$< 0.2 \pm 0.1^{e}$	3.5 ± 0.1^{c}	$< 0.2 \pm 0.0^{f}$	3.7 ± 0.1^{d}
2	$< 0.3 \pm 0.2^{g}$	4.4 ± 0.4^{c}	$< 0.3 \pm 0.2^{e}$	3.5 ± 0.2^{c}	$< 0.2 \pm 0.0^{f}$	4.4 ± 0.4^{cd}
3	$< 0.9 \pm 1.2^{fg}$	5.1 ± 0.3^{bc}	$< 0.7 \pm 1.2^{de}$	3.5 ± 0.2^{c}	$< 0.3 \pm 0.4^{f}$	5.1 ± 0.3^{c}
4	$< 0.4 \pm 0.3^{g}$	5.8 ± 0.6^{b}	$< 0.4 \pm 0.4^{e}$	4.0 ± 0.6^{c}	$< 0.2 \pm 0.0^{f}$	6.3 ± 0.5^{b}
5	NA[1]	7.1[2]	NA	4.7	NA	7.4
6	$< 0.8 \pm 1.0^{fg}$	8.3 ± 0.6^{a}	$< 0.8 \pm 1.0^{de}$	5.5 ± 0.4^{b}	$< 0.2 \pm 0.0^{f}$	8.3 ± 0.6^{a}
7	$< 1.7 \pm 1.7^{ef}$	8.7 ± 0.4^{a}	$< 1.3 \pm 1.2^{de}$	6.1 ± 0.4^{ab}	$< 1.2 \pm 1.8^{ef}$	8.7 ± 0.4^{a}
8	$< 2.7 \pm 1.4^{de}$	8.9 ± 0.4^{a}	$< 1.7 \pm 1.7^{d}$	6.7 ± 0.3^{a}	$< 2.3 \pm 1.5^{e}$	8.9 ± 0.4^{a}

[a–f]Marginal means within the same count type without a common superscript letter are different ($P < 0.05$).

[*]Marginal means with a less than symbol (<) indicate one or more of the samples within the treatment had plate counts below the analysis detection limit (0.2 log CFU/cm^2).

[1]Observed data for day 5 not available due to laboratory error.

[2]Observed data for day 5 not available due to laboratory error; values for INOC were estimated using the Biogrowth package (version 1.0.4; Garre, et al. 2023) in R (version 4.2.2).

Yu et al., 2017; Mancini et al., 2018; Ramanathan et al., 2021), and LL is generally considered a color-stable muscle (McKenna et al., 2005). The *a** values for DCON LL steaks remained relatively steady throughout the display period, only ever getting below 25 on day 8 of display (Table 1), demonstrating that DCON steaks remained bright cherry-red throughout the study. Conversely, the redness values for the INOC LL samples declined through the display period, dropping below 25 from day 5 onwards and were well below the consumer acceptance level (Holman et al., 2017) on the last day of display. The decreased redness of INOC compared with DCON LL steaks indicates that the presence or absence of bacteria may be playing a critical role in the color stability of beef LL steaks during aerobic retail display. These results illustrate a connection between bacterial growth and surface discoloration for beef LL steaks in aerobic packaging. Similarly, Yang et al. (2016) indicated that an increase in *Pseudomonas* spp. count corresponded with a

Table 7. Marginal means ± standard deviation (log CFU/cm^2) of aerobic plate counts (APC), lactic acid bacteria counts (LABC), and *Pseudomonas* spp. counts recovered from decontaminated (DCON; $n = 8$) and inoculated (INOC; $n = 8$) treatment groups of beef *psoas major* steaks during simulated retail display (3°C).

	APC		LABC		*Pseudomonas* spp.	
Day	DCON	INOC	DCON	INOC	DCON	INOC
0	<0.3 ± 0.3^{i*}	4.0 ± 0.1^{f}	< 0.2 ± 0.0^{g}	3.7 ± 0.1^{f}	< 0.2 ± 0.0^{i}	3.7 ± 0.1^{f}
1	<0.2 ± 0.0^{i}	4.5 ± 0.3^{ef}	< 0.2 ± 0.0^{g}	4.0 ± 0.2^{ef}	< 0.2 ± 0.0^{i}	4.3 ± 0.3^{ef}
2	<0.3 ± 0.4^{i}	5.6 ± 0.5^{de}	< 0.2 ± 0.0^{g}	4.8 ± 0.5^{de}	< 0.2 ± 0.0^{i}	5.6 ± 0.4^{de}
3	<1.0 ± 1.0^{hi}	6.9 ± 0.5^{cd}	< 0.5 ± 1.0^{g}	5.4 ± 0.5^{d}	< 0.3 ± 0.4^{hi}	6.9 ± 0.4^{cd}
4	<0.6 ± 0.8^{hi}	7.7 ± 0.4^{bc}	< 0.2 ± 0.0^{g}	5.9 ± 0.4^{cd}	< 0.5 ± 0.6^{hi}	7.7 ± 0.4^{bc}
5	<1.1 ± 1.3^{hi}	8.5 ± 0.4^{ab}	< 0.2 ± 0.0^{g}	6.7 ± 0.5^{bc}	< 1.0 ± 1.3^{hi}	8.5 ± 0.4^{ab}
6	<2.1 ± 2.1^{gh}	9.0 ± 0.3^{ab}	< 0.9 ± 1.2^{g}	7.5 ± 0.5^{ab}	< 1.8 ± 2.2^{gh}	9.0 ± 0.3^{ab}
7	<3.4 ± 2.1^{fg}	9.3 ± 0.5^{a}	< 1.0 ± 1.6^{g}	8.1 ± 0.7^{a}	< 2.8 ± 2.3^{fg}	9.2 ± 0.4^{a}

$^{a–i}$Marginal means within the same count type without a common superscript letter are different ($P < 0.05$).

*Marginal means with a less than symbol (<) indicate one or more of the samples within the treatment had plate counts below the analysis detection limit (0.2 log CFU/cm^2).

Table 8. Estimated growth kinetic parameters, derived from the Baranyi and Roberts (1994) model, of bacterial populations recovered from inoculated beef *longissimus lumborum* (LL; $n = 8$) and *psoas major* (PM; $n = 8$) steaks during simulated retail display (3°C).

		Growth parameters				
Bacterial count type	Muscle	Lag phase duration (days ± SE)	Maximum specific growth rate (μ_{max}; days^{-1} ± SE)	Y_0 (log CFU/cm^2)^a	Y_{end} (log CFU/cm^2)^b	R^2
APC	LL	2.2 ± 0.3	1.175 ± 0.114	3.9	8.9	0.958
	PM	-^{c}	0.975 ± 0.038	3.8	9.3	0.958
LABC	LL	3.3 ± 0.3	0.715 ± 0.056	3.5	-^{d}	0.939
	PM	-	0.644 ± 0.025	3.5	-	0.913
Pseudomonas spp.	LL	1.5 ± 0.3	1.120 ± 0.098	3.5	8.8	0.968
	PM	-	1.050 ± 0.037	3.5	9.2	0.968

APC: Aerobic plate count populations; LABC: Lactic acid bacteria count populations; SE: standard error.

[a]Lower asymptote estimated by the Baranyi and Roberts (1994) model.

[b]Upper asymptote estimated by the Baranyi and Roberts (1994) model.

[c]No measurable lag phase observed.

[d]No upper asymptote observed.

decrease in a^* values (redness) when highly marbled beef steaks were displayed in a 50% O_2 modified atmosphere packaging. In the present study, percent surface discoloration also indicated that LL INOC steaks had greater surface discoloration compared to DCON starting from day 6 (Table 4; Figure 2).

Bacteria rapidly catabolize substrates and excrete waste products during the exponential phase of their growth curve (Nychas et al., 2008). Six days of retail display, in the current study, could have provided enough time for bacteria to enter the exponential phase of growth, begin to catabolize the steak for nutrients, and for their metabolites to accumulate, resulting in surface color deterioration. Their consumption of proteins and lipids in meat and the production of microbial metabolites ultimately lead to the deterioration of a steak's surface, resulting in spoilage (Nychas et al., 2008), as observed in INOC steaks. Bacterial metabolites resulting in spoilage could include aldehydes, alcohols, and ketones, to name a few (Pellissery et al., 2020). However, there is limited evidence associating specific microbial metabolites with color changes in fresh beef. In the current study, the bacterial growth kinetics results showed that there was an estimated 1.5 d lag phase duration for the *Pseudomonas* spp. populations compared to a 3.3 d lag phase for the LABC populations (Table 8). Therefore, these bacterial populations entered the exponential phase of growth on ca. days 1 (*Pseudomonas* spp.) and 3 (LABC). Since the growth and resulting production of microbial metabolites greatly control the shelf-life of fresh meat, the longer lag phase allows for a longer shelf-life because

bacteria are minimally metabolically active during the lag phase (Nychas et al., 2008).

Microbial growth and color stability of beef psoas major steaks

The color-labile nature of PM steaks has been well documented (Seyfert et al., 2006; Joseph et al., 2012; Mancini et al., 2018; Ramanathan et al., 2021), and PM is routinely used as a color-labile muscle model in beef color research. Alongside metmyoglobin formation on the surface of PM steaks, the redness decreases rapidly (Joseph et al., 2012; Mancini et al., 2018), which results in PM displaying a relatively short shelf-life.

Initially, the *a** values (redness) of PM steaks of both treatments were similar and remained so until day 6 of display, after which the redness of INOC steaks increased compared to DCON (Table 2). Najar-Villarreal et al. (2021) conducted a meta-analysis and reported that the estimated consumer acceptable *a** value for PM was 20.99. Based on this, the level of redness of the steaks of both treatments in the current study may have dropped below levels acceptable to consumers by day 2 of display. Additionally, the percentage discoloration was much higher for INOC steaks (70.40%) compared to DCON steaks (26.19%) on day 4 of the retail display (Table 5; Figure 3), indicating that the INOC steaks discolored faster than the DCON steaks. The deterioration in lean color for both treatments with time was anticipated and agrees with previously reported data for beef PM color during retail display (Seyfert et al., 2006; Nair et al., 2018). However, the faster rate of discoloration on INOC PM steaks was a new finding, as far as the authors are aware. By day 4, there was an approximate 7 log CFU difference in APC between INOC and DCON PM steaks (Table 7), and the bacterial populations on INOC samples had been in exponential growth for more than 4 d. Furthermore, considering the much higher *a** values and lower surface discoloration for DCON versus INOC for PM steaks, it is reasonable to suggest that bacterial growth plays a role in surface color stability and discoloration.

Although there was not a statistical difference in *a** values between the DCON and INOC PM steaks on days 4 and 5, the *a** values were numerically lower for INOC steaks during these 2 d. Interestingly, on days 6 and 7, the *a** value of PM INOC steaks increased compared to the previous day's INOC *a** values and to PM DCON steaks at the same time point (Table 2). It is noteworthy that the increase in *a** values during the later portion of the retail display on INOC PM steaks coincided with slime formation on the surface of the steaks, and has been reported previously (Motoyama et al., 2010). Faustman et al. (1990) reported that *Pseudomonas* facilitated a color change of brown to red in ground beef meat extract, suggesting that the growth of *Pseudomonas* may have been responsible for the unanticipated color reversion. According to a study by Ayres (1960), bacterial slime formation on beef occurs when populations reach a concentration of 7–8 log CFU, which was reached around day 4 for PM INOC steaks in the current study (Table 7). Despite the increased redness of INOC steaks, when evaluating the decline in *a** values of DCON steaks by display day, it is apparent that DCON steaks remained redder compared to INOC PM steaks until the point of slime formation. Furthermore, the surface discoloration percentage was greater for PM INOC steaks compared to PM DCON steaks on days 4 and 5 (Table 5). In summary, these data showed the PM DCON steaks decreased in redness but had much less surface discoloration compared to PM INOC steaks.

The bacterial growth kinetics estimated using the Baranyi and Roberts (1994) primary model showed that there was not a measurable lag phase duration (i.e., <1 d) for the bacterial populations recovered from the INOC PM steaks. As previously mentioned, bacteria in the lag phase have substantially reduced metabolic activity and are not catabolizing much of their growing medium and not producing high quantities of metabolites (Nychas et al., 2008). However, with the short lag phase, bacteria on PM steaks could be rapidly catabolizing nutrients from the steak and producing metabolic waste. This is a similar finding as reported in Smith et al. (2024), where the authors reported a less than 24 h lag time for naturally occurring bacteria on aerobically packaged beef PM steaks, similar to the current study. The lack of a detectable bacterial lag phase suggests that PM is a very suitable substrate for microbial growth, and within a few days of growth, deleterious effects on color are observed, similar to the effects of growth observed in LL. This is highly suggestive that muscle specificity, specifically the PM and its biochemical properties, is interwoven with the negative consequences of microbial growth.

Qualitative comparison of longissimus lumborum and psoas major steaks

As the purpose of this study was not to compare the color stability of beef LL and PM, which has been done previously (Seyfert et al., 2006; Ramanathan et al.,

2021; Smith et al., 2024), we did not perform statistical comparisons between these 2 muscles. However, comparing these color-stable and color-labile muscle models qualitatively could provide insights into the interaction between bacterial growth and differential color stabilities of these muscles. In this study, PM was used as the color-labile model and LL as the color-stable model. Generally, color-labile muscles have a greater percentage of type I muscle fibers resulting in higher mitochondrial density, which contributes to a greater oxygen consumption rate, thus decreasing the color stability (McKenna et al., 2005). Without considering microbial influence, muscles similar to PM may be more biochemically predisposed to discolor rapidly compared to the color-stable muscles such as LL. However, based on the results of the current study, it is reasonable to speculate that muscles more intrinsically predisposed to rapid bacterial growth (such as PM) would have a much more rapid decrease in color stability compared to muscles that are color-stable and facilitate slower microbial growth such as LL.

The bacterial growth kinetics could also have had a role in the differential shelf-life of these 2 muscles. Bacterial populations on INOC PM samples experienced an estimated lag phase of less than 1 d and entered the exponential phase faster than populations on INOC LL samples (Table 8). Furthermore, INOC PM samples experienced discoloration at a considerable percentage of the steak surface after day 3 of retail display (Tables 5 and 8). In LL, bacterial populations had a lag phase of approximately 2–3 d, and surface discoloration began 3 d after entering the exponential phase (day 6 of retail display; Tables 4 and 8, respectively).

Regardless of muscle, once bacterial growth commenced during retail display, higher levels of *Pseudomonas* spp. were recovered than those of lactic acid bacteria. The dominance of *Pseudomonas* spp. in aerobically stored meat is not a new finding (Molin and Ternström, 1982; Labadie, 1999; Koutsoumanis et al., 2006). In the current study, *Pseudomonas* spp. and lactic acid bacteria were introduced onto the meat surface at similar levels (Tables 6 and 7). The predominance of *Pseudomonas* spp. could have been a driving force in the difference in color stability between the treatments (Bala et al., 1977; Chan et al., 1998). In fact, the greater surface discoloration and lower redness for INOC steaks compared to DCON steaks observed in the current study could be attributed to the aerobic *Pseudomonas* spp. growth. Steaks with higher microbial loads could also have greater lipid and protein oxidation compared with steaks that have lower microbial population levels (Robach and Costilow, 1961; Bala et al., 1977), which could have resulted in the color differences due to myoglobin oxidation (Faustman et al., 2010).

Conclusion

The results of this study demonstrated the impact of microbial growth on the color stability of beef LL and PM during aerobic retail display. The differences in bacterial growth kinetics between LL and PM could be playing a role in the muscle-specific color stability discrepancy between these muscles, although more research is needed. In general, the spoilage bacteria mixture used in this study grew faster on PM compared with LL, indicating that PM provides a highly hospitable environment for bacterial growth, which could be contributing to its rapid discoloration. These findings demonstrated that the role of microbial growth in fresh beef color should be further investigated and must be considered in fresh beef color stability research.

Acknowledgments

Research coordinated by the National Cattlemen's Beef Association, a contractor to the Beef Checkoff. The use of trade names in this publication does not imply endorsement or criticism of those products or other similar products not mentioned by Colorado State University.

Literature Cited

Argyri, A. A., A. Mallouchos, E. Z. Panagou, and G.-J. E. Nychas. 2015. The dynamics of the HS/SPME–GC/MS as a tool to assess the spoilage of minced beef stored under different packaging and temperature conditions. Int. J. Food Microbiol. 193:51–58. https://doi.org/10.1016/j.ijfoodmicro.2014.09.020

Atkinson, J. L., and M. J. Follett. 1973. Biochemical studies on the discoloration of fresh meat. Int. J. Food Sci. Tech. 8:51–58. https://doi.org/10.1111/j.1365-2621.1973.tb01688.x

Ayres, J. C. 1960. Temperature relationships and some other characteristics of the microbial flora developing on refrigerated beef. J. Food Sci. 25:1–18. https://doi.org/10.1111/j.1365-2621.1960.tb17930.x

Bala, K., R. T. Marshall, W. C. Stringer, and H. D. Naumann. 1977. Effect of *Pseudomonas fragi* on the color of beef. J. Food Sci. 42:1176–1179. https://doi.org/10.1111/j.1365-2621.1977.tb14454.x

Baranyi, J., and T. A. Roberts. 1994. A dynamic approach to predicting bacterial growth in food. Int. J. Food Microbiol. 23:277–294. https://doi.org/10.1016/0168-1605(94)90157-0

Carpenter, C. E., D. P. Cornforth, and D. Whittier. 2001. Consumer preferences for beef color and packaging did not affect eating satisfaction. Meat Sci. 57:359–363. https://doi.org/10.1016/S0309-1740(00)00111-X

Chan, W. K. M., S.-T. Joo, C. Faustman, Q. Sun, and R. Vieth. 1998. Effect of *Pseudomonas fluorescens* on beef discoloration and oxymyoglobin oxidation in vitro. J. Food Protect. 61:1341–1346. https://doi.org/10.4315/0362-028X-61.10.1341

De Filippis, F. D., A. L. Storia, F. Villani, and D. Ercolini. 2013. Exploring the sources of bacterial spoilers in beefsteaks by culture-independent high-throughput sequencing. PLOS ONE 8: e70222. https://doi.org/10.1371/journal.pone.0070222

Ederer, P., I. Baltenweck, J. N. Blignaut, C. Moretti, and S. Tarawali. 2023. Affordability of meat for global consumers and the need to sustain investment capacity for livestock farmers. Animal Frontiers 13:45–60. https://doi.org/10.1093/af/vfad004

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

Faustman, C., J. L. Johnson, R. G. Cassens, and M. P. Doyle. 1990. Observations on color reversion in beef and the influence of psychrotrophic bacteria. Fleischwirtschaft 70:676–679 (English). 70:689–692 (German).

Feuz, R., F. B. Norwood, and R. Ramanathan. 2020. Do consumers have an appetite for discolored beef? Agribusiness 36:631–652. https://doi.org/10.1002/agr.21651

Garre, A., J. Koomen, H. M. W. den Besten, and M. H. Zwietering. 2023. Modeling population growth in R with the biogrowth package. J. Stat. Softw. 107:1–51. https://doi.org/10.18637/jss.v107.i01

Glitsch, K. 2000. Consumer perceptions of fresh meat quality: Cross-national comparison. Brit. Food J. 102:177–194. https://doi.org/10.1108/00070700010332278

Godfray, H. C. J., P. Aveyard, T. Garnett, J. W. Hall, T. J. Key, J. Lorimer, R. T. Pierrehumbert, P. Scarborough, M. Springmann, and S. A. Jebb. 2018. Meat consumption, health, and the environment. Science 361. https://doi.org/10.1126/science.aam5324

Grebitus, C., H. H. Jensen, and J. Roosen. 2013a. US and German consumer preferences for ground beef packaged under a modified atmosphere – Different regulations, different behaviour? Food Policy 40:109–118. https://doi/10.1016/j.foodpol.2013.02.005

Grebitus, C., H. H. Jensen, J. Roosen, and J. G. Sebranek. 2013b. Fresh meat packaging: Consumer acceptance of modified atmosphere packaging including carbon monoxide. J. Food Protect. 76:99–107. https://doi.org/10.4315/0362-028X.JFP-12-045

Gunders, D. 2012. Wasted: How America is losing up to 40 percent of its food from farm to fork to landfill. Natural Resources Defense Council. https://www.nrdc.org/sites/default/files/wasted-food-IP.pdf. (Accessed 24 September 2023).

Holman, B. W. B., R. J. van de Ven, Y. Mao, C. E. O. Coombs, and D. L. Hopkins. 2017. Using instrumental (CIE and reflectance) measures to predict consumers' acceptance of beef colour. Meat Sci. 127:57–62. https://doi.org/10.1016/j.meatsci.2017.01.005

Hood, D. E. 1980. Factors affecting the rate of metmyoglobin accumulation in pre-packaged beef. Meat Sci. 4:247–265. https://doi.org/10.1016/0309-1740(80)90026-1

Hwang, B. K., H. Choi, S. H. Choi, and B. S. Kim. 2020. Analysis of microbiota structure and potential functions influencing spoilage of fresh beef meat. Front. Microbiol. 11:1657. https://doi.org/10.3389/fmicb.2020.01657

Jääskeläinen, E., P. Johansson, O. Kostiainen, T. Nieminen, G. Schmidt, P. Somervuo, M. Mohsina, P. Vanninen, P. Auvinen, and J. Björkroth. 2013. Significance of heme-based respiration in meat spoilage caused by *Leuconostoc gasicomitatum*. Appl. Environ. Microb. 79:1078–1085. https://doi.org/10.1128/AEM.02943-12

Joseph, P., S. P. Suman, G. Rentfrow, S. Li, and C. M. Beach. 2012. Proteomics of muscle-specific beef color stability. J. Agr. Food Chem. 60:3196–3203. https://doi.org/10.1021/jf204188v

Killinger, K. M., C. R. Calkins, W. J. Umberger, D. M. Feuz, and K. M. Eskridge. 2004. Consumer visual preference and value for beef steaks differing in marbling level and color. J. Anim. Sci. 82:3288–3293. https://doi.org/10.2527/2004.82113288x

King, D. A., M. C. Hunt, S. Barbut, J. R. Claus, D. P. Cornforth, P. Joseph, Y. H. Kim, G. Lindahl, R. A. Mancini, M. N. Nair, K. J. Merok, A. Milkowski, A. Mohan, F. Pohlman, R. Ramanathan, C. R. Raines, M. Seyfert, O. Sørheim, S. P. Suman, and M. Weber. 2023. American Meat Science Association guidelines for meat color measurement. Meat Muscle Biol. 6:12473,1–81. https://doi.org/10.22175/mmb.12473

King, D. A., S. D. Shackelford, and T. L. Wheeler. 2011. Relative contributions of animal and muscle effects to variation in beef lean color stability. J. Anim. Sci. 89:1434–1451. https://doi.org/10.2527/jas.2010-3595

Klont, R. E., L. Brocks, and G. Eikelenboom. 1998. Muscle fibre type and meat quality. Meat Sci. 49:S219–S229. https://doi.org/10.1016/S0309-1740(98)90050-X

Koutsoumanis, K., A. Stamatiou, P. Skandamis, and G.-J. E. Nychas. 2006. Development of a microbial model for the combined effect of temperature and pH on spoilage of ground meat, and validation of the model under dynamic temperature conditions. Appl. Environ. Microb. 72:124–134. https://doi.org/10.1128/AEM.72.1.124-134.2006

Labadie, J. 1999. Consequences of packaging on bacterial growth. Meat is an ecological niche. Meat Sci. 52:299–305. https://doi.org/10.1016/S0309-1740(99)00006-6

Laursen, G. B., L. Bay, I. Cleenwerck, M. Vancanneyt, J. Swings, P. Dalgaard, and J. J. Leisner. 2005. *Carnobacterium divergens* and *Carnobacterium maltaromaticum* as spoilers or protective cultures in meat and seafood: phenotypic and genotypic characterization. Syst. Appl. Microbiol. 28:151–164. https://doi.org/10.1016/j.syapm.2004.12.001

Leisner, J. J., B. G. Laursen, H. Prévost, D. Drider, and P. Dalgaard. 2007. *Carnobacterium*: Positive and negative effects in the environment and in foods. FEMS Microbiol. Rev. 31:592–613. https://doi.org/10.1111/j.1574-6976.2007.00080.x

Mancini, R. A., K. Belskie, S. P. Suman, and R. Ramanathan. 2018. Muscle-specific mitochondrial functionality and its influence on fresh beef color stability. J. Food Sci. 83:2077–2082. https://doi.org/10.1111/1750-3841.14219

McKenna, D. R., P. D. Mies, B. E. Baird, K. D. Pfeiffer, J. W. Ellebracht, and J. W. Savell. 2005. Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. Meat Sci. 70:665–682. https://doi.org/10.1016/j.meatsci.2005.02.016

Molin, G., and A. Ternström. 1982. Numerical taxonomy of psychrotrophic pseudomonads. Microbiology+ 128:1249–1264. https://doi.org/10.1099/00221287-128-6-1249

Motoyama, M., M. Kobayashi, K. Sasaki, M. Nomura, and M. Mitsumoto. 2010. *Pseudomonas* spp. convert metmyoglobin into deoxymyoglobin. Meat Sci. 84:202–207. https://doi.org/10.1016/j.meatsci.2009.08.050

Nair, M. N., S. Li, C. M. Beach, G. Rentfrow, and S. P. Suman. 2018. Changes in the sarcoplasmic proteome of beef muscles with differential color stability during postmortem aging. Meat Muscle Biol. 2. https://doi.org/10.22175/mmb2017.07.0037

Najar-Villarreal, F., E. A. E. Boyle, C. I. Vahl, Q. Kang, J. J. Kastner, J. Amamcharla, and M. C. Hunt. 2021. Determining the longissimus lumborum and psoas major beef steak color life threshold and effect of postmortem aging time using meta-analysis. Meat Muscle Biol. 5. https://doi.org/10.22175/mmb.12526

Nychas, G.-J. E., P. N. Skandamis, C. C. Tassou, and K. P. Koutsoumanis. 2008. Meat spoilage during distribution. Meat Sci. 78:77–89. https://doi.org/10.1016/j.meatsci.2007.06.020

Pellissery, A. J., P. G. Vinayamohan, M. A. R. Amalaradjou, and K. Venkitanarayanan. 2020. Chapter 17 - Spoilage bacteria and meat quality. In: A. K. Biswas and P. K. Mandal, editors, Meat quality analysis. Academic Press, Cambridge, MA. p. 307–334. https://doi.org/10.1016/B978-0-12-819233-7.00017-3

Ramanathan, R., L. H. Lambert, M. N. Nair, B. Morgan, R. Feuz, G. Mafi, and M. Pfeiffer. 2022. Economic loss, amount of beef discarded, natural resources wastage, and environmental impact due to beef discoloration. Meat Muscle Biol. 6:13218,1–8. https://doi.org/10.22175/mmb.13218

Ramanathan, R., M. N. Nair, Y. Wang, S. Li, C. M. Beach, R. A. Mancini, K. Belskie, and S. P. Suman. 2021. Differential abundance of mitochondrial proteome influences the color stability of beef longissimus lumborum and psoas major muscles. Meat Muscle Biol. 5. https://doi.org/10.22175/mmb.11705

Robach, D. L., and R. N. Costilow. 1961. Role of bacteria in the oxidation of myoglobin. Appl. Environ. Microb. 9:529–533. https:/doi.org/10.1128/am.9.6.529-533.1961

Robbins, K., J. Jensen, K. J. Ryan, C. Homco-Ryan, F. K. McKeith, and M. S. Brewer. 2003. Consumer attitudes towards beef and acceptability of enhanced beef. Meat Sci. 65:721–729. https://doi.org/10.1016/S0309-1740(02)00274-7

Seyfert, M., R. A. Mancini, M. C. Hunt, J. Tang, C. Faustman, and M. Garcia. 2006. Color stability, reducing activity, and cytochrome c oxidase activity of five bovine muscles. J. Agr. Food Chem. 54:8919–8925. https://doi.org/10.1021/jf061657s

Smith, C., L., S. V. Gonzalez, J. L. Metcalf, I. Geornaras, and M. N. Nair. 2024. Differences in spoilage microflora growth kinetics could be contributing to beef muscle-specific color stability. Meat Muscle Biol. 8:16915,1–14. https://doi.org/10.22175/mmb.16915

Thies, A. J., B. A. Altmann, A. M. Countryman, C. Smith, and M. N. Nair. 2024. Consumer willingness to pay (WTP) for beef based on color and price discounts. Meat Sci. 217:109597. https://doi.org/10.1016/j.meatsci.2024.109597.

USDA ERS. 2023. Statistics & Information. https://www.ers.usda.gov/topics/animal-products/cattle-beef/statistics-information/. (Accessed 17 November 2023).

Viana, E. S., L. A. M. Gomide, and M. C. D. Vanetti. 2005. Effect of modified atmospheres on microbiological, color and sensory properties of refrigerated pork. Meat Sci. 71:696–705. https://doi.org/10.1016/j.meatsci.2005.05.013

Wickramasinghe, N. N., J. Ravensdale, R. Coorey, S. P. Chandry, and G. A. Dykes. 2019. The predominance of psychrotrophic pseudomonads on aerobically stored chilled red meat. Compr. Rev. Food Sci. F. 18:1622–1635. https://doi.org/10.1111/1541-4337.12483

Yang, X., Y. Zhang, L. Zhu, M. Han, S. Gao, and X. Luo. 2016. Effect of packaging atmospheres on storage quality characteristics of heavily marbled beef longissimus steaks. Meat Sci. 117:50–56. https://doi.org/10.1016/j.meatsci.2016.02.030

Yu, Q., W. Wu, X. Tian, F. Jia, L. Xu, R. Dai, and X. Li. 2017. Comparative proteomics to reveal muscle-specific beef color stability of Holstein cattle during post-mortem storage. Food Chem. 229:769–778. https://doi.org/10.1016/j.foodchem.2017.03.004

Zhang, P., M. Gänzle, and X. Yang. 2019. Complementary antibacterial effects of bacteriocins and organic acids as revealed by comparative analysis of *Carnobacterium* spp. from meat. Appl. Environ. Microb. 85:e01227–19. https://doi.org/10.1128/AEM.01227-19