Differences in Spoilage Microflora Growth Kinetics Could Be Contributing to Beef Muscle-Specific Color Stability

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Abstract: The role of microbial communities on beef color stability during retail display is not fully understood. Therefore, this study aimed to characterize the physiochemical properties and microbial communities of color-stable (*longissimus lumborum* [LL]) and color-labile (*psoas major* [PM]) beef muscles during aerobic retail display. Paired USDA Select beef LL and PM ($n = 5$) were collected and aged (at 2°C) for 14 d. These were fabricated into 2.54-cm-thick steaks, packaged aerobically, and subjected to 7 d of retail display. Samples were evaluated daily for visual and instrumental color, percentage discoloration, water activity, pH, metmyoglobin reducing activity (MRA), and bacterial population levels using conventional culture-based methodologies. Additionally, 16S ribosomal RNA (rRNA) amplicon sequencing was performed to characterize microbial communities associated with the LL and PM steaks throughout storage. The percentage discoloration was greater ($P < 0.05$) in PM than LL after 2 d of retail display. Color stability, measured by MRA, was lower ($P < 0.05$) for PM compared with LL each day. Culture-based microbiological analysis revealed that bacterial populations of PM steaks either exhibited no lag phase or had a shorter lag phase than the populations on LL samples. By the end of the retail display, bacterial levels on PM steaks were 1.3 to 1.6 log CFU/cm^2 higher ($P < 0.05$) than those recovered from LL samples. The 16S rRNA gene sequencing showed no differences ($P \geq 0.05$) in the alpha or beta diversities for the microbial communities of PM and LL on the same display day. The results confirmed that PM steaks had less color stability and a faster increase in bacterial numbers compared with LL during display. These results suggest that when initial bacterial communities are similar, the differential growth kinetics of bacteria present on these 2 muscles could contribute to the difference in their color stabilities.

Introduction

In North America, about 16% of meat is lost at the distribution and consumer levels each year, primarily due to spoilage concerns, which equates to a loss of 18.6 kg per person (Gustavsson et al., 2011). When purchasing fresh beef, lean color is considered the most important criterion for consumers (Forbes et al., 1974; Killinger et al., 2004; Mwashiuya et al., 2018). Previous studies have suggested that once beef has reached a redness (*a**) value below 14.5 or has more than 20% discoloration, consumers regard this product as undesirable regardless of the microbial load (O'Keeffe and Hood, 1982; Holman et al., 2017). Although the color of fresh beef is not an indicator of microbial spoilage, beef discoloration is responsible for an annual loss of $3.73 billion in the United States (Ramanathan et al., 2022). Numerous studies have been conducted to understand the interactions between myoglobin chemistry and meat color (Renerre and Labas, 1987; Mancini and Hunt, 2005; Suman and Joseph, 2013; Ramanathan and Mancini, 2018). Despite this knowledge, there is still a fundamental lack of understanding of the differential rates of discoloration between muscle cuts from the same carcass.

For example, the beef *longissimus lumborum* (LL) retains its bright cherry red color for more than 6 d during retail display, whereas the *psoas major* (PM) only maintains a bright cherry red color for a little more than 24 h (O'Keeffe and Hood, 1982; Nair et al., 2018). Previous research has examined if the color stability differences between these 2 muscles can be explained by differences in pH, muscle fiber composition, mitochondrial functionality, proteomics, and metabolomics (Hunt and Hedrick, 1977; Wood et al., 2004; Mancini and Ramanathan, 2014; Ijaz et al., 2020). Still, we do not have a full understanding of the difference in color shelf life between these 2 economically important muscles.

One of the factors that determines the shelf life of meat is microbial growth. Meat provides an extremely suitable environment for bacteria because of its available nutrients and high water activity. Specifically, microbial metabolites produced during the exponential phase of growth can result in meat surface degradation, leading to the formation of slime, green color pigments, and putrid odors (Nychas et al., 2008). In general, meat does not display these defects until bacterial levels have reached approximately 7 to 8 log CFU/cm^2 (Vieira et al., 2009; Kameník, 2013). However, fresh beef steak shelf life is not entirely determined by the microbial load. Specifically, myoglobin oxidation, resulting in the formation of metmyoglobin, can lead to brown discoloration of meat during retail display (Mancini and Hunt, 2005). In fact, previous studies investigating the interaction between bacterial growth and beef color have concluded that the shelf life of meat is limited by surface discoloration before the meat is microbially spoiled (Hunt et al., 2004; Beggan et al., 2005; Yang et al., 2016). Conversely, studies have suggested that a high microbial load or bacterial growth on fresh beef during retail display could adversely impact meat color (Bala et al., 1977; Li et al., 2015). Therefore, the objective of this study was to explore the relationship between microbial growth and color shelf life of beef LL and PM during a 7-d retail display period by characterizing their microbial communities and physiochemical properties.

Materials and Methods

Meat collection and processing

Paired USDA Select beef striploins (LL, $n = 5$) and tenderloins (PM, $n = 5$) from the same side of individual beef carcasses were collected 24 h postmortem from a commercial beef harvesting facility in Colorado. The muscles were collected from animals of similar backgrounds and ages. Additionally, they were collected before the application of any wholesale cut antimicrobial intervention (standard industry practice) to avoid any potential confounding factors. The muscles were individually vacuum-packaged, transported on ice to the Colorado State University Global Food Innovation Center (Department of Animal Sciences, Fort Collins, CO), and aged at 2°C for 14 d in darkness. After aging, both muscle types from all 5 carcasses were removed from the bags and placed onto the same sanitized cutting surface and were deliberately brought into contact with each other to ensure a similar initial microbial population. To simulate retail industry practices, equipment and surfaces (i.e., knives, trays, gloves, cutting tables) were not sanitized in between the steak fabrication process, and the wholesale cuts were fabricated by alternating between LL and PM loins. All wholesale cuts were fabricated into 2.54-cm-thick steaks and placed onto foam trays lined with absorbent pads. Steaks were subsequently wrapped in a polyvinyl chloride overwrap film (O$_2$ transmission = 23,250 mL × m^2 × d^{-1}, 72 gauge; Resinite Packaging Film; Borden, Inc., North Andover, MA). Wrapped steaks were placed on the bottom deck of a multilevel retail display case with continuous lighting at 3°C ± 1°C (2,800 lx, 1810LX4000 LED fixture; Kason, Newnan, GA; color rendering index = 84, color temperature = 4,500 K) and allowed to bloom for 1 h prior to conducting initial analyses. Steaks were rotated every 12 h to ensure minimal differences in temperature and light intensity variations in the retail case. In a predetermined random order, 1 steak per loin (i.e., $n = 5$ each for LL and PM), at 24 ± 1 h intervals for 7 d, was used to assess the instrumental color, physiochemical properties, trained panelist visual color evaluation, culture-dependent enumeration of microbial populations, and 16S ribosomal RNA (rRNA) sequencing.

Instrumental color evaluation

Instrumental color measurement of Commission Internationale de l'Eclairage (CIE) lightness (*L**), redness (*a**), and yellowness (*b**) was performed using a HunterLab MiniScan LabScan EZ 4500 colorimeter (HunterLab, Reston, VA), using a 2.54-cm-diameter aperture with a 6-mm measurement port, illuminant A, and 10° standard observer (King et al., 2023). Three random locations on the light-exposed lean surface of each sample were scanned and averaged to

represent the lean color. The instrument was calibrated with white and black tiles prior to use.

Visual color evaluation

This work was approved by the Colorado State University Institutional Review Board (IRB #2929). Six to eight trained panelists evaluated meat color and percent surface discoloration. A continuous lean 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). Percent discoloration was on a continuous scale from 0% to 100%. Data were collected using order randomized surveys generated with an online survey software (Qualtrics, Provo, UT). Results for both were reported as estimated marginal means of panelist scores per loin per day.

Metmyoglobin reducing activity

Metmyoglobin reducing activity (MRA) was performed using methods from Zhai et al. (2019). A $5 \times 5 \times 1.5$ cm section was excised from the steak, ensuring the exclusion of external fat and connective tissues, and submerged for 20 min in a 0.3% sodium nitrite solution (Sigma-Aldrich, St. Louis, MO) at room temperature to produce metmyoglobin. Samples were blotted dry and vacuum-packaged, and the reflectance spectra from 400 to 700 nm on the light-exposed surface were recorded immediately. The vacuum-packaged samples were incubated at 30°C for 2 h to induce the reduction of metmyoglobin, and the reflectance data were collected again. The percentage of surface metmyoglobin (preincubation as well as postincubation) was calculated based on K/S ratios and according to established formulas (King et al., 2023). These values were used to calculate MRA.

Meat pH and water activity

To measure pH and water activity, approximately 20 g of lean tissue, avoiding connective tissue and external fat, from each sample was homogenized using a blender (Waring Laboratory Science, Stamford, CT). Duplicate 2.5 g portions of the meat homogenate were homogenized with 15 mL of distilled water using an immersion blender (PRO Scientific, Oxford, CT). An Orion Star A211 pH meter (Fisher Scientific, Pittsburgh, PA) fitted with an Orion 8157BNUMD Ross Ultra pH/ATC triode (Fisher Scientific) was used for measuring pH. With the remaining undiluted meat homogenate, water activity was measured, in duplicate, using an AquaLab 4TE water activity meter (Meter Group, Inc., Pullman, WA).

Culture-based enumeration of microbial populations

A 4×4 cm (16 cm^2) section of steak surface, approximately 1 mm thick, was aseptically excised using a sterile template and scalpel, avoiding external fat and connective tissues. The excised section of meat was placed into a filter-separated sterile 710-mL bag (Whirl-Pak, Pleasant Prairie, WI) containing 50 mL of maximum recovery diluent (MRD; Neogen Culture Media, Lansing, MI). Samples were gently hand-massaged for 60 s followed by manual shaking 60 times (for approximately 60 s) to detach bacterial cells. A 35 mL aliquot of the rinsate was collected for 16S rRNA (microbiome) analysis. These aliquots were centrifuged (Sorvall Legend X1R, Thermo Fisher Scientific, Karlsruhe, Germany) at $4,280 \times g$ for 20 min at 4°C. The supernatant was discarded, and the cell pellet, consisting of both bacterial and bovine cells, was frozen at −80°C until microbial DNA extraction.

The remaining meat sample rinsate was used for enumeration of aerobic microbial populations (aerobic plate counts [APC]), populations of *Pseudomonas* spp., lactic acid bacteria counts (LABC), and *Enterobacteriaceae*. Specifically, sample rinsates were 10-fold serially diluted in MRD, and appropriate dilutions were spread-plated, in duplicate, onto tryptic soy agar (TSA; Neogen Culture Media), and *Pseudomonas* agar base (Oxoid Ltd., Hampshire, UK) supplemented with *Pseudomonas* CFC supplement (comprising cetrimide, fucidin, and cephalosporin; Oxoid Ltd.). A pour plate overlay method was used with *Lactobacilli* MRS agar (Difco; Becton, Dickinson and Company, Franklin Lakes, NJ) for the enumeration of lactic acid bacteria. For the enumeration of *Enterobacteriaceae* populations, *Enterobacteriaceae* Petrifilm plates (3M *Enterobacteriaceae* Count Plates, St. Paul, MN) were used. Colonies were counted after incubation of plates at 25°C for 72 h (TSA and *Pseudomonas* agar), 25°C for 96 h (*Lactobacilli* MRS agar), or 35°C for 24 h (*Enterobacteriaceae* Count Petrifilm). Colony counts were converted to log CFU/cm^2.

For an estimation of the growth kinetic parameters of the microbial populations recovered with the culture-dependent analysis, the obtained microbial count data (log CFU/cm^2) were modeled as a function of time (i.e., days in retail display) using the Baranyi and Roberts (1994) model. This was accomplished with the Microsoft Excel predictor plug-in, DMFit

(v. 3.5), available from ComBase (https://www.combase.cc/index.php/en/). 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) representing the stationary phase of the bacterial growth curve.

Bacterial DNA extraction, library preparation, and sequencing and analysis

For 16S rRNA gene sequencing (microbiome), the frozen pellet for DNA extraction was resuspended in 4 mL of phosphate buffered saline (Sigma-Aldrich) and a 2 mL aliquot of the suspension was used for DNA extraction. Bacterial DNA was extracted using the DNeasy PowerFood Microbial Kit (Qiagen, Germantown, MD), following the manufacturer's instructions with minor modifications. Briefly, modifications included adding an additional 200 µL of lysis buffer, and samples were heated in a water bath at 65°C for 10 min to facilitate a complete bacterial cell lysis. Following mechanical cell lysis, samples were centrifuged for 15 min to ensure proper separation of cell debris and DNA. Lastly, DNA was eluted into 50 µL of elution buffer. Extracted DNA was quantified using a NanoDrop Lite ultraviolet spectrophotometer (Thermo Scientific, Pittsburgh, PA). The DNA concentration was standardized in all samples to 15 to 90 ng/µL prior to amplification.

Amplification and sequencing preparation were performed according to the Earth Microbiome Project (https://earthmicrobiome.org/) protocol. The DNA sequences were demultiplexed using QIIME 2 (v. 2022.11; Bolyen et al., 2019) and denoised using the DADA2 pipeline. After denoising, forward reads were trimmed at 13 base pairs, and all sequences were truncated at 250 base pairs to ensure quality. QIIME 2 with the SILVA taxonomical database was used to create a relative abundance feature table with taxonomy and phylogenetic tree. Chloroplasts, eukaryota, and mitochondria were removed prior to analysis. Additionally, the negative extraction controls contained less than 5,000 reads, and the positive controls did not have unexpected taxa present. Without evidence of contamination, the controls were removed from the dataset prior to analysis. Samples with less than 4,000 sequences were also excluded, leaving 64 out of 70 for analysis.

Sample sequences ranged from 4,125 to 52,576, with an average of 16,014 sequences and 18,024 amplicon sequence variants (ASVs). Diversity metrics were analyzed using the phyloseq package in R (v. 4.1.2; McMurdie and Holmes, 2013; R Core Team, 2021). Alpha diversity metrics were analyzed using a pairwise Wilcoxon rank-sum test, and beta diversity was analyzed using the cumulative sum squared with a permutational multivariate analysis of variance (PERMANOVA) with 9,999 permutations in the ADONIS package in R (Arbizu, 2017). Microbiota dispersion was analyzed using the PERMDISP function in the ADONIS package in R. For both diversity and relative abundance, the Benjamini-Hochberg multiple comparisons test adjustment was used. For all microbiome analyses, significance was set at $\alpha = 0.05$.

Statistical analysis

Water activity, pH, MRA, visual and instrumental color evaluation, and microbial population enumeration data were analyzed in a split-plot design. Muscle type, retail display day, and their interaction were the fixed effects, and the individual carcass was treated as a blocking factor. Results are reported as estimated marginal means, and an analysis of variance with Tukey's correction was used to determine significance at $\alpha = 0.05$.

Results

Instrumental and trained panelist color evaluation

Results of the instrumental and visual color evaluations of beef LL and PM during retail display are presented in Table 1. No interaction ($P \geq 0.05$) between muscle and retail display day was observed for any of the meat quality attributes. Additionally, there were no differences ($P \geq 0.05$) between $L*$ (lightness) values of LL and PM throughout the display days. Conversely, $a*$ (redness) values were higher ($P < 0.05$) for PM than LL for the first 2 d of retail display, after which $a*$ values of LL were higher ($P < 0.05$) than those of PM. The $b*$ value (yellowness) for PM was greater ($P < 0.05$) at the beginning of the display, but from day 2, the $b*$ values of the 2 muscles were similar ($P \geq 0.05$). Color panelists indicated that PM was darker ($P < 0.05$; Table 1) than LL on day 0 and each subsequent day of retail display. Within each muscle, however, lean darkness did not increase ($P \geq 0.05$) over the 7-d display period. After 24 h of simulated

Table 1. Marginal means with standard error of CIE *L** (lightness), *a** (redness), and *b** (yellowness), panelist lean color[1] and percentage discoloration of beef *longissimus lumborum* (LL) and *psoas major* (PM) during a 7-d simulated retail display (3°C) period

Assay	Muscle	Display day 0	1	2	3	4	5	6
L* value	LL	33.83 ± 0.43^{az}	33.94 ± 1.37^{az}	34.46 ± 0.42^{az}	34.38 ± 0.58^{az}	34.72 ± 1.38^{az}	34.47 ± 0.87^{az}	34.51 ± 0.62^{az}
	PM	33.14 ± 1.41^{az}	34.73 ± 1.56^{az}	33.78 ± 1.18^{az}	34.11 ± 1.48^{az}	33.35 ± 2.21^{az}	36.37 ± 1.3^{az}	34.19 ± 0.65^{az}
a* value	LL	19.28 ± 0.71^{az}	19.47 ± 1.08^{az}	19.20 ± 0.27^{ax}	20.21 ± 0.47^{ax}	19.14 ± 0.70^{ax}	19.50 ± 0.13^{ax}	19.17 ± 0.27^{ax}
	PM	23.68 ± 0.68^{ax}	21.33 ± 0.56^{ax}	17.18 ± 0.71^{bz}	17.54 ± 0.68^{bz}	16.34 ± 0.47^{bz}	15.23 ± 1.08^{bcx}	13.41 ± 0.62^{cz}
b* value	LL	14.94 ± 0.62^{az}	17.23 ± 0.8^{bz}	15.46 ± 0.26^{abz}	16.57 ± 0.45^{abz}	15.3 ± 0.75^{abz}	16.24 ± 0.21^{abz}	15.96 ± 0.27^{abz}
	PM	17.92 ± 0.52^{abx}	19.14 ± 0.69^{bx}	15.5 ± 0.33^{cz}	15.92 ± 0.43^{cz}	16.3 ± 0.4^{bcz}	15.61 ± 0.71^{cz}	15.81 ± 0.61^{cz}
Lean color score[1]	LL	3.19 ± 0.19^{ax}	3.35 ± 0.07^{ax}	3.34 ± 0.08^{ax}	3.35 ± 0.14^{ax}	3.62 ± 0.17^{ax}	3.55 ± 0.06^{ax}	3.55 ± 0.10^{ax}
	PM	5.04 ± 0.28^{abz}	4.44 ± 0.26^{az}	5.13 ± 0.25^{abz}	5.44 ± 0.30^{bz}	5.67 ± 0.27^{bz}	5.77 ± 0.18^{bz}	5.59 ± 0.22^{bz}
Percentage discoloration	LL	0.17 ± 0.12^{az}	0.36 ± 0.22^{az}	0.66 ± 0.14^{ax}	2.88 ± 1.07^{ax}	2.22 ± 0.37^{ax}	2.22 ± 1.11^{ax}	2.41 ± 0.30^{ax}
	PM	0.13 ± 0.13^{az}	2.03 ± 0.71^{az}	15.78 ± 3.87^{bz}	25.50 ± 4.13^{bz}	49.99 ± 3.02^{cz}	60.45 ± 6.50^{dz}	48.67 ± 3.40^{cz}

[a–d]Within each row (representing muscle type), marginal means without a common superscript letter are different ($P < 0.05$).

[x–z]Marginal means in the same column (representing display day) and 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, 8 = extremely dark red).

retail display, PM exhibited discernable surface discoloration, but it was not greater ($P \geq 0.05$) than that of LL until day 2 (Table 1). From day 2, surface discoloration for PM was greater ($P < 0.05$) than that of LL, with PM displaying over 15% surface discoloration compared with less than 1% for LL (Table 1). Surface discoloration remained greater ($P < 0.05$) for PM compared with LL for the remainder of the display period. Additionally, LL did not increase ($P \geq 0.05$) in percent surface discoloration throughout the retail display days.

pH, MRA, water activity

The pH, MRA, and water activity of beef LL and PM during retail display are presented in Table 2. The pH was greater ($P < 0.05$) in PM for the first 5 d of the retail display compared with that of LL, after which pH

values of PM and LL were similar ($P \geq 0.05$). On the other hand, MRA was higher ($P < 0.05$; Figure 1) for LL than PM across all days. Overall, PM had a sharp decrease ($P < 0.05$) in MRA after the first day of retail display, with it going down from 62.7% on day 0 to 28.8% 24 h later on day 1, and MRA remained low for PM for the next 5 d of display (Figure 1). The MRA for PM remained at or below 30% from display day 1 to the end of display. Water activity was greater than 0.98 for both muscles across all days, and there were no differences ($P \geq 0.05$) in water activity for either muscle across all display days (Table 2).

Culture-based analysis of microbial population levels

Results of the culture-based microbial analysis showed that LL and PM steaks had similar ($P \geq 0.05$)

Table 2. Mean ($n = 5$) values with standard error for pH and water activity for beef *longissimus lumborum* (LL) and *psoas major* (PM) muscles over a 7-d simulated retail display (3°C)

Assay	Muscle	Display day 0	1	2	3	4	5	6
pH	LL	5.59 ± 0.05^{az}	5.59 ± 0.03^{az}	5.54 ± 0.01^{az}	5.52 ± 0.01^{az}	5.56 ± 0.02^{az}	5.59 ± 0.01^{az}	5.55 ± 0.02^{az}
	PM	5.89 ± 0.05^{ax}	5.83 ± 0.07^{abx}	5.80 ± 0.04^{abx}	5.72 ± 0.03^{abcx}	5.76 ± 0.11^{abcx}	5.60 ± 0.01^{abcz}	5.66 ± 0.05^{abcz}
Water activity	LL	0.994 ± 0.001^{az}	0.993 ± 0.001^{az}	0.995 ± 0.002^{az}	0.995 ± 0.000^{az}	0.993 ± 0.000^{az}	0.994 ± 0.001^{az}	0.994 ± 0.001^{az}
	PM	0.995 ± 0.000^{az}	0.995 ± 0.001^{az}	0.996 ± 0.001^{az}	0.995 ± 0.000^{az}	0.994 ± 0.001^{az}	0.995 ± 0.000^{az}	0.994 ± 0.000^{az}

[a–d]Within each row (representing muscle type), marginal means without a common superscript letter are different ($P < 0.05$).

[x–z]Marginal means in the same column (representing display day) and within the same measurement without a common superscript letter are different ($P < 0.05$).
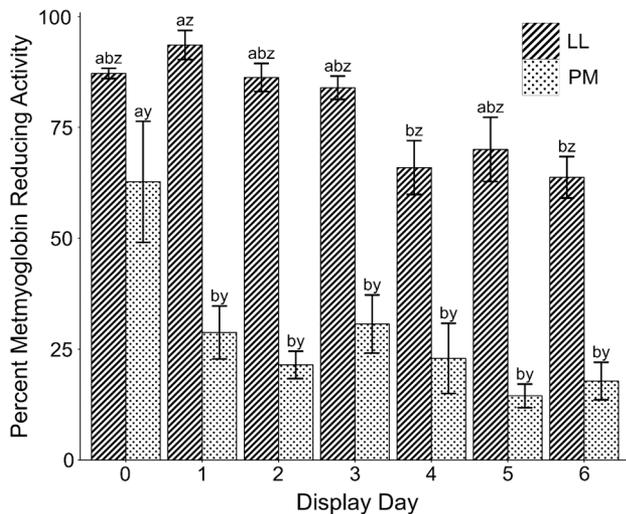
Figure 1. Mean ($n = 5$) metmyoglobin reducing activity (MRA) of beef *longissimus lumborum* (LL) and *psoas major* (PM) measured on each day over a 7-d retail display (3°C) period. [a,b]Bars within same muscle without a common letter superscript are different ($P < 0.05$). [y,z]Bars within the same display day without a common letter superscript are different ($P < 0.05$)

initial (day 0 of retail display) microbial contamination levels within each of the bacterial count types analyzed (Table 3). Irrespective of muscle, initial contamination levels were low, ranging from 1.6 to 2.0 log CFU/cm^2 for APC and LABC and <0.7 log CFU/cm^2 for *Pseudomonas* spp. counts. *Enterobacteriaceae* were not detected (0.2 log CFU/cm^2 detection limit) in any of the samples analyzed on day 0 as well as the majority of samples (81.4%; 57 out of 70) analyzed throughout the display period.

Growth of APC, LABC, and *Pseudomonas* spp. populations were obtained on steaks of both muscle

types during storage; however, initiation of bacterial growth occurred more rapidly on PM than on LL steaks. Although there was a difference ($P < 0.05$) between the APC of LL and PM samples by day 1, there was not a statistical ($P \geq 0.05$) increase from the initial population levels until day 2 for PM samples and 2 d later (on day 4) for LL samples. Similar findings were obtained for the LABC and *Pseudomonas* spp. counts, with statistical increases ($P < 0.05$) from day 0 levels occurring 1 d earlier on PM steaks than on LL steaks (Table 3). By the end of the retail display period, PM steaks had APC, LABC, and *Pseudomonas* spp. counts of 6.6, 6.9, and 5.4 log CFU/cm^2, respectively, whereas the LL samples were lower ($P < 0.05$) at 5.2, 5.6, and 3.8 log CFU/cm^2, respectively ($P < 0.05$).

The trends observed with the microbial count data were similar to the growth kinetics parameter results, which were estimated using the Baranyi and Roberts (1994) primary model. Notably, the APC and LABC populations on PM steaks exhibited no lag phase, whereas the same populations on LL samples had lag phases of 2.2 and 2.1 d, respectively (Figure 2; Table 4). *Pseudomonas* spp. populations exhibited a lag phase on both PM and LL samples, but it was approximately 2 times longer on LL steaks (i.e., 3.1 d for LL and 1.6 d for PM; Figure 2; Table 4). Interestingly, the μ_{max} (maximum specific growth rate in log CFU/cm^2/day) of the APC, LABC, and *Pseudomonas* spp. populations on LL steaks were slightly higher than those populations on the PM steaks (Table 4). More specifically, the μ_{max} of the APC, LABC, and *Pseudomonas* spp. populations on LL

Table 3. Mean ($n = 5$; log CFU/cm^2 ± standard deviation) aerobic plate counts (APC), *Pseudomonas* spp. counts, lactic acid bacteria counts (LABC), and *Enterobacteriaceae* counts for beef *longissimus lumborum* (LL) and *psoas major* (PM) over a 7-d simulated retail display (3°C) period

Bacterial count type	Muscle	Display day						
		0	1	2	3	4	5	6
APC	LL	1.6 ± 0.5^{az}	1.7 ± 0.3^{az}	1.8 ± 0.2^{az}	2.4 ± 0.6^{az}	3.4 ± 0.7^{bz}	4.1 ± 0.8^{bz}	5.2 ± 0.3^{cz}
	PM	2.0 ± 0.3^{az}	2.4 ± 0.3^{abx}	3.2 ± 0.6^{bx}	4.3 ± 0.9^{cx}	4.7 ± 0.6^{cdx}	5.2 ± 0.4^{dx}	6.6 ± 0.4^{ex}
Pseudomonas spp. Count	LL	$<0.5 \pm 0.3^{az,1}$	$<0.8 \pm 0.3^{az}$	$<0.7 \pm 0.3^{az}$	0.7 ± 0.7^{az}	1.8 ± 0.4^{bz}	2.8 ± 0.4^{cz}	3.8 ± 0.3^{dz}
	PM	$<0.7 \pm 0.3^{az}$	0.9 ± 0.3^{az}	1.4 ± 0.5^{ax}	2.4 ± 0.6^{bx}	3.0 ± 0.7^{bcx}	3.8 ± 0.4^{cx}	5.4 ± 0.4^{dx}
LABC	LL	1.8 ± 0.3^{az}	1.7 ± 0.3^{az}	2.2 ± 0.4^{abz}	2.7 ± 0.7^{bz}	3.8 ± 0.5^{cz}	4.3 ± 0.8^{cz}	5.6 ± 0.3^{dz}
	PM	2.0 ± 0.4^{az}	2.5 ± 0.2^{abx}	3.3 ± 0.5^{bx}	4.4 ± 0.8^{cx}	4.9 ± 0.6^{cdx}	5.4 ± 0.6^{dx}	6.9 ± 0.5^{ex}
Enterobacteriaceae Count	LL	$<0.2 \pm 0.0^{az}$	$<0.2 \pm 0.0^{az}$	$<0.2 \pm 0.0^{az}$	$<0.2 \pm 0.0^{az}$	$<0.2 \pm 0.0^{az}$	$<0.3 \pm 0.1^{az}$	$<0.2 \pm 0.0^{az}$
	PM	$<0.2 \pm 0.0^{az}$	$<0.2 \pm 0.0^{az}$	$<0.3 \pm 0.2^{az}$	$<0.6 \pm 0.6^{ax}$	$<0.5 \pm 0.6^{az}$	$<0.3 \pm 0.1^{az}$	1.3 ± 0.3^{bx}

[a–e]Within each row (representing muscle type), marginal means without a common superscript letter are different ($P < 0.05$).

[x–z]Marginal means in the same column (representing display day) and within the same bacterial count type without a common superscript letter are different ($P < 0.05$).

[1]At least 1 of the 5 samples analyzed had a count that was below the microbial analysis detection limit of 0.2 log CFU/cm^2; therefore, the mean is reported as less than the mean.
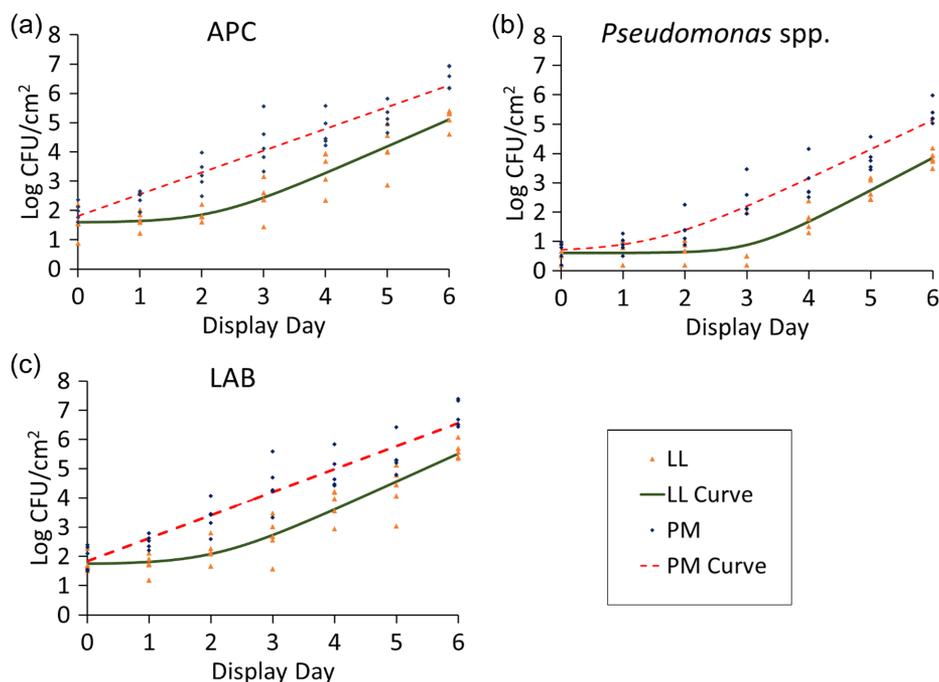
Figure 2. Baranyi and Roberts (1994) estimated growth curves for aerobic plate counts (APC), *Pseudomonas* spp., and lactic acid bacteria (LAB), determined from the log colony-forming units (CFU)/cm^2 plate counts on beef *longissimus lumborum* (LL) and *psoas major* (PM) steaks from each day of a 7-d retail display (3°C) period.

Table 4. Estimated growth kinetic parameters, derived from the Baranyi and Roberts (1994) model, of bacterial populations recovered from beef *longissimus lumborum* (LL) and *psoas major* (PM) during simulated retail display (3°C) for 7 d

		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 ± 0.5	0.925 ± 0.136	1.6	—[d]	0.870
	PM	—[c]	0.743 ± 0.044	1.8	—	0.893
Pseudomonas spp.	LL	3.1 ± 0.3	1.106 ± 0.140	0.6	—	0.899
	PM	1.6 ± 0.5	0.998 ± 0.120	0.7	—	0.914
LABC	LL	2.1 ± 0.5	0.955 ± 0.136	1.8	—	0.879
	PM	—	0.788 ± 0.047	1.8	—	0.892

APC = aerobic plate count; LABC = lactic acid bacteria count; 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 lag phase observed.

[d]No upper asymptote observed.

samples was 1.3, 1.2, and 1.1 times higher, respectively, than on PM samples.

Microbial community differences

There were 1,111,678 total sequences for the 16S rRNA gene sequencing, with 18,024 ASVs and a mean of 16,014 sequences per sample (from 64 total samples). The predominant phyla identified were *Firmicutes* and *Proteobacteria*. The most abundant taxa across both muscles and all display days were in the family *Lactobacilliceae* followed by the order *Pseudomonales* (Figure 3). The 16S rRNA gene sequencing lacked enough sequencing depth to classify these to lower levels of taxonomy, such as genus.

There were no differences ($P \geq 0.05$) in alpha diversity, determined by Faith's phylogenetic diversity, Shannon's index, or Simpson's index, between muscles
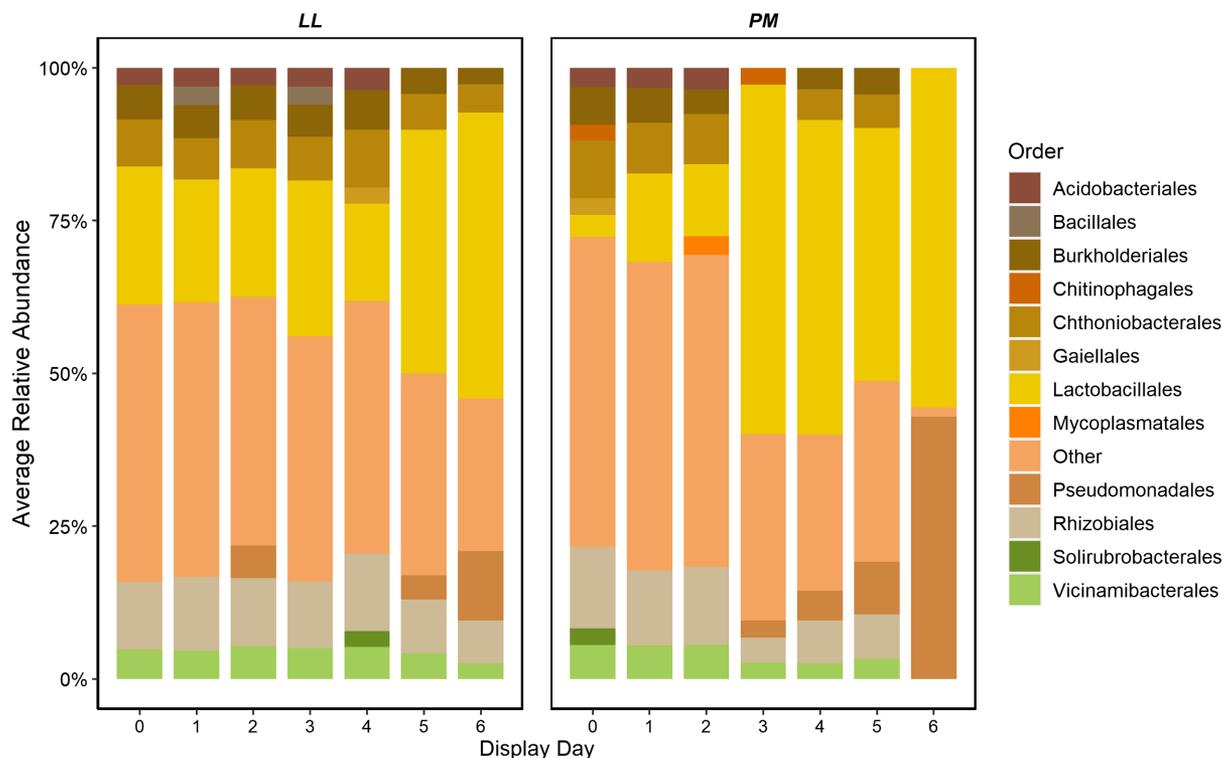
Figure 3. Relative abundance taxonomy plot grouped by beef *longissimus lumborum* (LL) and *psoas major* (PM) organized at the order level over a 7-d simulated retail display (3°C) period. *The "Other" category included highly diverse and unassigned taxa.

on the same day. However, there were differences between display days ($P < 0.05$) in Faith's phylogenetic diversity and Shannon's index, as well as differences between the muscles, across all display days. Additionally, there were no differences ($P \geq 0.05$; Figure 4) in weighted UniFrac, unweighted UniFrac, or generalized UniFrac (beta diversity) using PERMANOVA

between muscles on the same display day. Moreover, there were no differences ($P \geq 0.05$) in beta diversity in display day or muscle type when analyzed with PERMANOVA. However, PERMDISP showed differences in microbial dispersion between muscles ($P < 0.05$) and between days ($P < 0.05$) for generalized UniFrac. Still, PERMDISP analysis showed no differences ($P \geq 0.05$) in microbiota dispersion between muscles on the same day for weighted, unweighted, and generalized UniFrac analyses.
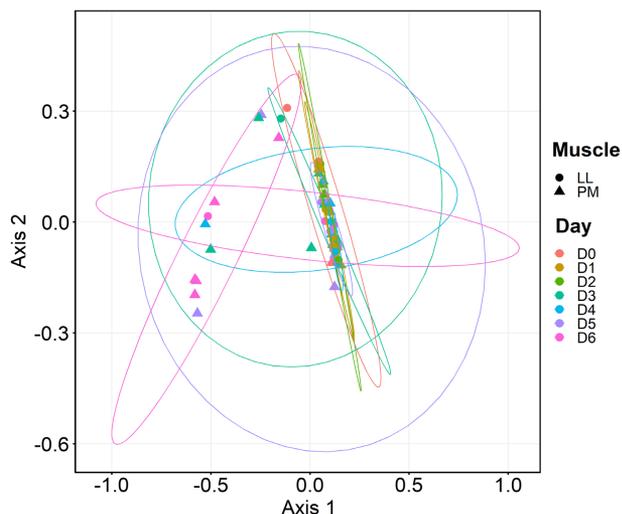
Discussion

Instrumental and visual color

In the present study, redness (a^* value) was greater for PM than LL initially (day 0); however, from day 2, this trend reversed, and PM had lower a^* values than LL (Table 1). Similar findings have been reported by other researchers as well, with PM having greater initial redness compared with LL, only for the trend to be reversed on the subsequent retail display days (Seyfert et al., 2006; Joseph et al., 2012; Nair et al., 2018). Moreover, Seyfert et al. (2006) reported that PM had surface discoloration (evaluated by panelists) at 24 h of retail display, whereas the LL did not begin to

Figure 4. Unweighted UniFrac principal coordinates analysis of beta diversity for microbial populations collected from beef *longissimus lumborum* (LL) and *psoas major* (PM) steaks over a 7-d simulated retail display (3°C) period.

discolor until 96 h. Although the PM in the current study did not have statistically significant ($P \geq 0.05$) surface discoloration compared with LL in the first 24 h, panelists reported that PM had discernable discoloration (1% to 3%) within 24 h. Furthermore, PM was discolored greater than 20% within 3 d of display, indicating that by the third day, the instrumental redness, measured as a^* value of the steak, alone may not be able to represent the consumer acceptability of the steaks. In contrast, LL in all the aforementioned studies maintained relatively steady a^* values throughout the display periods, similar to this study. Chan et al. (1995) reported that PM was at approximately 60% discolored by day 4 of aerobic display, similar to current findings. The authors also reported that a^* values for LL and PM were the same on day 0 but by day 2 of display, the a^* values for PM had greatly decreased and were much lower compared with LL.

Metmyoglobin reducing activity

MRA is one of the primary ways that meat maintains a cherry red color during retail display. This is accomplished by reducing metmyoglobin to deoxymyoglobin, which can then be oxygenated to oxymyoglobin (Echevarne et al., 1990; Joseph et al., 2012). In color-labile muscles such as PM, MRA declines quickly during retail display, and the ability of the meat to return from brown to red is substantially diminished, greatly reducing the retail shelf life. Conversely, in color-stable muscles such as LL, the MRA remains higher for a longer duration, resulting in meat that remains brighter red for longer during retail display. In this study, the color-labile PM had a sharp decline in MRA after 24 h in retail display (Figure 1). This finding is in agreement with previous studies that reported a sharp decline in MRA of PM within the first 24 h (Joseph et al., 2012; Canto et al., 2016; Mancini et al., 2018; Ramanathan et al., 2021). The MRA for LL remained above 80% until the fifth day (day 4 in Figure 1), after which it decreased slightly for the remainder of the display (Figure 1). In contrast to findings reported by O'Keeffe and Hood (1982), in which MRA for LL decreased daily in a more linear progression, MRA for LL did not decrease steadily throughout the display period in this study. This may be due to the difference in temperature of retail display conditions between the 2 studies, with O'Keeffe and Hood (1982) using a slightly higher temperature (5°C) compared with that of the current study (3°C), and microbial growth was not monitored. Moreover, in a study investigating the differences in the mitochondrial activity

between these same 2 muscles, the MRA of the PM and LL behaved in a similar fashion to the current study, with PM demonstrating a sharp decline in MRA after 24 h (Ramanathan et al., 2021). Joseph et al. (2012) also reported MRA was lower for PM at days 0, 5, and 9 of retail display, compared with LL. The MRA results and the color evaluation during retail display reaffirm that beef PM is a color-labile muscle compared with LL.

Meat pH and water activity

The pH of muscle has long been known to play an important role in meat color. Higher pH can result in a darker-colored lean as well as provide a more conducive environment for spoilage microflora growth (Newton and Gill, 1981). Investigating the impacts of pH on beef PM and LL color stability, Wu et al. (2020) reported that pH was not impactful on steak color stability but rather on steak color intensity, as determined by greater calculated chroma values. Furthermore, the authors reported that PM had a normal pH of 5.75 and LL at 5.52, which is similar to the data presented in Table 2. The pH of LL was lower ($P < 0.05$) than PM for most of the display period (Table 2). Interestingly, the recovered APC, LABC, and *Pseudomonas* spp. populations on LL steaks in the present study had extended lag phases but slightly higher maximum specific growth rates compared with the populations on the PM steaks (Table 4). This may suggest that meat pH could be one of the many factors affecting the growth kinetics of spoilage bacteria associated with these 2 muscles. The water activity of the muscles fell within the normal range expected for these muscles (Fennema and Carpenter, 1984) and did not change ($P \geq 0.05$) during retail display (Table 2).

Culture-based microbial population levels and estimated bacterial growth kinetics

The typical bacterial growth curve consists of 4 different phases: lag, exponential, stationary, and death, with the latter 2 having less significance in meat shelf life (Maier and Pepper, 2015). The lag phase is characterized as little to no doubling of bacteria, and the cell metabolites produced by bacteria are minimal. However, when bacteria enter the exponential phase, they utilize the most nutrients from the substrate and produce the most metabolic compounds (Maier and Pepper, 2015). These compounds are partially responsible for the deleterious effects on meat quality, such as surface discoloration, malodors, and slime formation

(Bala et al., 1977; Leisner et al., 1995; Nychas et al., 2008).

The LL and PM steaks in the current study had similar levels of APC, LABC, *Pseudomonas* spp. counts, and *Enterobacteriaceae* counts at the beginning (day 0) of the retail display (Table 3). This was anticipated because the LL and PM loins were intentionally brought into contact with one another prior to fabricating them into steaks, and the same knife was used to cut the steaks. In this way, we attempted to minimize/eliminate differences in starting microbial levels and community compositions as potential variables for microbial growth differences obtained between the muscles during retail display. A notable difference between the muscles in relation to the recovered microbial populations was the lag phase duration of the APC, LABC, and *Pseudomonas* spp. populations. The APC and LABC populations on PM samples grew without a lag phase, or in the case of the *Pseudomonas* spp. populations, with a shorter lag phase than the populations on LL samples (Table 4). It is possible that these differences in lag times could be one of the explanations for the differences ($P < 0.05$) in microbial numbers recovered from PM and LL during the display period and for the 1.4 (APC), 1.3 (LABC), and 1.6 (*Pseudomonas* spp.) log CFU/cm^2 higher ($P < 0.05$) bacterial concentrations on PM than on LL steaks on the last day of display (Table 3). Populations on LL had a slightly higher maximum specific growth rate (μ_{max}) than those on PM. However, this was not enough to result in higher population counts by the end of the retail display period for LL compared with PM (Table 3). Moreover, higher final microbial populations for PM compared with LL have been regularly reported, regardless of environment or treatment (Chan et al., 1995; Hunt et al., 2004), suggesting that muscle-specific factors play a key role in discoloration and microbial growth kinetics between these muscles.

Overall, the microbial flora associated with PM entered the exponential growth phase faster than the populations associated with LL, suggesting that PM could be a more suitable substrate for microbial growth. It is reasonable to anticipate that factors including pH, carbohydrate content, and organic acids are influencing the faster increases in microbial levels on the PM versus the LL because previous studies have indicated that these factors could influence microbial growth (Nychas et al., 2008; Rojo 2010; Abraham et al., 2017; Yu et al., 2019). For example, Abraham et al. (2017) reported that PM had more carbohydrate metabolites in the form of organic acids than LL, whereas the LL had a greater pyruvate concentration during a 7-d simulated retail display.

Irrespective of the muscle, samples analyzed on day 0 had similar APC and LABC, indicating that the APC population was most likely composed of predominantly lactic acid bacteria. This was not surprising because the loins had been aged for 14 d (2°C) under vacuum-packaged conditions prior to the start of the experiment. It is generally accepted that *Pseudomonas* spp. are the dominant spoilage microorganisms of aerobically stored meat products (Dainty and Mackey, 1992; Nychas et al., 2008; Wickramasinghe et al., 2019). However, in the current study, this was not the case, likely due to competition with the lactic acid bacteria population which, as already mentioned, was the dominant population on the steaks at the beginning of retail display, which was also observed in the microbiome analysis (Figure 3). Similar to previous reports (Smolander et al., 2004; Nychas et al., 2008; Djordjevic et al., 2016), our results showed that members of the *Enterobacteriaceae* family did not make up a significant portion of the final populations on the aerobically packaged steaks. Furthermore, *Enterobacteriaceae* are routinely associated with meat spoilage and as food safety indicator organisms, although they are not necessarily considered spoilage organisms. Despite this, *Enterobacteriaceae* have the potential to decrease steak color stability because they catabolize amino acids and excrete volatile organic compounds that could result in surface greening in meat (Chaves-López et al., 2006; Li et al., 2015; Djordjevic et al., 2016; Stellato et al., 2016).

16S rRNA gene sequencing

The 2 most abundant bacterial taxa identified on beef LL and PM during retail display in this study were both *Firmicutes* (Figure 3) and included the orders *Lactobacillales* and *Bacillales*. Unsurprisingly, these taxa were of the family *Lactobacillaceae*. Lactic acid bacteria are a large group of bacteria with 15 known genera and thousands of species. The lactic acid bacteria commonly associated with fresh beef include organisms such as *Carnobacterium* spp. and *Leuconostoc* spp. (Egan, 1983; Chenoll et al., 2007). This result was anticipated because lactic acid bacteria were the predominant organisms on the steaks, as observed from the microbial population counts (Table 3).

The 16S rRNA gene sequencing also confirmed that the genus *Pseudomonas* was the third most abundant taxa identified in this study. Although unknown

from the data in this study, *Pseudomonas fragi*, *P. fluorescens*, and *P. lundensis* are well known to be aerobic meat spoilage organisms (Dainty and Mackey, 1992; Wickramasinghe et al., 2019). Furthermore, *Pseudomonas* species are more diverse in their energy metabolism compared with the other predominant bacterial spoilage organisms (lactic acid bacteria) found in this study. Although LAB are most commonly carbohydrate heterofermenters, *Pseudomonas* spp. are more metabolically diverse (Rojo, 2010). For example, *Carnobacterium* spp. will preferentially use carbohydrates and arginine as an energy source (Leisner et al., 2007), whereas *P. fragi* is a proteolytic and lipolytic organism, meaning it derives nutrients from catabolizing proteins and lipids (Ercolini et al., 2010). However, previous studies investigating the metabolites found in both LL and PM showed that PM is less carbohydrate (i.e., glucose, ribose) rich compared with LL, but PM has greater amounts of tricarboxylic acid cycle intermediates such as citrate, aconitate, and fumarate (Abraham et al., 2017; Yu et al., 2019), adding further complexity to the muscle-specific microbial growth kinetics that has not been elucidated.

As meat spoils, microbial diversity decreases, or a select few taxa become dominant in abundance (Fougy et al., 2016). Although not statistically significant, the microbiome analysis does reveal a slightly faster decrease in microbial diversity for PM compared with LL. This minor difference can most easily be seen beginning on day 3, when the predominance of the orders of *Lactobacillales* and *Pseudomonales* on PM are more evident (Figure 3). By the last day of retail display, these 2 orders (*Lactobacillales* and *Pseudomonales*) make up approximately 95% of the relative abundance of taxa on PM, which is not observed for LL.

The lack of differences in microbial diversity metrics between muscles on the same day is unsurprising and could be attributed to multiple factors. The most important aspect of this is the fact that all strips and loins were comingled before fabrication. This was done to ensure that both LL and PM had similar microbial communities and microbial loads at the start of the study. Furthermore, even though both muscle cuts had significantly different pH values, they were only 0.3 units apart initially. Likewise, the water activities were similar ($P \geq 0.05$) and within normal fresh beef ranges. Steaks were also kept at the same display temperature and same packaging types. It is possible that the differing metabolites present in each steak may be directly influencing the differential abundance of bacteria on these 2 muscles. These observations suggest that PM and LL provide different nutrients for the microbes (Lambert et al., 1991; Abraham et al., 2017; Yu et al., 2019; Yang et al., 2022).

The 16S rRNA gene sequencing is a well-rounded tool for investigating the microbial ecology of bacterial communities during meat spoilage. However, 16S rRNA gene sequencing lacks the resolution to identify most individual organisms to the species and/or strain levels (Doulgeraki et al., 2012; Gwak and Rho, 2020). Knowing the exact species and strains dominating the spoilage process and their metabolic needs, combined with understanding the differences in the habitat provided by different muscles, may help explain further the potential impact of microbial growth on meat color stability.

Conclusions

The results of this study confirmed that beef LL and PM had different color stabilities, with PM being less color stable than LL during retail display. Additionally, LL steaks exhibited a slower initial bacterial growth for a greater amount of time (longer lag phase) during retail display than PM steaks. Furthermore, our results indicate that when bacterial communities (microbiome) were similar between LL and PM, the bacterial functionality and their growth kinetics might be contributing to their differential color stability.

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