Meat and Muscle Biology™

A Survey of the Microbial Communities of Commercial Presliced, Packaged Deli-Style Ham Throughout Storage

Rebecca A. Furbeck, Chad G. Bower, Samodha C. Fernando, and Gary A. Sullivan*

Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE 68583-0908, USA
*Corresponding author. Email: gary.sullivan@unl.edu (Gary A. Sullivan)

Abstract: The goal of this study was to evaluate the variation in spoilage microbiota associated with sliced, prepackaged deli-style ham from varying processing environments available in the retail market in the United States. Three different brands of presliced ham, water added were purchased at local markets and evaluated every 2 wk beginning 4 wk prior to the sell-by date until 4 wk beyond the sell-by date. Analysis of 16S ribosomal RNA genes using operational taxonomic units showed that Brand A had a different bacterial community structure compared with Brands B and C, according to unweighted ($P = 0.006$) and weighted ($P < 0.001$) UniFrac distance matrices. Brand A had a greater proportion of sequence reads mapping to *Carnobacterium*, *Bacillus*, and *Prevotella*, whereas B and C had greater proportions of *Pseudomonas*, *Photobacterium*, and *Lactococcus*. Brand A also had a lower salt concentration ($P < 0.007$), greater moisture percentage and less fat percentage ($P < 0.012$), and increased aerobic plate count ($P = 0.017$). Differences in spoilage microbiota can in part be attributed to the factors involved with different processing locations, as shown by 3 different brands of ham, as well as slight differences in formulation including salt concentration and organic acid use.

Key words: ham, spoilage, microbiota, bacterial community
Meat and Muscle Biology 6(1): 15446, 1–11 (2022) **doi:10.22175/mmb.15446**
Submitted 22 June 2022 Accepted 1 August 2022

Introduction

Meat spoilage is characterized as a change in the product rendering it unacceptable to the consumer, whether from chemical, biological, or physical change. Bacterial spoilage manifests itself as visible growth, textural changes, or off-odors and flavors caused by bacterial growth (Gram et al., 2002). Refrigeration and packaging type are 2 of the biggest contributors in selective growth of microorganisms (Doulgeraki et al., 2012); however, when placed under similar storage conditions, more precise factors may cause significant changes in the spoilage community, thus altering shelf life as well as the severity of spoilage defects. Lactic acid bacteria are typically identified as the primary contributors to spoilage in cooked meats stored under refrigeration and modified atmosphere packaging (Geeraerts et al., 2017, 2018); however, there may still be great variability of the bacterial community composition within lactic acid bacteria. Additionally, members of the genus *Pseudomonas* have been identified as specific spoilage organisms (SSOs) in vacuum-packaged products, disrupting the notion that pseudomonads are obligate aerobes (Bower et al., 2018). These findings surmount the fact that the microbial community of processed meats is more complex and adaptable than previously understood. With such mixed results regarding the dominant spoilage communities of cooked meats, steps should be taken to further characterize the predicted microbiome associated with cooked meat products under similar storage conditions.

The purpose of this study was to evaluate the spoilage microbiota of case-ready sliced and packaged ham available in the retail market. Sources of SSOs and factors modulating their growth rate are variable and contested. Miller and McMullen (2015) suggest that when comparing products of varying sodium concentration, the genera present

© 2022 Furbeck, et al.
This is an open access article distributed under the CC BY license (https://creativecommons.org/licenses/by/4.0/)

on meat samples is specific to individual processing facilities. In cooked sausage, however, Hultman et al. (2015) suggest that the spoilage microbiome is more similar to that of the meat batter than of the processing environment. Tracing route of contamination and identifying processes that modulate the bacterial community of processed meats may allow for "precision shelf-life extension." By knowing which taxa are present in specific systems, best practices can be enacted for microbial control. The aim of this study was to determine differences in the microbiota of products from the same product category (presliced ham, water-added product in gas flushed packaging) based on the variation between the postlethality processing environments of various manufacturers in the United States.

Materials and Methods

Sample procurement

Prepacked, sliced ham samples were purchased at a local grocery store and selected from products on the retail shelf. Three different brands of smoked ham were evaluated (A, B, C) originating from 3 different establishments. All 3 products were labeled as "Ham, Water Added" and were presliced in case-ready packages. In the interest of maintaining brand anonymity, Table 1 contains select functional ingredients of each individual brand that may affect microbial growth and/or community composition. Three separate replications were purchased for each brand, with one package removed at each sampling time for analysis. A replication consisted of products of the same brand, establishment number, and sell-by date (to have been produced on the same day in the same plant). Furthermore, each of the 3 replications, respective to each brand, were from the same establishment number to ensure replications were from the same processing plant within brand, but each replicate was a unique sell-by date. Products were stored in the original packaging at the Loeffel Meat Laboratory in a covered plastic lug at approximately 1°C (± 3°C) until their respective sampling time. Samples were evaluated according the sell-by date of each replication at the following intervals: 4 wk prior to sell-by (−4), 2 wk prior to sell-by (−2), sell-by date (0), 2 wk after sell-by (+ 2), and 4 wk after sell-by (+ 4).

Initial physicochemical analyses

Water activity, salt concentration, and proximate composition were evaluated at the initial (−4 wk) sampling time only. Samples used for water activity and salt concentration were homogenized using a food processor (Black + Decker Handy Chopper, Black + Decker, Baltimore, MD). Water activity was measured using an Aqualab water activity meter (Decagon Devices, Pullman, WA). Salt concentration was measured as described by Sebranek et al. (2001) using QuanTab high range chloride titration strips (Hach Company, Loveland, CO). Moisture, fat, protein, and ash was determined on pulverized samples. Samples were manually diced, submerged in liquid nitrogen until completely frozen, and pulverized using a Hobart commercial blender (Model 51BL32, Waring Commercial, Torrington, CT). In duplicate, 2 g of pulverized tissue was used to quantify moisture and ash content using a LECO thermogravimetric analyzer (Model TGA701, LECO Corporation, St. Joseph, MI). Using triplicate 2 g samples in a filter paper thimble, total fat was determined as outlined by AOAC (1990) using the Soxhlet extraction procedure. In duplicate, protein content was measured using a LECO nitrogen/protein analyzer (Model FP-528, LECO Corporation).

Longitudinal physicochemical analyses

Objective color and pH were evaluated at each sampling time. In duplicate, pH was measured using an Orion 410A+ pH meter (Thermo Scientific, Waltham, MA) on a slurry of 10 g of ham sample in 90 ml of double distilled water. Objective color (L^*, a^*, b^*) was measured using a colorimeter (Chroma Meter CR-400, Konica Minolta Sensing Americas, Ramsey, NJ) using a 2° standard observer with an 8 mm aperture and a D65 illuminant, calibrated with a white tile (Y:93.15, x:0.3165, y:0.3330). A total of 6 readings were taken

Table 1. Package ingredient statements, listed in alphabetical order, of the 3 brands (A, B, C) of sliced, prepackaged ham used in the study

Brand	A	B	C
Ingredients	Dextrose, modified corn starch, salt, sodium phosphate, sodium propionate, sodium erythorbate, sodium nitrite, sugar, water	Dextrose, potassium lactate, salt, sodium diacetate, sodium erythorbate, sodium nitrite, sodium phosphates, water	Dextrose, salt, sodium erythorbate, sodium nitrite, sodium phosphates, water

from 2 slices from each sample and averaged for color values.

Microbial analyses

For each respective sampling, one package was removed from storage and processed for analyses. Approximately 30 to 40 g of each sample was aseptically transferred from the retail package into a Whirl-Pak bag (Nasco, Fort Atkinson, WI), weight recorded, combined with 50 ml of sterile BBL peptone water (Becton, Dickinson and Company, Franklin Lakes, NJ), and homogenized using a bag blender (bioMérieux, Durham, NC) for 3 min. Duplicate 2 ml samples of homogenate were collected for microbial community analysis and was stored at −20°C until used for DNA extraction. Aerobic plate counts (APC) and anaerobic plate counts (AnPC) were performed using the homogenized samples. Ten-fold serial dilutions were conducted on homogenate as counts increased over shelf life to ensure accurate enumeration and then plated using the 50 μl E-Mode of an Eddy Jet spiral plater (IUL, Barcelona, Spain) on brain heart infusion agar (Thermo Fisher Scientific, Waltham, MA). Plates were then incubated at 37°C for 48 h for APC and in an anaerobic chamber containing BD GasPak EZ sachets for AnPC (BD, Franklin Lakes, NJ).

16S ribosomal RNA amplicon sequencing

Bacterial community analysis using high-throughput sequencing of the 16s ribosomal RNA (rRNA) gene was performed on each sample collection using the MiSeq Illumina Sequencing Platform as outlined by Kozich et al. (2013). Microbial DNA extraction from homogenized meat samples were performed using the Epicentre QuickExtract DNA extraction kit (Epicentre, Madison, WI). Sequencing library preparation follows protocols used in Bower et al. (2018). Approximately 1 to 5 ng of extracted DNA was amplified via polymerase chain reaction (PCR) with universal primers targeting the V4 region of 16S rRNA. Following amplification, PCR products were analyzed on a 1.5% agarose gel to confirm correct product size and amplification. Products were normalized using an Invitrogen SequalPrep Normalization Kit (Thermo Fisher Scientific). Barcoded PCR products were pooled and purified using the MinElute PCR Purification Kit (Qiagen, Germantown, MD) and further gel purified using the Pippin Prep system (Sage Science, Beverly, MA). Final concentration of the 16S rRNA libraries was determined using Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA), and the 16S libraries were sequenced using the Illumina MiSeq platform (Illumina, San Diego, CA) using the V2 500 cycle kit.

Processing of sequencing data was performed as described previously (Paz et al., 2016), using the bioinformatics pipeline Quantitative Insights Into Microbiological Ecology (QIIME; Caporaso et al., 2010). Sequences shorter than 245bp and longer than 275bp were removed and remaining sequences were trimmed to 251bp. Sequences were binned into operational taxonomic units (OTUs) at 97% similarity using the UPARSE pipeline (USEARCH v8.1; Edgar, 2010). Representative sequences from each OTU were assigned taxonomy using the UCLUST consensus taxonomy assigner method using Greengenes database release 119 as reference sequences (McDonald et al., 2012). The rarefaction of the OTU table was performed using QIIME (Caporaso et al., 2010) to a depth of 3,000 reads/sample. Samples under this threshold were removed from analysis. Good's coverage test was performed to ensure adequate sampling depth was achieved. Alpha diversity matrices (Chao1 and observed OTUs) were calculated using QIIME. The difference in bacterial communities (beta diversity) among treatments was determined using the QIIME pipeline using distance matrices (weighted UniFrac, unweighted UniFrac) from the rarefied OTU table. The raw sequence data generated and analyzed during this study are available under BioProject accession number PRJNA722526 from the National Center for Biotechnology Information sequence read archive.

Statistical analyses

Physicochemical and microbial growth data were analyzed using R (R Core Team, 2017). For salt, water activity, and proximate composition (measured Day 0 only), data were analyzed using R (lm and anova functions), and means were separated using the *agricolae* package (De Mendiburu, 2017). For pH, color, APC, and AnPC, data were analyzed as a 3 (brand) by 5 (storage time) interaction, with storage time as a repeated measure with an independent covariance structure using the *nlme* package (Pinheiro et al., 2017). Means were separated using the *lsmeans* package in R (Lenth, 2016). Significance was determined at $\alpha = 0.05$ throughout the study.

Interactions and main effects on mean alpha diversity were calculated using R (anova function) with storage time as a repeated measure (R Core Team, 2017). Pairwise comparisons on significant ($P < 0.05$) interactions and main effects of Chao1 and observed

OTUs were performed using the *lsmeans* package in R (Lenth, 2016). To reduce variation between replications, the OTU table was filtered to include only OTUs present in all 3 replications. This filtered OTU table was used for subsequent analysis. Bacterial community composition differences were estimated using the weighted and unweighted UniFrac distance matrices as input for permutational multivariate analysis of variance in the *vegan* package in R (Oksanen et al., 2019) to analyze interactions and main effects. Significance was declared at $P \leq 0.05$ throughout the study.

Results

Results for physiochemical metrics are displayed in Table 2, with additional storage time by brand interaction effect described in Table 3. Measures of meat pH and objective color (CIE L^*, a^*, b^*) were recorded throughout storage time. There was a brand by storage time interaction for pH ($P = 0.021$), where Brand B at Week 4 had decreased pH compared with other treatments (Table 3). There were no main effects or interaction for L^* or a^* ($P \geq 0.244$), but there was a main effect of brand for b^* ($P = 0.017$), where Brand A displayed greater b^* (yellowness) than both Brands B and C (Table 2). Salt and water activity were measured on the initial week of sampling (Week −4) only. Both salt and water activity were different between brands ($P \leq 0.007$), where Brand A had a lower salt concentration than B and C and Brand B had lower water activity than A and C.

Table 2. Least squared means for main effect of brand on chemical and microbiological analysis of retail ham products (brands labeled A, B, C)

Measured trait	Brand A	Brand B	Brand C	SEM	*P* value
Salt %	1.74^b	2.49^a	2.59^a	0.13	0.007
Water activity	0.98^b	0.96^a	0.98^b	0.001	< 0.001
Protein %	15.98	15.68	16.80	0.48	0.304
Moisture %	78.18^b	74.65^a	74.66^a	0.33	< 0.001
Fat %	2.64^b	5.16^a	4.96^a	0.45	0.012
Ash %	3.2^b	4.5^a	3.59^b	0.11	< 0.001
pH†	6.43	6.22	6.44	0.04	
L***	67.34	68.37	67.53	0.57	0.405
a*	9.38	10.29	9.58	0.35	0.182
b*	6.26^b	5.34^a	5.54^a	0.16	< 0.001
APC	2.97^b	0.4^a	0.36^a	0.34	< 0.001
AnPC†	3.19	0.87	0.17	0.36	

†Indicates a significant ($P < 0.05$) brand by storage time interaction and therefore main effects cannot be analyzed.

a,bMeans in the same row lacking a common superscript are significantly different ($P < 0.05$);

AnPC = anaerobic plate count; APC = aerobic plate count; SEM = standard error of the overall mean.

Table 3. Least square means for interaction effect of brand by storage time for pH, L^*, a^*, and b^* of retail ham products (brands labeled A, B, C)

Brand	Storage time (week)	pH	L^*	a^*	b^*
A	0	6.29^{ab}	66.68	10.80	6.40
	2	6.44^b	66.87	8.86	5.98
	4	6.44^b	67.08	9.20	6.13
	6	6.45^b	68.72	8.34	6.42
	8	6.51^b	67.33	9.69	6.35
B	0	6.22^{ab}	68.78	10.27	5.77
	2	6.43^b	67.57	10.67	5.44
	4	5.82^a	68.59	9.63	4.81
	6	6.31^b	68.62	10.33	5.28
	8	6.34^b	68.31	10.52	5.42
C	0	6.44^a	66.53	9.57	5.57
	2	6.45^a	66.56	10.31	5.71
	4	6.43^a	66.65	9.95	5.88
	6	6.40^a	68.46	9.31	5.57
	8	6.47^a	69.45	8.77	4.98
SEM		0.08	1.28	0.79	0.35
***P* value**		0.021	0.918	0.594	0.520

a,bMeans in the same row lacking a common superscript are significantly different ($P < 0.05$).

SEM = standard error of the overall mean.

Proximate composition was measured on one sample from each brand and replication. Moisture, fat, and ash were all significantly different between brands ($P < 0.012$), whereas there were no differences in protein ($P = 0.304$). Brand A had a greater moisture and a lower fat content than Brands B and C, whereas Brand B had a greater ash percentage compared with A and C.

Means for APC and AnPC are presented in Figure 1. There was a brand by storage time interaction for AnPC ($P = 0.032$), but no interaction for APC ($P = 0.441$). For AnPC, Brand A generally increased throughout storage time, ranging from 0.89 log CFU/g at Week −4 to 5.13 log CFU/g at Week + 4. Brand B remained under 2.00 log CFU/g throughout storage time, and Brand C remained under 0.90 log CFU/g. There was a brand effect on APC ($P = 0.017$), where Brand A had the greatest mean APC (2.97 log CFU/g) across all sampling times, whereas Brands B and C were less with mean values of 0.40 and 0.36 log CFU/g, respectively.

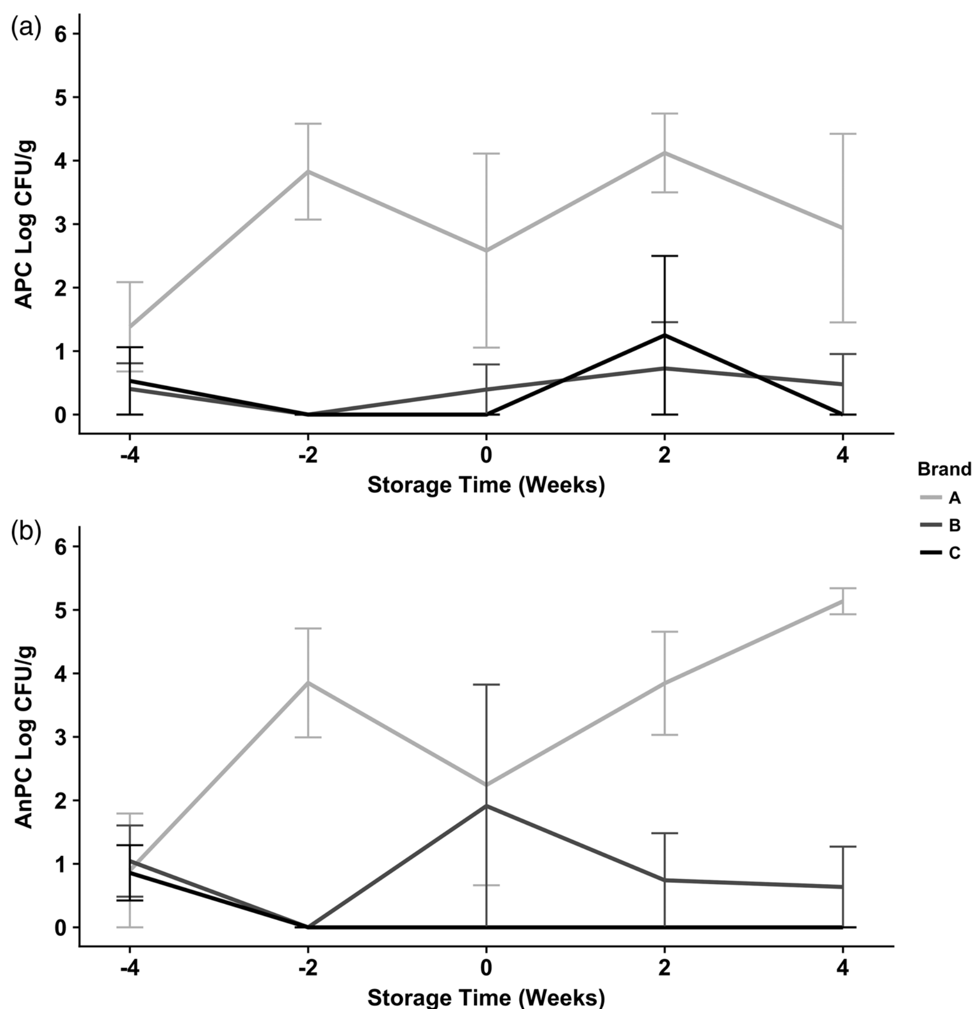

Figure 1. (a) Aerobic plate counts and (b) anaerobic plate counts of 3 brands of ham (A, B, C) throughout storage time.

In order to ensure adequate sampling depth, goods coverage was performed on the rarefied OTU table, and revealed that the depth used was able to characterize ≥ 95.8% of the total bacterial community. Diversity estimates Chao1 and observed OTUs were analyzed to determine differences in community richness or the number of different species in a sample (Figure 2). There was a brand by storage time interaction for Chao1 ($P = 0.043$); however, there were no significant interactions or main effects for observed OTUs ($P >$ 0.099). Using the weighted and unweighted UniFrac distance matrices, overall differences in bacterial community structure were determined. There was a main effect of brand on the weighted ($P < 0.001$) and unweighted ($P = 0.006$) UniFrac, where Brands B and C had a more similar community structure than Brand A, as shown in the principal component analysis visualization of weighted UniFrac distances in Figure 3. Brand A is separated in the top-right quadrant, whereas B and C are clustered closer on the left, illustrating that sliced hams produced in different environments can have microbiomes consisting of different community structures, but similar taxa can arise across different locations as well. To investigate which taxa are responsible for these shifts, relative abundance of taxa at the family and genus level was generated (Figure 4). Main contributors to the observed community differences are Brand A having greater proportion of *Carnobacterium*, *Bacillus*, and *Prevotella* than both B and C and B and C having greater proportions of *Pseudomonas*, *Photobacterium*, and *Lactococcus* compared with Brand A.

Discussion

Physiochemical attributes suggest that tested formulations of the tested hams were generally of similar function and composition; however, the exact ingredients added and ingoing concentrations may have influenced microbial populations. Lower fat percentage and higher

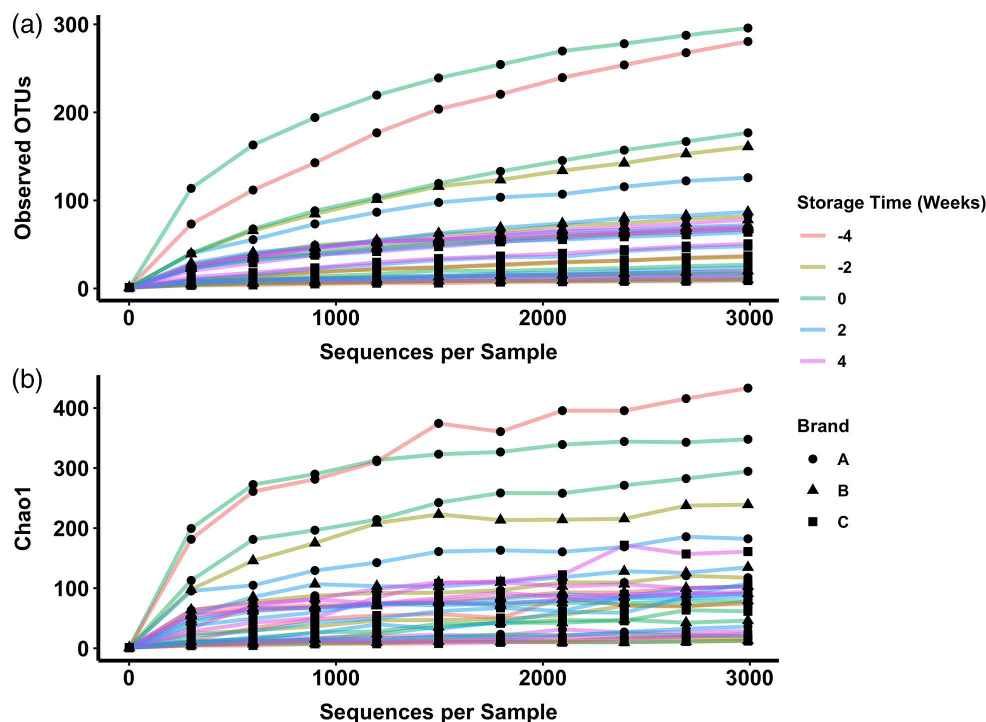

Figure 2. (a) Observed operational taxonomic units (OTUs) and (b) Chao1 estimates of community richness at various sampling depths for different ham brands (A, B, C) and weeks of sampling of sliced, prepackaged deli ham. All individual samples are displayed, colored by storage time with the marker shape representing brand. All samples were rarefied to an even depth of 3,000 reads.

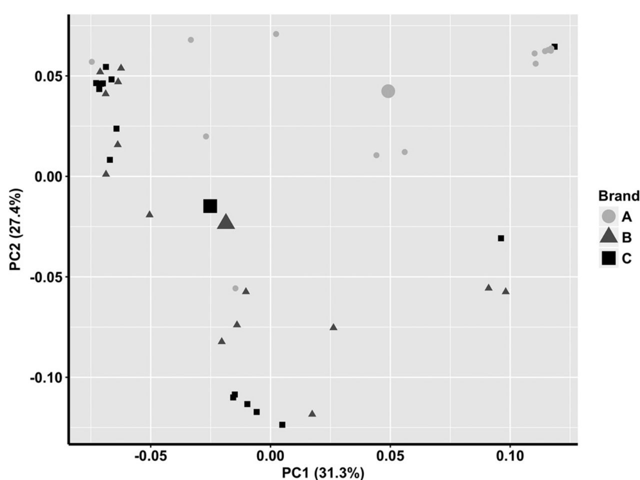

Figure 3. Principal coordinate analysis plot of all samples using a weighted UniFrac distance matrix. Small markers identify individual samples, whereas large markers indicate treatment means. Relative distance between samples indicates dissimilarity between overall bacterial community structure for different ham brands (A, B, C).

moisture percentage in Brand A is likely a reflection of the muscle composition of the ingoing meat block. Ham pH fluctuated over the course of the study in each brand, as displayed in Table 3. Increases in pH can be explained by the protein degradation and accrual of ammonia and biogenic amines during spoilage (Zhang and Guo, 2016), whereas decreases, such as those midstorage in Brand B, could be attributed to acids generated by fermentative organisms. A lack of brand or storage time effect on L^* and a^* suggests that ingoing nitrite concentrations were viable for cured color development and protection against cured meat fading. Increased ash content in Brand B is likely a reflection of its additional weight from sodium/potassium-containing antimicrobial agents and salt, compared with Brand A with less salt (Table 2) and Brand C with less added antimicrobials (Table 1).

Alongside this antimicrobial amount difference, lower salt concentration in Brand A is of considerable interest. Brand A had greater bacterial growth as well as a significantly different bacterial community structure compared with Brands B and C. Given that Brand A had less salt than both B and C, it is possible that the increased growth and the shift in community structure seen in Brand A are related to the difference in salt concentration. Salt is one of the main preservative ingredients added to meat products and as such typically decreases bacterial growth with increased concentrations (Borch et al., 1996; Bower et al., 2018). Furthermore, Brand A contained sodium propionate, an antimicrobial, whereas Brand B contained potassium lactate and sodium diacetate antimicrobials, and C contained no organic acid. Organic acids are one of the more commonly used antimicrobial agents to prevent

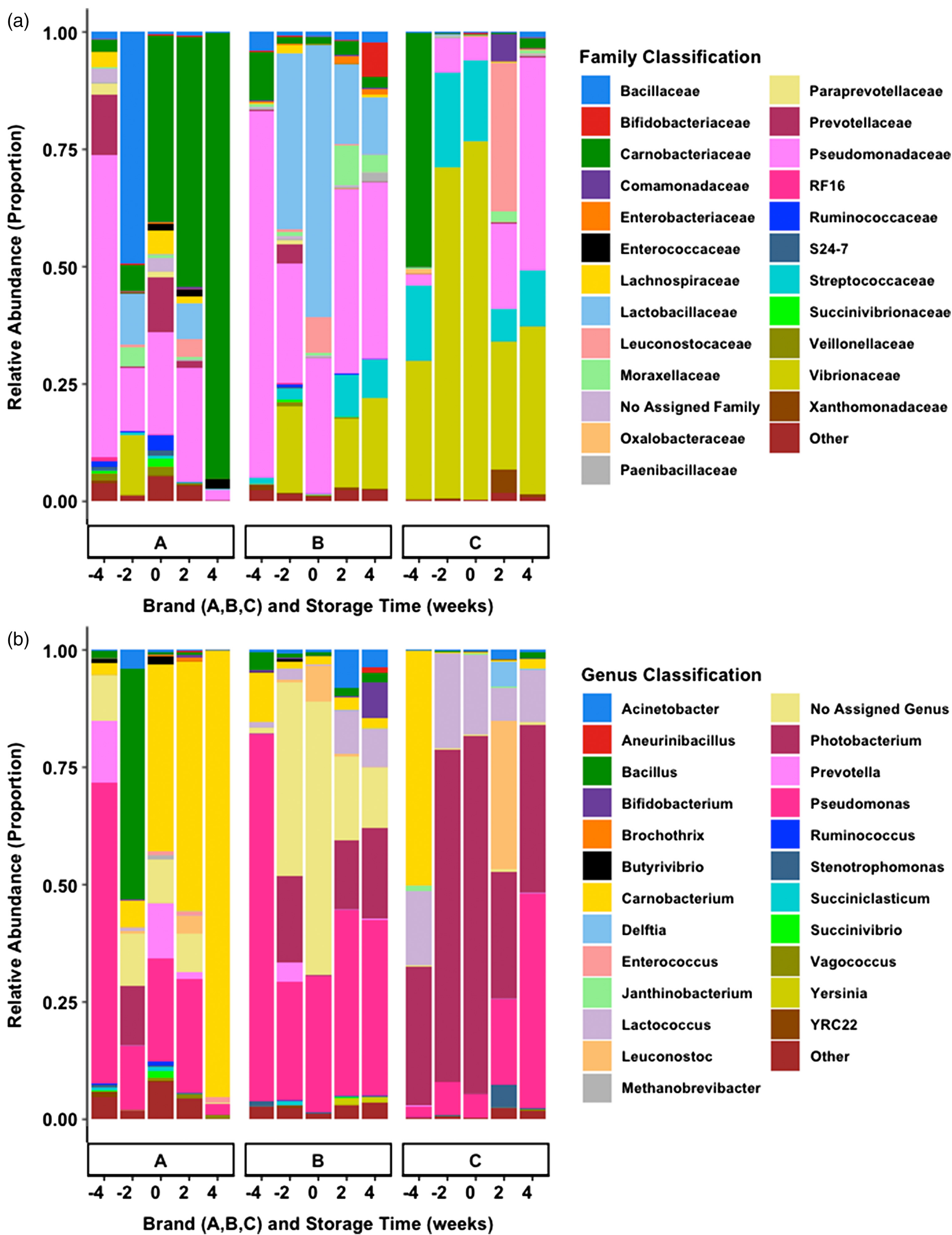

Figure 4. Relative abundance (proportion) of (a) family and (b) genus classification of bacterial community according to brand of sliced, prepackaged deli ham. The top 24 most prevalent genus according to maximum relative abundance across all 3 treatments are represented.

the growth of *Listeria monocytogenes* in ready-to-eat products but also inhibit other organisms and may shift the bacterial community structure (Benson et al., 2014; Ahmed et al., 2015). Thus, differences in organic acids could in part explain some of the differences seen in the current study. The Week + 4 AnPC count increase in Brand A could be attributed to *Carnobacterium* spp. anaerobic lifestyle and permeance in the context of this ingredient system. It is important to note, however, that spoilage is a complex phenomenon based on consumer objections to product odor, color, and exudate, and microbial plate counts are only a proxy for these objections and may not truly describe consumer attitudes.

Regardless of product composition, hams generally showed a decrease in alpha diversity metric Chao1. Brand A did have more diverse bacterial composition compared with B and C; however, in all cases, bacterial populations concentrated into fewer taxa after "sell-by" date. It has been observed that spoilage coincides with microbial succession and loss of diversity (Johansson et al., 2020), potentially making this measure valuable in more accurate determination of "sell-by dates."

Another explanation of the differences observed is simply the difference between the postlethality environment in which each of the brands was handled and packaged. Similar to how terroir plays a large role in the microbiome associated with wine and grape production (Canfora et al., 2018), it is likely that a processing plant environment contributes a unique initial contaminating bacterial community structure that shapes the eventual spoilage microbiome.

The cooking process inactivates many microorganisms present on the raw meat; therefore, it is important to consider the introduction of spoilage organisms on products after thermal processing. In the case of the studied hams, products would be sliced and packaged prior to distribution and thus would each be handled in a different, unique postlethality processing environment. It has been shown that *Pseudomonas* is commonly found on the contact surfaces of meat slicers (Mertz et al., 2014), which could contribute to the predominance of the taxa in this study. Furthermore, model systems have indicated that sanitation practices on slicing lines, as well as types of products sliced on said line, can influence the microbial load and shelf life of meat products; microbes from fermented products can migrate to nonfermented goods manufactured in the same facility without thorough sanitation between batches, leading to spoilage (Holley, 1997). It is possible that the *Lactobacillaceae* in Brand B are indigenous contaminants from the postprocess environment that are equipped for survival in the cold-stored modified atmosphere conditions and thus flourish as SSOs, as observed in other instances (Pothakos et al., 2015).

This study also serves to disrupt conventional notions regarding the taxa of the meat microbiome. Traditional wisdom would suggest that the conditions and environment of cooked ham either in vacuum packaging or low-oxygen modified atmosphere packaging would suppress the growth of *Pseudomonas* because they were believed to be obligate aerobes (Walker, 1980; Sun and Holley, 2012; Rossaint et al., 2015). It has been a recent surprise that hams in this study along with other studies of vacuum-packaged meats evaluated using 16s rRNA sequencing and traditional plating methods have revealed pseudomonads as a considerable proportion of the microbial community at time of spoilage (Wang et al., 2017; Hilgarth et al., 2019). It is clear that pseudomonads are more complex and adaptable than they are given credit; generalizations of behavior at the genus level have led to a misunderstanding regarding the environments in which they can flourish. This large genus does contain obligate aerobes but also contains obligate respirators or anoxic persisters that can grow without the presence of oxygen, utilizing additional fermentative pathways (Kolbeck et al., 2021). It has even been observed that *Pseudomonas fragi* strains in vacuum-packaged beef utilize more protolytic behavior and less aerobic respiration behaviors than counterparts stored aerobically (De Filippis et al., 2018). Although suppression may be observed in some strains, such as those in Brand B, vacuum or MAP packaging may not be an adequate solution for all pseudomonads, like those of Brand C. Beyond the scope of storage, metabolically repressed pseudomonads in MAP systems may gain function and quickly spoil products once the package is opened and exposed to oxygen, issuing a further line of consideration for microbiome characteristics in the context of consumer usage. These findings further illustrate the importance of understanding the microbiome of specific meat products and processing environments when devising shelf-life extension strategies.

One additional taxon of note is *Photobacterium*. Although this genus is generally associated with fish, recent works utilizing next generation sequencing have been uncovering *Photobacterium* as a sizable portion of the spoilage microbiome of some meats (Fuertes-Perez et al., 2019). *Photobacterium* are notability difficult to culture, so these organisms were likely underrepresented in studies utilizing traditional plate media commonly used to isolate organisms from meat surfaces, as shown by Hilgarth et al. (2018).

Unculturability is a widespread phenomenon, with many organisms deemed unable to readily grow in laboratory settings, leading to gaps in understanding regarding microbial diversity. The fact that these organisms were not widely recognized as a part of the meat microbiome until recently displays the capability that next generation sequencing has in finding problematic spoilage microbiota and possibly under characterization of microbial communities as a result of lack of diversity of locations sampled. *Photobacterium* spp. were a substantial portion of the bacterial community in Brand C, less portion in Brand B, and minimally in Brand A, as shown in Figure 4. Facility C was the closest (less than 10 miles) to a large freshwater lake and operated in a county that used said lake as its main water source, Brand B was near an estuary (less than 20 miles), and Brand A was more than 500 miles from any major water bodies. Considering the genus is generally associated with aquatic environments, it is possible these bacteria entered the food system via these nearby sources or employees that interfaced with these waters. Each processing plant contains a unique microbiome influenced by their operations and surroundings; scientists and processors should consider the utilization of precise sequencing methods to identify issues without the cumbersome preparation and incomplete view traditional methods impose.

Conclusions

The results of this study surmount the fact that bacteria of the meat spoilage microbiome are more adaptable and diverse than traditional methodologies describe. Technologies utilized to modulate bacterial communities and delay their growth must consider efficacy in the context of the system applied. In the context of the studied presliced ham, spoilage microbiomes were generally dominated by *Pseudomonas*, *Photobacterium*, *Lactococcus*, or *Carnobacterium*; however, the proportion of these taxa and other accessorial taxa varied across brands. Factors that influence microbial composition in ready-to-eat products are numerous but could include geographical location, sanitation practices, employee hygiene practices, temperature of processing and storage environments, or line speed and postlethality exposure time, among others. Although it is difficult to identify any one factor individually, the results presented indicate that one or a combination of these factors influence the microbial community to cause differences between brands. Products made with similar ingredients and through similar processes still have unique spoilage bacterial communities, which are likely impressed on them from the environment in which they were handled and packaged.

Acknowledgments

No external funding supported this research. Samodha C. Fernando, author of this publication, has disclosed a significant financial interest in NuGUT LLC. In accordance with its Conflict of Interest policy, the University of Nebraska-Lincoln's Conflict of Interest in Research Committee has determined that this must be disclosed. The rest of the authors have nothing to disclose.

Literature Cited

Ahmed, O. M., P. Pangloli, C.-A. Hwang, S. Zivanovic, T. Wu, D. D'Souza, and F. A. Draughon. 2015. The occurrence of *Listeria monocytogenes* in retail ready-to-eat meat and poultry products related to the levels of acetate and lactate in the products. Food Control. 52:43–48. https://doi.org/10.1016/j.foodcont.2014.12.015.

AOAC. 1990. AOAC: Official methods of analysis. 15th ed. Association of Official Analytical Chemists, Arlington, VA.

Benson, A. K., J. R. D. David, S. E. Gilbreth, G. Smith, J. Nietfeldt, R. Legge, J. Kim, R. Sinha, C. E. Duncan, J. Ma, and I. Singh. 2014. Microbial successions are associated with changes in chemical profiles of a model refrigerated fresh pork sausage during an 80-day shelf life study. Appl. Environ. Microb. 80:5178–5194. https://doi.org/10.1128/AEM.00774-14.

Borch, E., M.-L. Kant-Muermans, and Y. Blixt. 1996. Bacterial spoilage of meat and cured meat products. Int. J. Food Microbiol. 33:103–120. https://doi.org/10.1016/0168-1605(96)01135-X.

Bower, C. G., R. E. Stanley, S. C. Fernando, and G. A. Sullivan. 2018. The effect of salt reduction on the microbial community structure and quality characteristics of sliced roast beef and turkey breast. LWT-Food Sci. Technol. 90:583–591. https://doi.org/10.1016/j.lwt.2017.12.067.

Canfora, L., E. Vendramin, B. Felici, L. Tarricone, A. Florio, and A. Benedetti. 2018. Vineyard microbiome variations during different fertilization practices revealed by 16s rRNA gene sequencing. Appl. Soil Ecol. 125:71–80. https://doi.org/10.1016/j.apsoil.2017.12.019.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods. 7:335–336. https://doi.org/10.1038/nmeth.f.303.

De Filippis, F., A. La Storia, F. Villani, and D. Ercolini. 2018. Strain-level diversity analysis of *Pseudomonas fragi* after in situ pangenome reconstruction shows distinctive spoilage-associated metabolic traits clearly selected by different storage conditions. Appl. Environ. Microbiol. 85:e02212–18. https://doi.org/10.1128/AEM.02212-18.

De Mendiburu, F. 2017. agricolae: statistical procedures for agricultural research. https://cran.r-project.org/package=agricolae.

Doulgeraki, A. I., D. Ercolini, F. Villani, and G.-J. E. Nychas. 2012. Spoilage microbiota associated to the storage of raw meat in different conditions. Int. J. Food Microbiol. 157:130–141. https://doi.org/10.1016/j.ijfoodmicro.2012.05.020.

Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 26:2460–2461. https://doi.org/10.1093/bioinformatics/btq461.

Fuertes-Perez, S., Hauschild, P., Hilgarth, M., and R. F. Vogel. 2019. Biodiversity of *Photobacterium* spp. isolated from meats. Front. Microbiol. 10:2399. https://doi.org/10.3389/fmicb.2019.02399.

Geeraerts, W., V. Pothakos, L. De Vuyst, and F. Leroy. 2017. Diversity of the dominant bacterial species on sliced cooked pork products at expiration date in the Belgian retail. Food Microbiol. 65:236–243. https://doi.org/10.1016/j.fm.2017.03.007.

Geeraerts, W., V. Pothakos, L. De Vuyst, and F. Leroy. 2018. Variability within the dominant microbiota of sliced cooked poultry products at expiration date in the Belgian retail. Food Microbiol. 73:209–215. https://doi.org/10.1016/j.fm.2018.01.019.

Gram, L., L. Ravn, M. Rasch, J. B. Bruhn, A. B. Christensen, and M. Givskov. 2002. Food spoilage—interactions between food spoilage bacteria. Int. J. Food Microbiol. 78:79–97. https://doi.org/10.1016/S0168-1605(02)00233-7.

Hilgarth, M., S. Fuertes-Pèrez, M. Ehrmann, and R. F. Vogel. 2018. An adapted isolation procedure reveals *Photobacterium* spp. as common spoilers on modified atmosphere packaged meats. Lett. Appl. Microbiol. 66:262–267. https://doi.org/10.1111/lam.12860.

Hilgarth, M., E. M. Lehner, J. Behr, and R. F. Vogel. 2019. Diversity and anaerobic growth of *Pseudomonas* spp. isolated from modified atmosphere packaged minced beef. J. Appl. Microbiol. 127:159–174. https://doi.org/10.1111/jam.14249.

Holley, R. A. 1997. Impact of slicing hygiene upon shelf life and distribution of spoilage bacteria in vacuum packaged cured meats. Food Microbiol. 14:201–211. https://doi.org/10.1006/fmic.1996.0089.

Hultman, J., R. Rahkila, J. Ali, J. Rousu, and K. J. Björkroth. 2015. Meat processing plant microbiome and contamination patterns of cold-tolerant bacteria causing food safety and spoilage risks in the manufacture of vacuum-packaged cooked sausages. Appl. Environ. Microbiol. 81:7088–7097. https://doi.org/10.1128/AEM.02228-15.

Johansson, P., E. Jääskeläinen, T. Nieminen, J. Hultman, P. Auvinen, and K. J. Björkroth. 2020. Microbiomes in the context of refrigerated raw meat spoilage. Meat Muscle Biol. 4(2). https://doi.org/10.22175/mmb.10369.

Kolbeck, S., M. Abele, M. Hilgarth, and R. F. Vogel. 2021. Comparative proteomics reveals the anaerobic lifestyle of meat-spoiling *Pseudomonas* species. Front. Microbiol. 12:664061. https://doi.org/10.3389/fmicb.2021.664061.

Kozich, J. J., S. L. Westcott, N. T. Baxter, S. K. Highlander, and P. D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl. Environ. Microbiol. 79:5112–5120. https://doi.org/10.1128/AEM.01043-13.

Lenth, R. V. 2016. Least-squares means: the R package lsmeans. J. Stat. Softw. 69:1–33. https://doi.org/10.18637/jss.v069.i01.

McDonald, D., M. N. Price, J. Goodrich, E. P. Nawrocki, T. Z. DeSantis, A. Probst, G. L. Andersen, R. Knight, and P. Hugenholtz. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J. 6:610–618. https://doi.org/10.1038/ismej.2011.139.

Mertz, A. W., O. K. Koo, C. A. O'Bryan, R. Morawicki, S. A. Sirsat, J. A. Neal, P. G. Crandall, and S. C. Ricke. 2014. Microbial ecology of meat slicers as determined by denaturing gradient gel electrophoresis. Food Control. 42:242–247. https://doi.org/10.1016/j.foodcont.2014.02.027.

Miller, P., X. Liu, and L. M. McMullen. 2015. Microbiota of regular sodium and sodium reduced ready-to-eat meat products obtained from the retail market. Can. J. Microbiol. 154:150–154. https://doi.org/10.1139/cjm-2014-0630.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2019. vegan: community ecology package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan.

Paz, H. A., C. L. Anderson, M. J. Muller, P. J. Kononoff, and S. C. Fernando. 2016. Rumen bacterial community composition in Holstein and Jersey cows is different under same dietary condition and is not affected by sampling method. Front. Microbiol. 7:1206. https://doi.org/10.3389/fmicb.2016.01206.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2017. nlme: linear and nonlinear mixed effects models. https://cran.r-project.org/package=nlme.

Pothakos, V., F. Devlieghere, F. Villani, J. Björkroth, and D. Ercolini. 2015. Lactic acid bacteria and their controversial role in fresh meat spoilage. Meat Sci. 109:66–74. https://doi.org/10.1016/j.meatsci.2015.04.014.

R Core Team. 2017. R: a language and environment for statistical computing. R package version 3.1-131. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/.

Rossaint, S., S. Klausmann, and J. Kreyenschmidt. 2015. Effect of high-oxygen and oxygen-free modified atmosphere packaging on the spoilage process of poultry breast fillets. Poultry Sci. 94:93–103. https://doi.org/10.3382/ps/peu001.

Sebranek, J., S. Lonergan, M. King-Brink, E. Larson, and D. Beerman. 2001. Meat science and processing. 3rd ed. Zenda, WI: Peerage Press.

Sun, X. D., and R. A. Holley. 2012. Antimicrobial and antioxidative strategies to reduce pathogens and extend the shelf life of fresh red meats. Compr. Rev. Food Sci. F. 11:340–354. https://doi.org/10.1111/j.1541-4337.2012.00188.x.

Walker, H. W. 1980. Effects of microflora on fresh meat color. In: *Reciprocal meat conference proceedings*, vol. 33. American Meat Science Association, Kearney, MO. p. 33–36.

Wang, G., M. Li, F. Ma, H. Wang, X. Xu, and G. Zhou. 2017. Physicochemical properties of Pseudomonas fragi isolates response to modified atmosphere packaging. FEMS Microbiol. Lett. 364:fnx106. https://doi.org/10.1093/femsle/fnx106.

Zhang, H., Wu, J., and X. Guo. 2016. Effects of antimicrobial and antioxidant activities of spice extracts on raw chicken meat quality. Food Science and Human Wellness. 5:39–48. https://doi.org/10.1016/j.fshw.2015.11.003.