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The effect of salt reduction on the microbial community structure and quality characteristics of sliced roast beef and turkey breast

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ABSTRACT

Sectioned and formed turkey breast and roast beef were manufactured with four salt concentrations (1.0%, 1.5%, 2.0%, and 2.5%, meat block basis). After cooking, chilling, and slicing, samples were evaluated throughout 18 weeks of storage for various quality parameters, and microbial community changes. Microbial community changes were analyzed using 16S rRNA gene sequencing for the V4 region. Bacterial richness decreased over storage time ($P = .040$) and as salt concentration increased ($P = .021$). Microbial communities were dominated by bacteria from family Pseudomonadaceae, regardless of treatment or storage time. Salt reduction had greater negative effects on cooking yield ($P < .001$), hardness ($P = .006$), cohesiveness ($P = .031$), and chewiness ($P = .007$) in beef samples compared to turkey. Results from this study indicate that *Pseudomonas* are dominant spoilage organisms found in cooked meat microbial communities during storage. Furthermore, quality aspects were more negatively affected by salt reduction in sliced roast beef compared to sliced turkey breast.

1. Introduction

Sodium intake has been a health concern for many years, however, pressure to further reduce sodium in the diet has resurfaced and increased the demand for lower sodium foods. Excessive sodium intake can be detrimental to human health and is a contributor to high blood pressure and increased risk for heart disease (Karppanen & Mervaala, 2006). Salt, sodium chloride, is a multi-functional ingredient essential to the texture, flavor, bind, water holding capacity, and microbial stability of processed meats. Reducing salt concentration in meat products with no replacement can negatively affect sensory properties and consumer acceptability (Aaslyng, Vestergaard, & Koch, 2014; Tobin, O'Sullivan, Hamill, & Kerry, 2012). Salt also enhances the perception of meat flavor. Furthermore, salt extracts protein, increases muscle to muscle bind, and improves water and fat binding (Ruusunen & Puolanne, 2005). These functions can somewhat be replicated with less added sodium chloride, however, it may require the use of multiple ingredients or processing techniques in order to replace each of the functions of salt and can be associated with negative quality characteristics.

One of the main and certainly oldest function of salt is preservation. A reduction in salt causes a more rapid growth of the natural flora of cooked meats resulting in a shortened shelf life (Whiting, Benedict, Kunsch, & Woyochik, 1984). Furthermore, it is thought that salt may shift spoilage bacterial communities, which could favor the growth of more salt tolerant, slower growing bacteria such as lactic acid bacteria (LAB) that will tend to produce less offensive sour and acid aromas, rather than putrid or cheesy odors (Borch, Kant-Muermans, & Blixt, 1996). Researchers have recently studied the microbial communities of various processed meat products with various methods. Benson et al. (2014) studied microbial succession in fresh pork sausage using the Roche-454 platform, while Miller, Liu, and Mcmullen (2015) studied the microbiota of reduced sodium RTE meat products using Sanger Sequencing of isolated cultures in combination with DGGE analysis. Past applications of the Illumina sequencing platform have yielded results of similar quality to the 454 platform while providing considerably greater sequencing coverage (Kozich, Westcott, Baxter, Highlander, & Schloss, 2013). Using this platform, we hope to gain a more in depth understanding of the role microbial communities play in the spoilage of cooked meat products. The aim of this study was to determine the effects of formulated salt concentration on the microbial community, mapped using 16S rRNA sequencing, shelf life, and quality characteristics in uncured deli turkey and roast beef.

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2. Materials and methods

2.1. Deli meat processing

For three independent replications produced on separate days, boneless beef top round (*semimembranosus*) were obtained from the Loeffel Meat Laboratory (University of Nebraska, Lincoln, NE) and boneless, skinless, turkey breast (*pectoralis*) were purchased from a wholesale distributor and delivered to the Loeffel Meat Laboratory for processing. All meat was stored frozen until use, and tempered at 2 °C for 3–4 days prior to processing. Turkey and beef muscles were trimmed of external fat and connective tissue and separately ground through a 12.7 mm plate using a Hobart Meat Grinder (Model #4734; Hobart MFG. Co., Troy, OH). Within a species, meat was mixed by hand to ensure a uniform mixture of the base meat block prior to portioning into 9.1 kg batches to manufacture products with different salt concentrations.

Within each replication, products were manufactured for each of two meat species (beef [B] and turkey [T]) and four different formulations were used for salt concentration on meat block: 1.0% NaCl, 1.5% NaCl, 2.0% NaCl, and 2.5% NaCl. A brine for 25% extension was formulated to contain the target salt concentration, 1.0% sugar, and 0.35% sodium phosphate (Brifisol 85 Instant, Bk Giulini, Ladenburg, Germany) on a meat block basis, and water was added as balance. Ground meat and brine were added to a vacuum tumbler (Model DVTS R2-250; Daniels Food Equipment, Parkers Prairie, MN) and were tumbled under vacuum (66.7 kPa) at 4 °C for 90 min. Each treatment batch was stuffed using a vacuum stuffer (Vemag Robot 1000 DC; Reiser, Canton MA) into fibrous casings (90 mm x 24" pre-stuck, Kalle, Gurnee, IL) and casings were pulled and clipped using a Tipper Clipper (Model PR465L; Tipper Tie, Inc., Apex, NC). Chubs within treatment were weighed and thermally processed in an Alkar smokehouse (Alkar-RapidPak Inc., Lodi, WI) to an internal temperature of 71 °C, followed by a 30 min cold water shower. Products were chilled overnight at 4 °C.

The day after processing, turkey and roast beef rolls were weighed, casings were removed, and product was sliced (Bizerba Model SE12; Bizerba, Balingen, Germany) into 2 mm slices for microbiological sampling and 13 mm slices for quality and texture analysis. One slice from each of the two chubs per treatment was placed into each 3 mil std barrier nylon/PE vacuum pouch, vacuum sealed (Multivac Model C500; Multivac Inc., Kansas City, MO), and placed in a covered plastic lug and stored at 4 °C until sampling. Water activity and salt content were measured on the day of slicing and shelf life parameters were measured every two weeks starting on the day of slicing until 18 w shelf life.

2.2. Microbial analysis

Microbial analysis was performed by sampling from a package of two slices per treatment starting on the day of slicing (week 0) and continuing every two weeks until week 18. During packaging, two slices of each meat sample were pre-packaged individually corresponding to each day of sampling. At sampling on each day a pre-packaged sample was removed and processed. During sampling, samples were aseptically transferred from the vacuum pouch into a WhirlPak bag (Nasco, Fort Atkinson, WI), combined with 50 ml of sterile BBL Peptone water (Becton, Dickinson and Company, Franklin Lakes, NJ) and placed in a bag blender (bioMerieux Inc., Durham, NC) for 3 min to homogenize the sample. Two, 2 ml samples of homogenate was collected for community analysis and was stored at −20 °C until used for DNA extraction. Additionally, aerobic plate counts (APC) were performed using the homogenized samples. An Eddy Jet spiral plater (IUL, S.A., Barcelona, Spain) was used to plate 50 μl of homogenate on Brain Heart Infusion agar (BHI) plates (Thermo Fisher Scientific, Waltham, MA) and were incubated at 37 °C for 48 h. For Anaerobic plate counts (AnPC), samples were prepared as described for APC and were incubated at 37 °C for 48 h in an anaerobic box containing BD GasPak EZ sachets to create an anaerobic environment (BD Medical Technology, Franklin Lakes, NJ). After 48 h of incubation, colonies were counted manually as described by the EddyJet owner's manual. Bacterial counts were converted to $\log_{10}$ colony forming units (CFU)/gram of sample.

2.3. Analysis of bacterial communities

Bacterial community analysis using high throughput sequencing of the 16s rRNA gene was performed on samples collected at two week intervals from week 0 to week 14 using the MiSeq Illumina Sequencing Platform as outlined by Kozich et al. (2013). Based on APC results, shelf life of the products in this study was determined to approximately 10 weeks, therefore community analysis was performed at two week intervals throughout shelf life and up to 4 weeks beyond the usable shelf life. Microbial DNA extraction from homogenized meat samples were performed using a modified protocol of the Epicentre QuickExtract DNA extraction kit. Briefly, 1 ml sample was centrifuged at 10,000 × *g* for 10 min at 20 °C, supernatant was removed, and 500 μl of QuickExtract solution (Epicentre, Madison, WI) was added to the pellet. Following addition of lysis solution, samples were vortexed, incubated at 65 °C for 10 min, vortexed again, and incubated at 98 °C for 2 min. The resulting DNA was used for Polymerase chain reaction (PCR). PCR amplification was performed in a 20 μl reaction that contained 1X Terra PCR Direct Buffer (Clontech Laboratories Inc., Mountain View, CA), 0.75 U Terra PCR Direct Polymerase Mix (Clontech Laboratories Inc., Mountain View, CA), approximately 1–5 ng of extracted DNA, and 0.5 μM barcoded universal primers as described by Kozich et al. (2013). PCR reaction was performed in a Veriti 96 well thermocycler (Thermo Fisher Scientific, Walther, MA), where samples were subjected to the following PCR cycle: initial denaturation at 98 °C for 2 min, followed by 30 cycles of 98 °C for 30s, 58 °C for 30 s, and 68 °C for 45 s, and a final extension of 68 °C for 4 min. 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 Sequal Prep Normalization Kit (Thermo Fisher Scientific, Walther, MA) according to the manufacturer's protocol for binding, washing, and elution steps to yield ~25 ng DNA per well. Barcoded PCR products were pooled and gel purified using the Pippin Prep system (Sage Science, Inc., 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, Inc., San Diego, CA) using the V4 500 cycle kit. Analysis of sequencing data was performed as described previously (Paz, Anderson, Muller, Kononoff, & Fernando, 2016), using the bioinformatics pipeline Quantitative Insights Into Microbiological Ecology (QIIME; Caporaso et al., 2010). Briefly, sequences shorter than 245 bp and longer than 275 bp were removed and remaining sequences were trimmed to 251 bp. Sequences were binned into operational taxonomic units (OTUs) at 97% similarity using the UPARSE pipeline (USEARCH v8.1). Representative sequences from each OTU were assigned taxonomy using the UCLUST consensus taxonomy assigner (QIIME default) method using Greengenes database release 119 as reference sequences.

2.4. Cooking yield, water activity, salt content, and pH

Cooking yield was determined by weighing each treatment batch (2 chubs/stick) prior to cooking, and again after chilling overnight prior to slicing. Cooking yield was calculated as cooked weight as a percentage of raw weight: $Cooking\ yield = (cooked\ weight)/(raw\ weight)*100$.

Samples used for water activity and salt concentration were homogenized using a food processor (Black & Decker Handy Chopper, Black & Decker Inc., Baltimore, MD). Water activity was measured the day of slicing using an Aqualab water activity meter (Decagon Devices, Inc., Pullman, WA) according to manufacturer's specifications.