#### 2019 Reciprocal Meat Conference – Processing, Ingredient Technology and Packaging

Meat and Muscle Biology<sup>TM</sup>



## Spoilage Microbiota of Beef Throughout Various Phases of Processing

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**Keywords:** beef, microbiota, spoilage Meat and Muscle Biology 3(2):42-43

# **Objectives**

This study aimed to evaluate the spoilage microbiota of beef throughout various processing steps and identify key differences in the microbiome associated with each phase of processing.

## **Materials and Methods**

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In each of three replicates, products representing each phase of processing were made from the same uniform meat block (beef shoulder clods): T1-ground beef; T2-fresh sausage; T3-cooked links; T4-beef franks; T5sliced bologna; T6-bologna with HPP treatment; T7bologna with lactate/diacetate. Raw treatments were evaluated every 3 d for 21 d, and cooked treatments were evaluated every 14 d for 112 d. Heat treated products were cooked to an internal temperature of 71°C and chilled overnight at 4°C. Parameters for HPP were 600 MPa for 3 min. Aerobic (APC), anaerobic (AnPC), lactic acid bacteria (LAB), and psychrotrophic (PPC) plate counts were measured. Microbial communities were evaluated using high throughput 16S rRNA gene sequencing on the Illumina MiSeq platform. Reads were processed using QIIME, binned into operational taxonomic units (OTUs) at 97% similarity, and assigned taxonomy using the Greengenes database as reference. Alpha and  $\beta$  diversity of bacterial communities were analyzed using QIIME and R. Alpha diversity was estimated using observed OTUs and Chao1 estimates, and  $\beta$  diversity was determined using the weighted and unweighted UniFrac distance matrices (Fig. 2). Raw and cooked samples were analyzed independently for plate counts and  $\alpha$  diversity.

## Results

There was a treatment by storage time interaction for AnPC in cooked samples (P = 0.003), where T3, T4, and T7 increased from Day 28 and 42. In raw samples, there was a main effect of storage time on APC, AnPC, LAB, and PPC (P < 0.001), where growth increased over time. In cooked samples, there was a main effect of storage time on APC, LAB, and PPC, and a main effect of treatment for APC and LAB (P < 0.030). Higher APC and LAB counts were observed in T5,



#### PCoA Plot of Weighted (a) and Unweighted (b) UniFrac Distance Matrices

Figure 2. PCoA Plot of Weighted (a) and Unweighted (b) UniFrac Distance Matrices.

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while a general increase in APC, LAB, and PPC was seen throughout storage time. There were main effects of treatment and storage time on Chao1 and Observed OTUs in raw samples (P < 0.023) and a main effect of treatment in cooked samples (P < 0.009). In raw samples, bacterial richness was greater in T2 compared to T1, and generally decreased throughout storage time. In cooked samples, richness was the greatest in T3 and T4, the least in the T5, and T6 and T7 were intermediate. There were main effects for treatment and storage time on the bacterial community structure according to the weighted UniFrac distance matrix (P < 0.004)and a treatment by storage time interaction for the unweighted UniFrac distance matrix (P = 0.031). For the weighted UniFrac, T1 and T5 samples formed a cluster relatively separate from the other treatments, while T2

formed an additional cluster by itself. For the unweighted UniFrac, T1, T2, and T5 formed a cluster separate from the other samples, with increased storage times being further separated from the other samples.

## Conclusion

Results from this study indicate that the microbiota of cooked, sliced, bologna is somewhat similar to that of raw ground beef, whereas fresh sausage, cooked links, and bologna with HPP and antimicrobial treatments are different from the former. Treatments where microbial growth was reduced had a significantly different microbial composition compared to those with greater amounts of growth.