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MARINATION INHIBITS MICROBIAL GROWTH AND MODIFIES MICROBIOME SUCCESSION OF REFRIGERATED CHICKEN BREAST CUTS WITHOUT AND WITH WOODEN BREAST MYOPATHY

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I. INTRODUCTION

Wooden breast (WB) is a common defect affecting the musculoskeletal tissue of broilers that mainly causes the breast muscle to become stiff and sometimes exude a serous liquid [1]. Among other, this condition results in poor meat quality, briefly, an increased pH and decreased water-holding capacity. These parameters are involved in bacterial growth in foods, and can promote it, which raises concerns about the shelf-life of affected breasts. In contrast, marination can prolongate shelf-life through microbial growth inhibition. This study aims to identify the bacteria involved in the spoilage of chicken breasts cuts without and with WB and treated or not with a marinade through 16S amplicon sequencing, a culture-free technique that has the potential to identify bacteria, assess their abundance and compare microbial communities between treatments and along storage time.

II. MATERIALS AND METHODS

Cubes of aprox. 25 g were cut from normal (N) and WB breasts. Half of each were mixed with an oil-based red pepper marinade (M) including lactate in a tumbler for 10 minutes, leaving half not marinated (C) and making 4 batches: NC, WBC, NM, WBM). Individual cubes were vacuum packaged and stored at +3.9 ± 0.8 ºC for 17 days. On days 1, 8, 14 and 17 of storage, 3 replicates of each combination were diluted 1/10 with a peptone saline solution (1% peptone, 0.85% NaCl) in a filter bag and homogenized using a Smasher blender. Plate count enumeration was performed for total viable counts (TVC), Enterobacteriaceae, lactic acid bacteria (LAB), *Brochothrix* spp. and *Carnobacterium* spp. For metagenomics, aliquots of the homogenates were centrifuged (200 x g, 3 minutes). The supernatant was centrifuged again to precipitate the cells (10000 x g, 3 minutes), and the resulting pellet was stored at -20 ºC until extraction with the DNeasy PowerFood Microbial Kit (Qiagen). 16S rRNA gene amplification and sequencing was performed by the DNA Sequencing and Genomics Laboratory (supported by HiLIFE and Biocenter Finland funding), Institute of Biotechnology, University of Helsinki. The V3-V4 regions of the 16S rRNA gene were amplified using universal bacterial primers (341F1-4 and 785R1-4) and the Illumina MiSeq was used for sequencing. Bioinformatic analyses were conducted using R Statistical Software (v. 4.4.3; R Core Team 2021). Before loading the sequences, primers were removed from raw reads using cutadapt (version 5.0). Reads were processed through DADA2 (v. 1.34.0) pipeline for filtering, denoising and microbial inference. Amplicon sequence variants (ASV) were used for taxonomic assignment using the SILVA database (version 138.2). The phyloseq package was used for further data management (version 1.50.0). Chloroplasts, mitochondria and non-identified variants at the Phylum level were removed from the dataset before abundance per sample was calculated as the number of sequencing reads that corresponded to a certain ASV in each sample. Plots were generated using phyloseq and ggplot2 (version 3.5.1) after sub-setting the samples by marination and merging the taxa under 5000 reads to the “others” category.

III. RESULTS AND DISCUSSION

Plate count results showed that initial levels were maintained throughout the storage for marinated samples, implying that marination inhibited microbial growth. In contrast, in non-marinated cuts, a relevant growth of 5-6 logs for TVC, 3-5 log for *Brochothrix* spp. and LAB, and 4-5 log for Enterobacteriaceae and *Carnobacterium* spp. was observed during storage. Growth was generally slowed by the presence of WB ($p < 0.05$), with the most noticeable differences between NC and WBC at day 8 of storage (aprox. 1 log), except for Enterobacteriaceae, that showed the same levels in WB and CO. Marination also affected the composition of the microbial communities, as seen by 16S amplicon sequencing results (Figure 1), where genera relative abundance was maintained through the storage period, with *Pantoea* spp. being most abundant along with other gram-negative bacteria such as *Erwinia* spp.,

Pseudomonas spp. or *Xanthomonas* spp. However, in some replicates, *Latilactobacillus* spp. was also detected at the end of storage time. Growth inhibition in marinated breasts could be attributed to the presence of lactate [2] and lower pH, conditions under which *Latilactobacilus* spp. is able to thrive [3]. In contrast, in non-marinated meat, *Lactobacillus* spp. and *Limosilactobacillus* spp. were present at the initial time, but were substituted by other genera along storage, as observed by other authors in similar cuts [4]. Dominance of different genera in successive storage times does not appear to have a clear pattern and no relevant differences were observed between normal and WB samples. The most common genera among replicates was a mixture of *Carnobacterium* spp., *Hafnia* spp. and *Pseudomonas* spp. at different abundances. However, in other replicates, *Photobacterium* spp. showed an abundance of more than 70%. *Photobacterium* spp. has recently emerged as an important meat spoiler and has been previously detected in high abundances in refrigerated chicken cuts [4]. *Yersinia* spp. prevailed only in one replicate at 17 days. Other important genera observed in the chicken samples after 8 days of storage include *Lactoccoccus* spp., which is present since day 14 and dominates over one replicate, *Serratia* spp., and *Vagoccocus* spp., all common chicken spoilers [3,4].

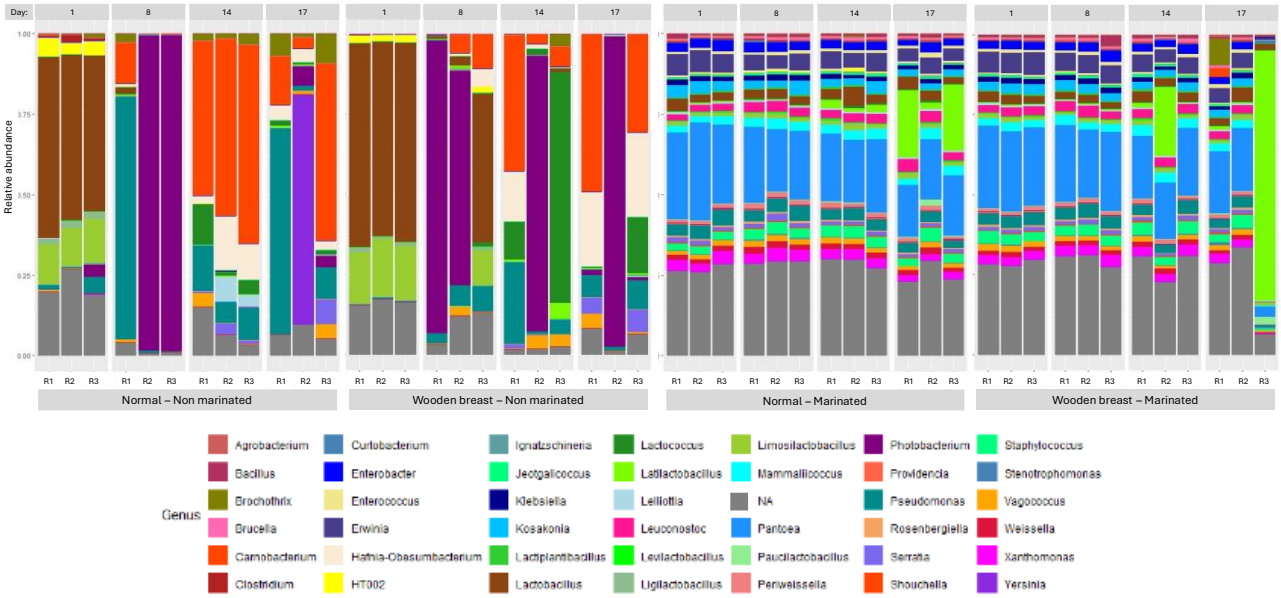


Figure 1. Relative abundance of bacterial genera in control and WB at 1, 8, 14 and 17 days of storage in non-marinated and marinated.

IV. CONCLUSION

The presence of WB myopathy did not negatively affect shelf-life from the microbiological point of view. Marination extended the shelf-life of chicken breast cuts without and with WB. The use of 16S amplicon sequencing allowed for a thorough investigation of the spoilage processes, detecting genera that are usually missed in conventional plate-count enumeration such as *Photobacterium* spp., and allowing to characterize the evolution of the microbial communities along refrigerated storage.

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