THIRD WAVE BIOACTIVES®

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GENUS-LEVEL MICROBIAL COMMUNITY PROFILING DURING SHELF-LIFE OF RAW PLANT-BASED PATTIES

INTRODUCTION

Demand for plant-based alternatives to traditional meat products has grown rapidly over the past several years, in part due to the health and environmental impact concerns of customers. The food industry has responded by diversifying not only applications and flavors but storage, packaging, and point of sale, with many retailers offering refrigerated options in addition to frozen. Combined with a consumer base that is increasingly clean-label conscious, this presents novel shelf-life challenges to investigate and overcome.

In addition to studying how fast these product spoil, investigating the identity and proliferation of the microbial populations and how these communities are affected by different packaging and natural preservative options can provide insights on how best and most effectively to prolong product quality and shelf-life.

METHODOLOGY

All patties were commercially produced with one of the following natural preservatives: 1% bioVONTAGE® 7374 cultured dextrose (BV7374), 0.5% reFRESH® 386 cultured onion juice (RF386), or no preservative (Control). All treatment types were packed in non-MAP (Non-Modified Atmospheric Packaging) trays or vacuum-sealed bags. All samples were refrigerated at 4°C for the duration of the study. At each time point (Day 1, Day 7, Day 14, and Day 21) a 22g sample from each treatment and packaging type was taken, diluted with 198mL of sterile, buffered peptone (0.1%) and masticated. The samples were further serially diluted in sterile peptone and plated on Tryptic Soy Agar (TSA) for Aerobic Total Plate Count (APC), DeMan, Rogosa, and Sharpe Agar (MRSA) for Lactic Acid Bacteria (LAB) and Potato Dextrose Agar (PDA) acidified with 10% Tartaric Acid for Yeast and Mold (YM). TSA plates were incubated aerobically at 32°C for 48 hours, MRSA plates were incubated anaerobically at 32°C for 48 hours, and PDA plates were incubated aerobically at 28°C for 72 hours. The pH at each time point was measured using the masticated sample in peptone.

In addition, 10mL masticated samples were pelleted via centrifugation and stored dry at -20°C for downstream microbiome analysis. The gDNA was extracted using the MPBio Fast DNA Spin Kit and sent to the Roy J. Carver Biotechnology Center at the University of Illinois Urbana-Champagne for genomic sequencing via Illumina MiSeq. Primers for V4, ITS3-4 and NLAB2F/WLAB1R were used for genomic analysis and relative abundance was identified at genus level.

RESULTS & DISCUSSION

Microbial Analysis in Non-MAP samples: In the Control samples, the APC and LAB counts increased to 10^5 CFU/g at day 14. In comparison, the APC and LAB counts in BV7374 samples remained below 10^4 CFU/g and RF386 samples remained below 10^3 CFU/g through day 14. Yeast was detected on day 14 in only the RF386 sample.

All non-MAP samples showed visible mold growth at day 21 and were not tested further.

Microbial Analysis in Vacuum samples: The APC and LAB counts in the Control increased to 10^6 CFU/g on day 14 and 10^8 CFU/g on day 21. In comparison, the APC counts did not rise above 10^4 CFU/g in BV7374 and 10^3 CFU/g in RF386 samples. The LAB levels remained below 10^3 CFU/g in both the BV7374 and RF386 samples throughout the study. Visible mold was present only on the Control sample at day 21.

Microbiome Analysis: Aerobic and Anaerobic organisms were diverse in all treatments and packaging types through day 7. However, on day 14, the microbiome of the Control sample was dominated by *Leuconostoc* (>98%) in non-MAP and *Pseudomonas* (>90%) in vacuum-packed. This was consistent with the increase in APC and LAB during microbial analysis on the same days. The microbial communities in BV7374 and RF386 remained diverse under both packaging conditions within all days tested. The genera *Lacticaseibacillus, Geobacillus, Bacillus, Enterococcus,* and *Cutibacterium* were the most prominent of both treatments throughout the study, and no one genus was found to comprise more than 60% of the microbial load within BV7374 or RF386 treated samples at any time point.

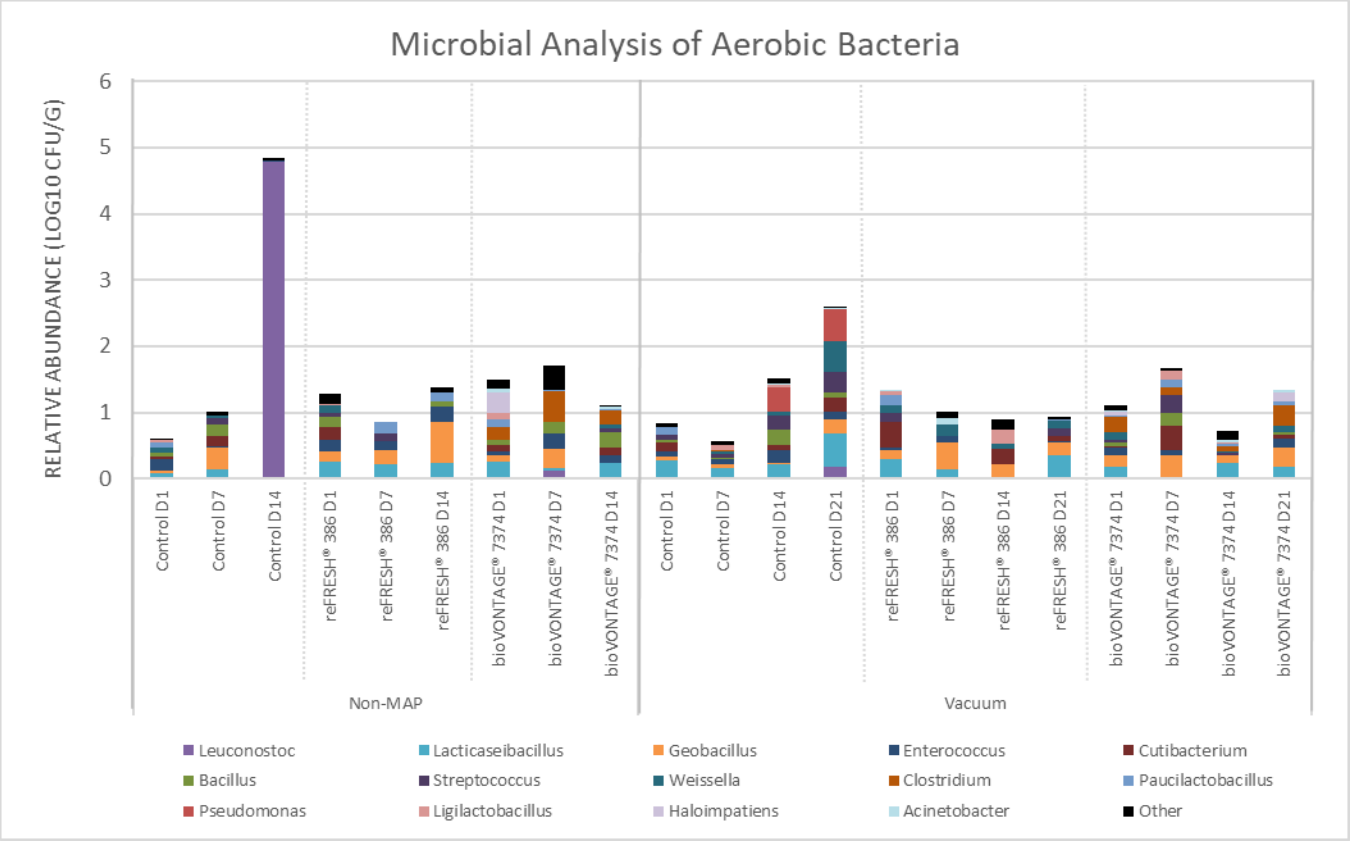
The fungal microbiome had fewer significant trends between packaging and treatment types, likely due to the relatively low fungal counts throughout the study and samples with visual mold not having been processed (as in the case of the would-be day 21 non-MAP samples). *Cladosporium* was the most represented moiety present in the vacuum-packed samples, comprising much of the fungal microbiome of all samples by day 21 (data not shown).

CONCLUSION

The treatment and packaging method affected the microbial composition and community of the raw, plant-based patties tested in this study. Natural preservatives bioVONTAGE 7374 at 1% (BV7374) and reFRESH at 0.5% (RF386) exhibited significantly less microbial growth compared to the untreated Control samples in non-MAP samples and vacuum-packed samples. Though visual mold growth was observed in all non-MAP samples on day 21, in vacuum-packed samples visual mold was only observed in untreated Control samples on day 21, suggesting that BV7374 and RF386 could have some ability to inhibit this type of spoilage when combined with modified packaging solutions. Considering these products are not Ready-to-Eat and are intended to be cooked to an internal temperature of 74°C prior to consumption, either natural preservative treatment can extend the refrigerated shelf life of these plant-based patties, particularly when combined with modified packaging.

The investigation of the identity of the microbial communities revealed several interesting trends. Two very different bacterial dominated the populations in Control samples: gram-positive *Leuconostoc* in non-MAP samples and gram-negative *Pseudomonas* in vacuum packed samples. Both genera are known spoilage agents of refrigerated meat products, observed in previous work with chicken as well as commercial, refrigerated plant-based meat alternatives. In addition to the lower plate counts observed in BV7374 and RF386 samples, the bacterial microbiomes of all these treated samples remained more diverse, with neither *Leuconostoc* nor *Pseudomonas* comprising even a slight majority at any time point. This suggests that these treatments may aid in promoting a competitive environment that can further delay spoilage. Similar trends linking microbiome diversity to environment balance have been observed in natural soil systems, the human GI tract, and in past spoilage studies. These trends have been observed as well in meat product studies, where the relative abundance of bacterial genera often correlates directly to the relative abundance and speed of the respective metabolic processes responsible for the degradation of the food matrix and the buildup of the potentially harmful metabolites.

For further information on this study and to learn more about reFRESH® and other fermented ingredients, please contact Third Wave Bioactives, LLC.



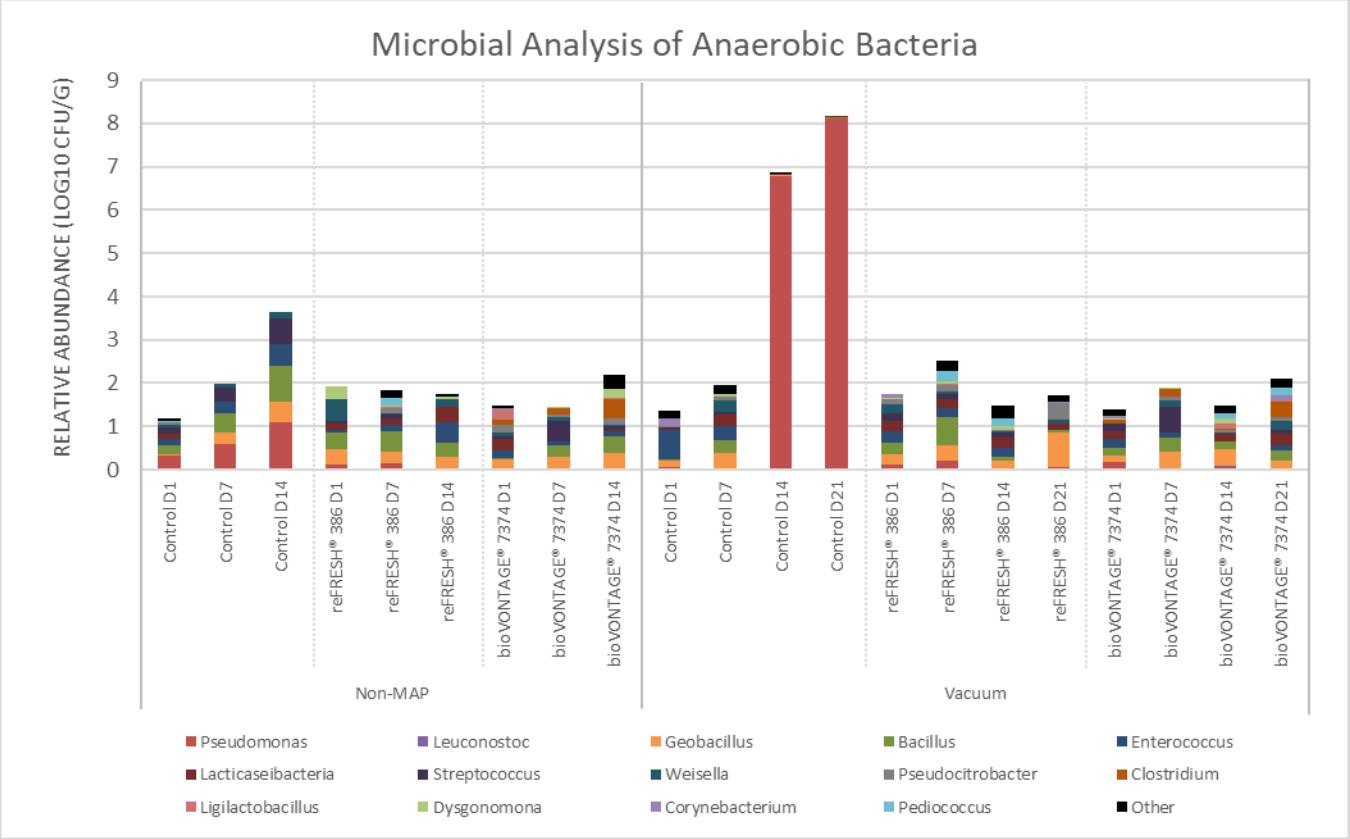


FIGURE 1. MICROBIAL COUNT AND COMPOSITION OF SAMPLES AS A FUNCTION OF PLATE COUNT AND PERCENTAGE OF GENUS DNA TO TOTAL SAMPLE GDNA AT EACH TIME POINT.

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