THIRD WAVE BIOACTIVES®

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MICROBIAL SUCESSION OF ENHANCED RAW CHICKEN MARINATED WITH FERMENTED FOOD INGREDIENTS

INTRODUCTION

Moisture enhanced meat products (raw meat that has been marinated, injected or tumbled with salt, water, flavoring and other ingredients) are a growing part of the food supply in the United States. This process adds flavor, increases water content and helps to produce juicer, more flavorful products, once cooked[1]. Understanding how the ingredients and formulations of these systems impact the overall microbial shelf-life and quality of these foods is an important step in providing high-quality meats. This study aimed to investigate the addition of various natural antimicrobials and their impact on microbial and sensory qualities of raw marinated chicken. Further, the study utilized both traditional, as well as genomic, tools to better understand how the microbial populations evolved over time.

METHODOLOGY

Raw chicken breast fillets were marinated in a solution of water, salt, rice starch, and a commercial blend of vinegar and lemon juice (Control). Treated chicken fillets received the addition of either 1.25% bioVONTAGE® 5117, or 1.25% proVONTAGE™ 463. The chicken breasts were marinated and vacuum-sealed the day after harvesting and received by the lab for analysis four days later. All samples were stored at refrigerated temperatures (4-6°C) for the duration of the study.

Chicken breast fillets from each treatment were analyzed in duplicate for sensory (visual and odor), pH, and microbial analysis on the day of arrival and again every 2-4 days until 21 days post-marinade for Control samples and 28 days post-marinade for the Treated samples. Microbial analysis consisted of total Aerobic Plate Count (APC) to enumerate the total microbial population and Lactic Acid Bacteria (LAB) to identify bacteria that are capable of growing on a more selective medium in an anaerobic environment.

In addition, samples were pelleted via centrifugation, gDNA was extracted, and the 16s rRNA genes were amplified via PCR. PCR products were sent to the Roy J. Carver Biotechnology Center at the University of Illinois Urbana-Champagne for genomic sequencing via Illumina MiSeq. Genomic analysis was conducted using standard processes and the relative abundance of sequences were identified at the genus or species level (when possible). Taxonomic classifications were plotted using bar charts.

RESULTS & DISCUSSION

Visual & Sensory: The marinade within the Control and proVONTAGE treated samples were similar in color and consistency, whereas the marinade of the bioVONTAGE treated samples was tannish in color. These differences remained throughout the study. The Control had a noticeable and offensive odor by day 10, which persisted and was more pronounced through day 21. There was no

abnormal odor from the chicken of either treatment until day 24 for bioVONTAGE and day 28 for proVONTAGE.

pH Analysis: The initial pH of the raw chicken averaged 6.1 in the Control and 5.8 in the bioVONTAGE and proVONTAGE treated chicken. The Control chicken had a dramatic drop in pH by day 10 and ended 0.2 pH units below the starting pH by day 21 (Figure 1). The bioVONTAGE treated chicken stayed within 0.07 units of the starting pH until day 21 and proVONTAGE treated chicken stayed within 0.04 units of the starting pH throughout the 28 days of the study.

Microbial Analysis: The initial APC ranged from $5x10^4$ - $6x10^5$ CFU/g for all treatments (Figure 2). The Control samples reached >$1x10^7$ CFU/g by day 10, whereas the bioVONTAGE treated chicken stayed near the starting level through day 17 before slowly increasing through day 28 to $3x10^6$ CFU/g and the proVONTAGE treated chicken remained near starting levels through day 28 and never trended towards spoilage levels at any time.

The initial LAB levels in all treatments averaged 1-2 logs lower than the APC levels, with a similar trend seen in the Control chicken with LAB levels >$1x10^7$ CFU/g by day 10 (Figure 2). The LAB levels in the bioVONTAGE treatment remained at or below starting levels until day 21 before increasing to $1x10^6$ CFU/g through day 28. The LAB levels in the proVONTAGE treatment remained steady until day 21 when they reached $6.5x10^4$ CFU/g and stayed at, or below, this level through day 28.

Microbiome Analysis: As expected, the microbial succession (Figure 3) showed clear differences in the population of bacteria between the treatments and over the course of the 28d study. Day 4 samples of all three treatments had populations of *Ralstonia*. This was the dominate organism in the Control, whereas both natural treatments also had populations of *Lactobacillus*. The presence of *Ralstonia spp* in these initial samples is interesting, as this has not been associated with raw meat or poultry, previously. However, with the advent of improved sequencing and classification systems, it has been reported in the medical field that likely some *Ralstonia* cultures have historically been misidentified as *Pseudomonas spp*, which are well known to be a common organism found in raw poultry[2,3]. *Ralstonia* was historically classified under the Genus *Pseudomonas* and remains possible that a similar phenomenon has occurred in the published literature for meat and poultry, as well. The microbial succession in the Control samples was very rapid and, by day 7, this initial *Ralstonia* group was surpassed by a much larger outgrowth of *Photobacterium*, which dominated the microbiome of the Control through the end of the study. *Photobacterium* has previously been associated with raw poultry[4] and was partially linked to sensory defects in raw pork[5]. This organism is capable of growing at refrigerated temperatures and is known to be relatively tolerant to salt, which was in the marinade. Although present, *Photobacterium* was not a major component of either the bioVONTAGE or proVONTAGE treatments at any point during the shelf-life. *Ralstonia*, however, was present in both treatments and was the dominate group for proVONTAGE samples. Overall, the bioVONTAGE and proVONTAGE treatments saw a steady shift to include more species of *Lactobacillus* and *Leuconostoc*, which was not unexpected, as both groups have been associated with raw poultry[6]. Although these groups were eventually able to proliferate in the samples, their overall abundance remained low enough to avoid perceivable defects.

CONCLUSION

The use of traditional microbial techniques, like plating and sensory analysis, remains effective in understanding how food formulations and ingredients will impact the shelf-life of various foods. The additional microbiome analysis gives us a deeper understanding of how these formulations and ingredients provide protection. While it's generally accepted that the addition of organic acids and other ingredients can delay the outgrowth of microbes and extend shelf-life, this research further elucidates how certain technologies drive protection and support greater diversity over time. Microbial diversity has been linked to "healthier" environments in soil, the human gastrointestinal tract, and other niches. It stands to reason, then, that the greater diversity seen in the succession of both the proVONTAGE and bioVONTAGE treatments might also contribute to improving the shelf-life of raw chicken, when compared to the Control, which was dominated by one group of bacteria.

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

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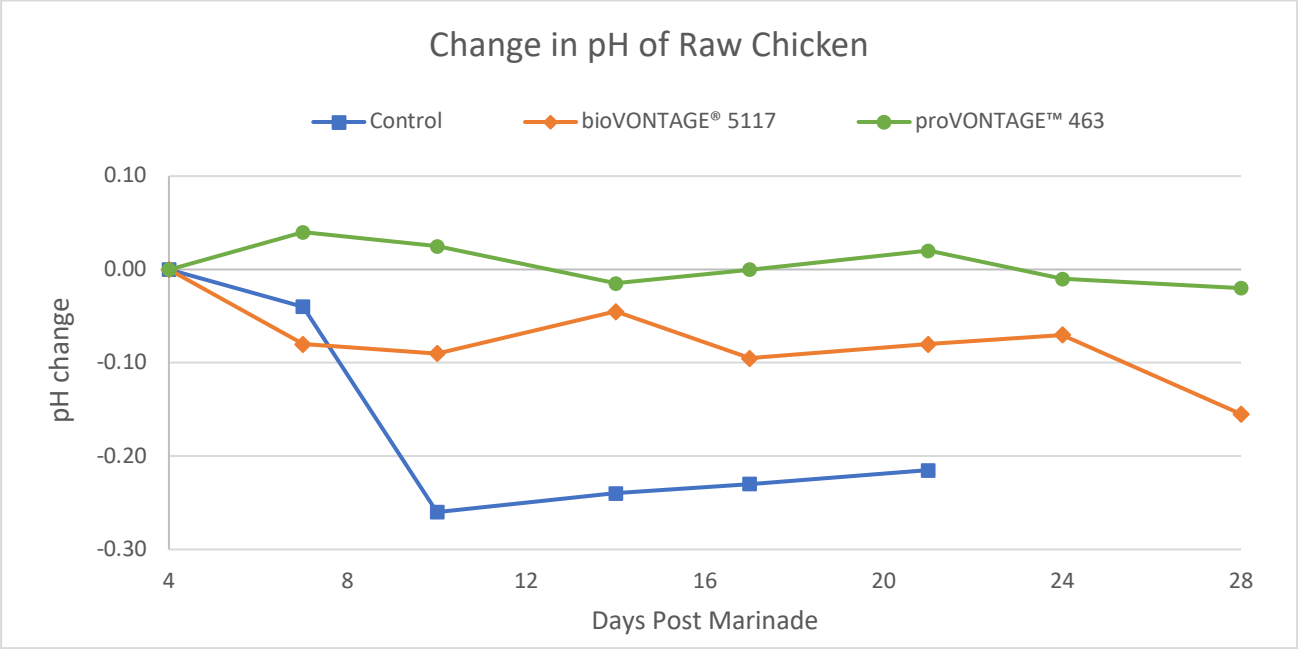

FIGURE 1. AVERAGE CHANGE IN PH OF DUPLICATE CHICKEN SAMPLES FOR EACH TREATMENT OVER TIME.

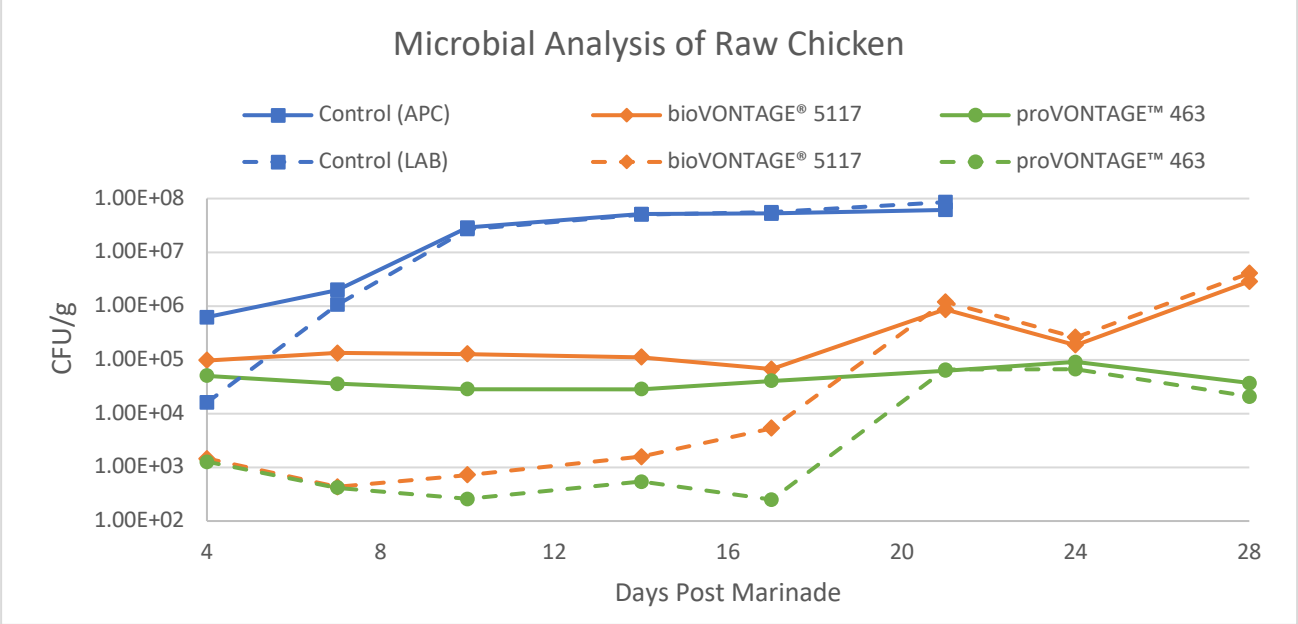

FIGURE 2. AVERAGE AEROBIC PLATE COUNT (SOLID LINES) AND LACTIC ACID BACTERIA (DOTTED LINES) IN DUPLICATE CHICKEN SAMPLES FOR EACH TREATMENT OVER TIME.

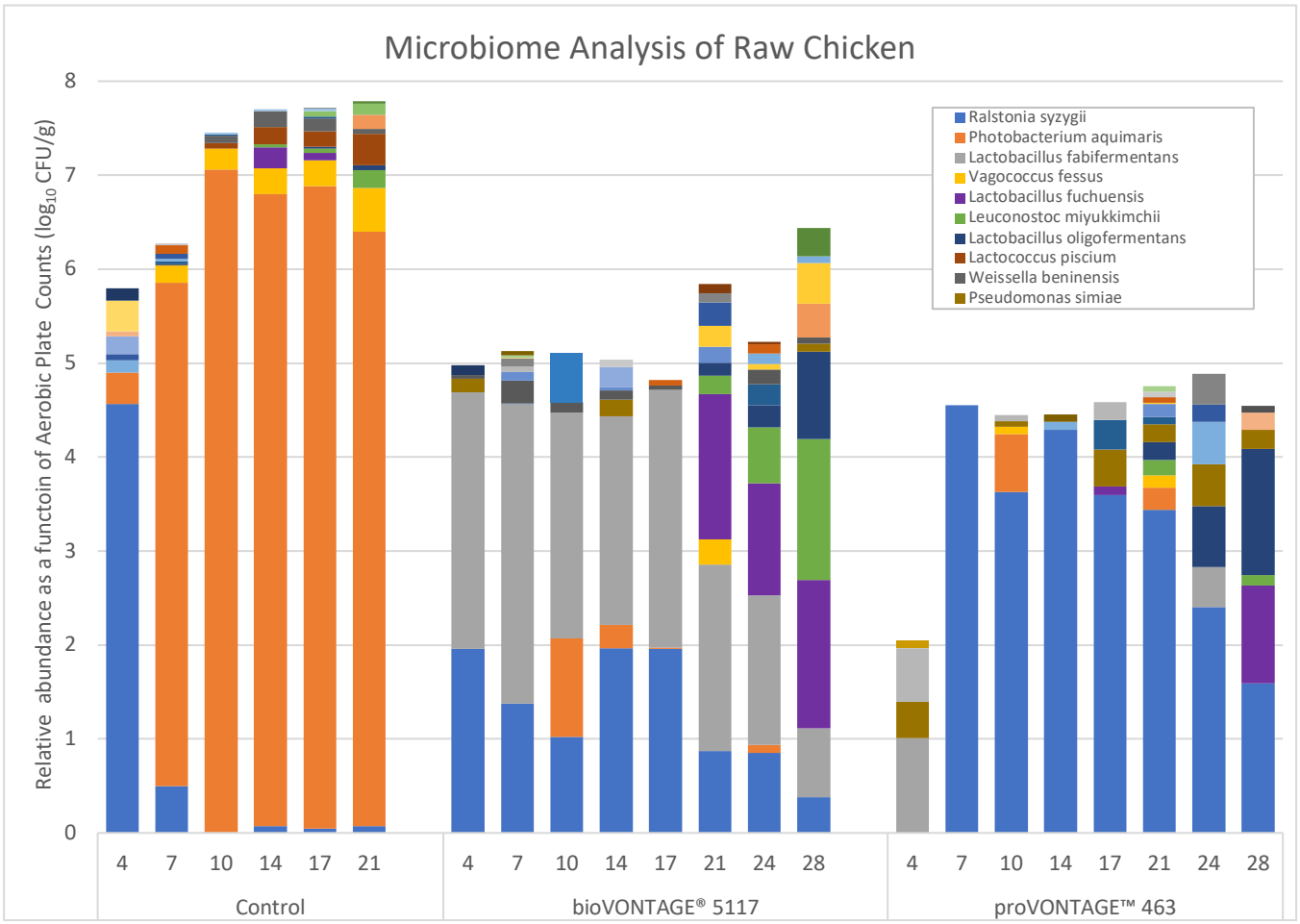

FIGURE 3. MICROBIAL COMPOSITION BASED ON RELATIVE 16S RDNA SIGNAL OF TOTAL POPULATION WITH BAR HEIGHT REPRESENTING APC LEVELS BY TREATMENT OVER TIME

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