THIRD WAVE BIOACTIVES™

White Paper June 2018

MICROBIAL ECOLOGY OF REFRIGERATED CHICKEN SOUP WITH NATURALLY FERMENTED FOOD INGREDIENTS

INTRODUCTION

Earlier this year, Panera bread announced that its retail line of fresh refrigerated soups had surpassed $100 million in sales in 2017, making it the first brand to reach this level in the growing refrigerated soup space[1]. Also, IRI, a Chicago-based market research firm, reported that the whole fresh soup category grew 18% in 2017 as just one of the several retail categories seeing tremendous growth as consumer shoppers reach for fresh and healthy convenience foods[2]. While some of the refrigerated soups today use high-pressure pasteurization or aseptic packaging to maintain quality, this is not the best option for everyone. Hence, some manufactures are turning to naturally fermented food ingredients which can be considered as clean label alternatives to help maintain the quality of their products.

In this study, researchers from Eurofins and Third Wave Bioactives collaborated to compare the microbial succession of refrigerated chicken noodle soup. The soup was produced with various cultured ingredients, marketed as having some benefits in refrigerated soup, with the purpose of better understanding how these ingredients not only prevent microbial spoilage populations, but also dive deeper into how the microbial populations evolved over time.

METHODS

Chicken noodle soup was cooked from scratch in a commercial kitchen and treatments were added prior to the cooking process. The treatments included a no preservative control, Cultured Dextrose #1 (0.3%) (bioVONTAGE® from Third Wave), Cultured Dextrose #2 (0.3%), and Nisin Preparation (400 ppm), a standardized blend of the purified natural antimicrobial nisin, and salt[3].

Once the treatments were sufficiently incorporated, and the soups cooked, the soup was chilled, divided into individual sample cups, and shipped overnight on ice to Third Wave Bioactives for microbial analysis. On sampling days, the entire contents of one cup from each treatment was tested in duplicate on De Man, Rogosa and Sharpe agar (MRS) for the recovery of lactic acid bacteria and Tryptic Soy agar (TSA) for enumeration of total bacteria. In addition, samples from the 10^{-1} dilution were pelleted and were stored for gDNA extraction and downstream analysis by Eurofins. After DNA extraction, PCR was conducted to amplify the 16s rRNA genes which were further purified and sequenced on an Illumina MiSeq. Genomic analysis was conducted using standard processes and the relative abundance of sequences were identified at the species (when possible) or genus level of taxonomic classification and was plotted using bar charts.

RESULTS and DISCUSSION

Microbial plate counts on MRS agar indicated that, by day 17, the Control sample had begun to support the outgrowth of lactic acid bacteria (Figure 1) and this increase was continued through day 27, at which time the samples for that treatment were deemed spoiled and removed from further testing. All of the other treated samples had low LAB levels, ~100 CFU/g, on each testing day.

Unlike the microbial findings on MRS, which showed relatively few differences between treatments, there were distinct variances in the total bacteria levels found on TSA (Figure 2). The Nisin and Cultured Dextrose #2 treated samples saw a rapid increase in total bacteria at day 9 and grew to ~100,000,000 CFU/g by day 17, before leveling off at day 27. The Control samples stayed relatively level until day 17 and continued to increase through day 27, following the same trend seen in the MRS data. The Cultured Dextrose #1 treated samples had LAB levels ~100 CFU/g on each testing day.

The genomic profiles for all four treatments were similar to each other on day 1, which would be expected, and showed a predominance of *Photobacterium* (Figure 3). This psychrotrophic organism has been of increasing interest to food scientists as it has been associated with the spoilage of various refrigerated meat products[4,5,6], however, this microorganism would not have been recovered with the microbiological methods used in this study. The *Photobacterium* community was quickly replaced, as early as day 9, by *Pseudomonas spp.* in both the Nisin and Cultured Dextrose #2 samples, and correlates to the increase seen in total bacteria in both of these treatments. By day 13, the Control sample population was replaced with *Leuconostoc spp*. which correlates to the increase seen in LAB and total bacteria as *Leuconostoc* is a facultative anaerobe and can switch to aerobic respiration if oxygen is present.

Interestingly, the genomic profile in the Cultured Dextrose #1 samples show little change in the relative abundance of *Photobacterium spp*. for the duration of the trial and found a relatively low predominance of *Pseudomonas spp.*. In support of this, there was little change in the level of LAB and total bacteria recovered from the Cultured Dextrose #1 samples.

CONCLUSION

Psuedomonas is a common spoilage organism, so it was not surprising to see this microbe appear in the genomic profiling of the soup, and the lack of this microbe in the control sample may be due to the outgrowth of *Leuconostoc,* which may have created enough competitive exclusion to delay or prevent the outgrowth of *Pseudomonas* and other microorganisms. The Nisin and both of the Cultured Dextrose treatments were selected for their ability to control LAB, which could allow an opening for additional microbes to take residence. The low levels of bacteria and the little change in microbial succession of the Cultured Dextrose #1 ingredient may suggest that it contains an additional component capable of limiting the outgrowth of *Pseudomonas spp.* which was prevalent in the Nisin and Cultured Dextrose #2 samples.

Cultured food ingredients such as Nisin, cultured dextrose, fermented whey, and others can be used effectively as part of a broader food quality system to ensure that consumers get safe and suitable foods. The data from this study further supports this conclusion and demonstrates that, more specifically, these products could be used in refrigerated soups to maintain quality during shelf-life. It also shows that selecting the proper ingredient is critical as the selection and use of these products can change the microbial succession within that food system.

The use of genomic ecology can further enhance and support traditional plating techniques and facilitate a better understanding of how these populations are evolving. Overall, understanding how cultured products are influencing microbial populations in foods is an important step in selecting the most suitable protective ingredient for each food application.

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

Special thanks given to the team at Eurofins, Greg Siragusa, Jodi Benson and Bryan Dieckelman, for their support of the Microbial Ecology and Succession work.

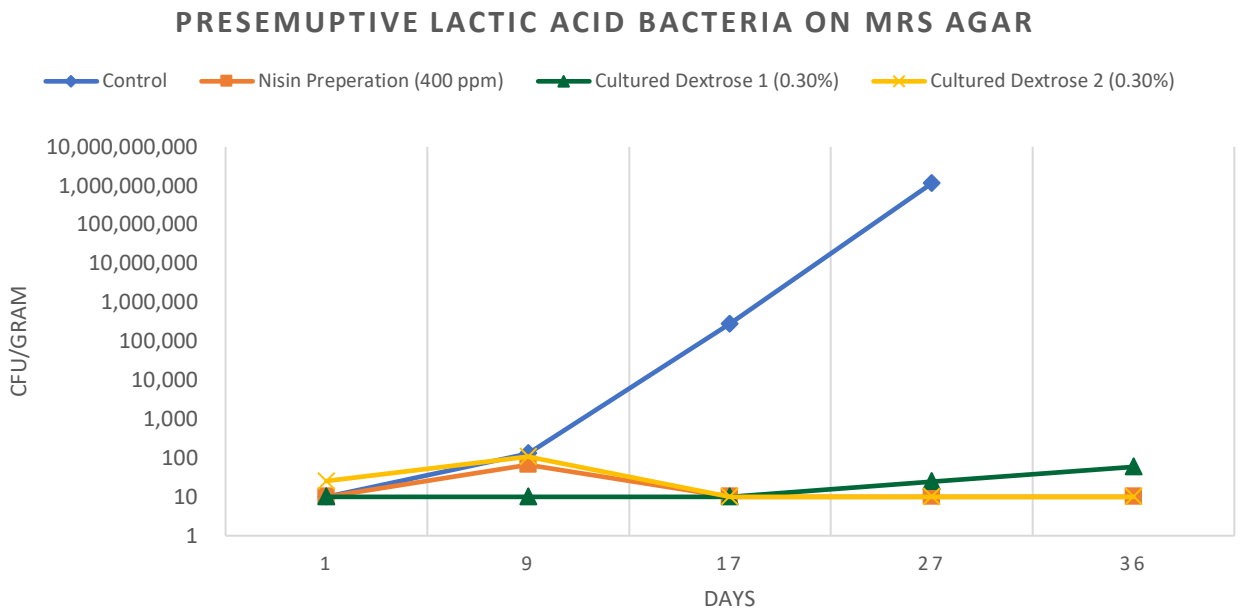

FIGURE 1. PRESUMPTIVE LACTIC ACID BACTERIA PLATE COUNTS ON DE MAN, ROGOSA AND SHARPE AGAR (MRS).

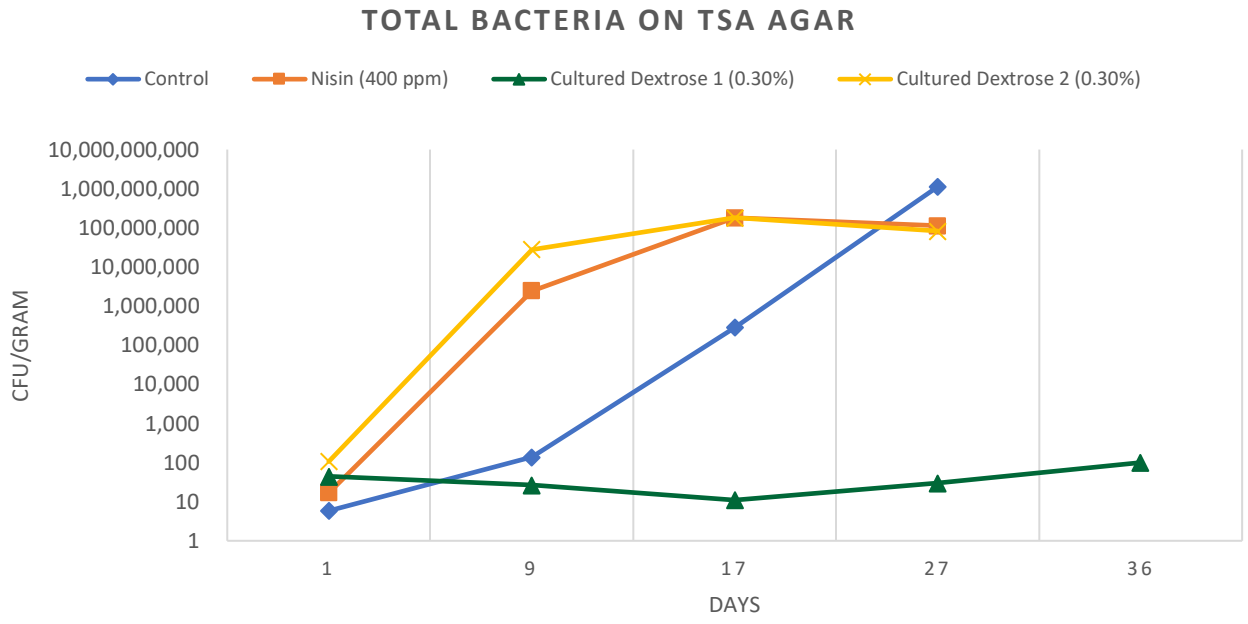

FIGURE 2. TOTAL BACTERIAL PLATE COUNTS ON TRYPTIC SOY AGAR (TSA)

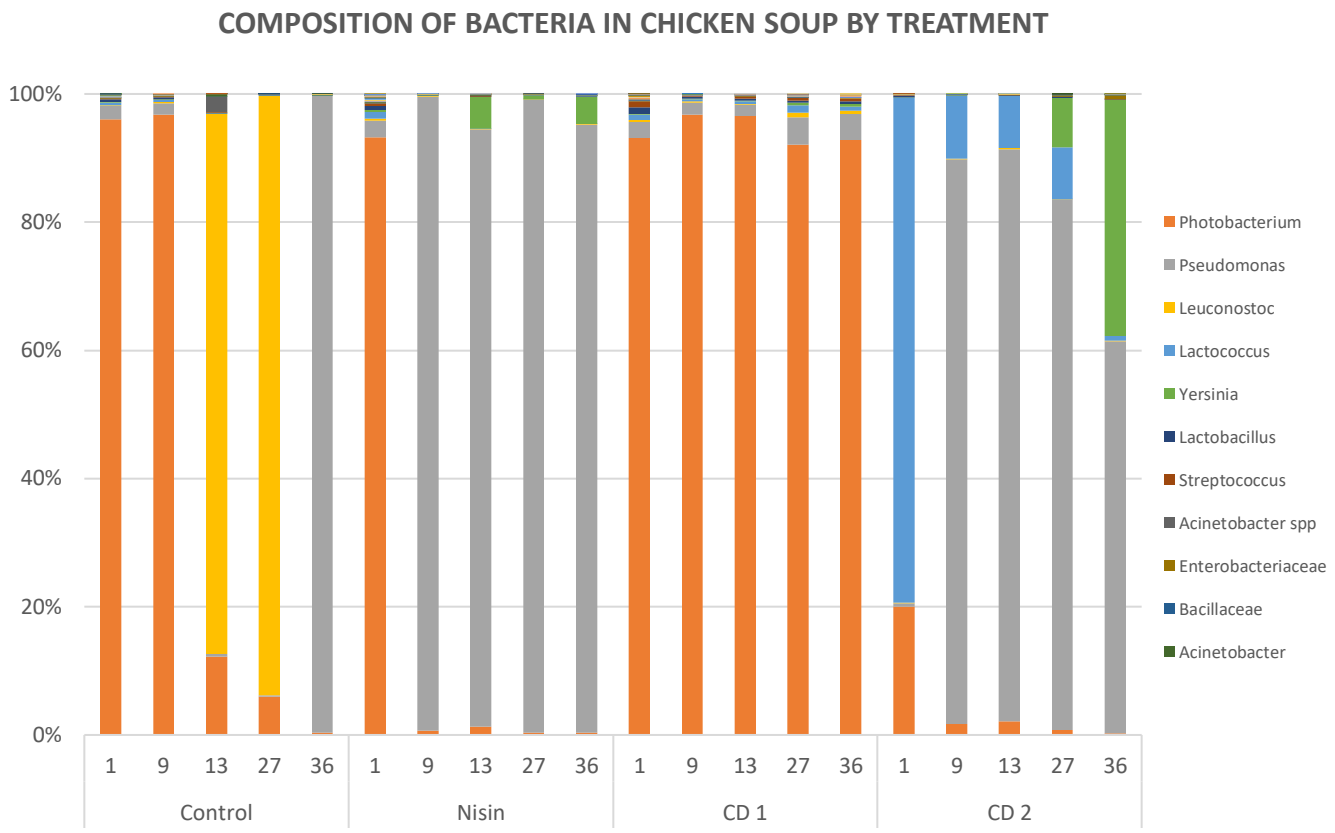

FIGURE 3. MICROBIAL COMPOSITION BASED ON RELATIVE 16S RDNA SIGNAL

1. Panera at Home Hits $100 Million in Annual Retail Sales for its Refrigerated Soups. *Panera Bread* March 22, 2018 https://globenewswire.com/newsrelease/2018/03/22/1444180/0/en/Panera-at-Home-Hits-100-Million-in-Annual-Retail-Sales-for-its-Refrigerated-Soups.html

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