INFLUENCE OF FERMENTATION BASED SOLUTION (NOURISHIELD® 2000L) AND PROTEIN SOURCE ON PLANT PROTEIN MICROBIOME

Rebecca Furbeck[1*], Matthew McCusker[1], Nicolette Hall[1], Eelco Heintz[2], Saurabh Kumar[1]

[1]Kerry, Beloit, Wisconsin, United States

[2]Niacet-A Kerry Company, Tiel, Netherlands

*Corresponding author email: rebecca.furbeck@kerry.com

I. INTRODUCTION

As plant protein products evolve, food processors face challenges similar to those of conventional meat systems, such as shelf life, food safety, cold-chain management and sustainability concerns. Regardless of substrate, microbial spoilage of commercial product contributes to food waste. Processors may need to consider the antimicrobial systems used in meat systems into the plant protein matrices. Thus, the objective of this study was to determine influence of Nourishield® 2000L antimicrobial system efficacy in controlling microbial shelf life in plant protein.

II. MATERIALS AND METHODS

Plant protein matrices, representative of those used in commercially available chicken analogue nuggets were produced at an industrial facility. Two batches of each treatment (a no-antimicrobial pea, no-antimicrobial soya, and soya formulation with 2.25% Nourishield 2000L) were made in duplicate on separate days and shipped refrigerated to the laboratory for analysis. At each plating time point, sample was diluted, stomached, and plated onto tryptic soy agar for aerobic plate counts. Plates were incubated at 37°C for 48 hours. Samples were considered spoiled at 6 log CFU/g.

Additionally, samples were collected and frozen at each sampling point for 16S rDNA bacterial community profiling. A “start” sample was taken the first day of sampling, “end” point when samples spoiled, and “post” one week after spoilage threshold was reached. DNA was extracted, library prepped as described in Kozich *et al.* [1], sequenced with the v2 500 Illumina prep kit (Illumina, San Francisco, CA, USA). Reads were paired, trimmed, and filtered in DADA2 [2] to ensure proper region and quality, and assigned taxonomy with SILVA database v138 [3]. Differentially abundant features (Pea vs Soya; Soya vs Durafresh UC Plus Soya) determined with Linear discriminant analysis Effect Size [4]. Significance was established at LDA scores > 4 and $P < 0.05$.

III. RESULTS AND DISCUSSION

Nourishield samples gained 7 days of measurable shelf life compared to control treatment (Table 1). Without antimicrobial, *Pseudomonas* spp. tended to dominate as specific spoilage organism, significantly more abundant than in Nourishield samples (LDA = 5.61, P = 0.0250, Figure 1.

Table 1. Shelf-Life and Physicochemical Properties of Plant Protein Products

Formula Run	Shelf Life (days)	Starting pH	Water Activity	Moisture %	Salt (% NaCl)
No antimicrobial – Pea Test 1	7	6.01	0.99	73.23	1.84
No antimicrobial – Pea Test 2	7	6.20	0.99	72.33	1.51
No antimicrobial – Soya Test 1	7	6.02	0.98	66.67	1.43
No antimicrobial – Soya Test 2	8	5.93	0.98	64.00	1.57
2.25% Nourishield 2000L – Soya Test 1	14	5.60	0.98	66.90	1.58
2.25% Nourishield 2000L – Soya Test 2	15	5.67	0.98	65.47	1.57

The presence of an antimicrobial treatment shifted composition to *Lactococcus* (LDA = 5.85, P = 0.0039). Pea substrate had more *Lactobacillus* (LDA = 5.40, *p* = 0.0039) present than soy substrate. These taxa are similar to those found in traditional meat systems. The top three genuses in pea were *Pseudomonas*, *Weisella*, and *Lactobacillus*. Soy most abundant are *Lactococcus*, *Pseudomonas*, and *Thermobrachium*, and Nourishield *Lactococcus*, *Thermobrachium*, and *Anoxybacillus*.

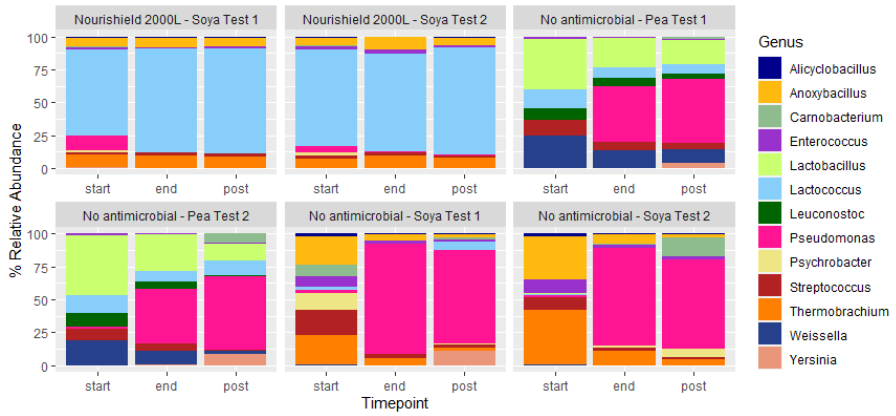

Figure 1. Bar plot of top twenty-five most abundant amplicon sequence variants in plant proteins over shelf-life

IV. CONCLUSION

Both protein matrix and antimicrobial treatment influence the microbial communities of plant protein products. While the initial background of plant-based meat contains genera not associated with meat, the observed specific spoilage organisms of *Pseudomonas* and lactic acid bacteria found in this study, are also common spoilers of traditional meat systems. Results suggest antimicrobial efficacy of Nourishield® 2000L utilized in traditional meats fully translates to plant-based protein matrices for shelf-life extension.

ACKNOWLEDGEMENTS

We would like to thank Steven Ricke, Dana Dittoe, and Jessica Brown from the Food Research Institute at the University of Wisconsin-Madison for their technical support in sequence analysis.

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