RESEARCH IN PROCESS

The effects of storage conditions and the microbiome of non-traditional salad ingredients on the fate of *Listeria monocytogenes*

SUMMARY

Consumer demand for bagged salad has moved beyond shredded iceberg and chopped romaine to more nutritionally dense greens with bold flavors. Many of these new ingredients have not normally been consumed raw or may not have even been widely consumed. This project will investigate the fate of *Listeria monocytogenes* on non-traditional salad ingredients under ideal, abusive and "real-world" storage conditions, and the influence of the produce microbiome on *L. monocytogenes* behavior. Beet greens, kale, Brussels sprouts, and broccoli stalk will be inoculated with *L. monocytogenes* and incubated at 4, 12, 22 and 35°C. *L. monocytogenes* populations and the microbiome will be monitored over the incubation period. To further assess *L. monocytogenes* growth risk, products will be tested under simulated storage and distribution conditions using *L. innocua*. Producers will be able to use this data to develop management strategies to minimize food safety risk.

OBJECTIVES

1. Determine the ability of *L. monocytogenes* to grow, survive or die off in fresh-cut broccoli stalk, Brussels sprouts, kale, and beet greens at recommended and abusive storage temperatures.
2. Determine if the growth characteristics of *L. innocua* on the above products are equivalent to *L. monocytogenes*.
3. Determine the diversity and dynamics of microbial communities (microbiome) present on the selected produce during storage conditions, and determine correlates of community structure with changes in *L. monocytogenes* populations.
4. Determine the growth potential of *L. innocua* on the selected products under simulated storage and distribution conditions.

METHODS

Listeria Challenge Studies

Fresh-cut beet greens, Brussels sprouts, kale, and broccoli stalk will be challenged with *Listeria* (Figure 1). Each product will be inoculated with a cocktail of *L. monocytogenes* or *L. innocua*, placed into polypropylene bags, sealed, and incubated at 4, 12, 22, or 35°C and held for 25 days, 7 days, 48 h, or 12 h, respectively. At each sampling time, two inoculated samples will be enumerated for *Listeria*.

Microbiome Testing and Analysis

Samples for DNA extraction will be collected from *L. monocytogenes* inoculated and uninoculated produce from each temperature condition at the beginning, middle, and end of incubation. After high-throughput 16S rRNA gene sequencing of the V4 variable region, microbiomes will be compared using PRIMER 6 statistics software.

Storage and Distribution Simulation

Inoculated samples will be prepared as described above and tested using an adapted version of the ISTA 3F general simulation performance test procedure (Figure 2).

Produce (Storage Time)	Headspace gases %O_2 and %CO_2							
	4°C				12°C			
	% O_2	%CO_2	% O_2	%CO_2	% O_2	%CO_2	% O_2	%CO_2
Kale	***50 g***		***100 g***		***50 g***		***100 g***	
Day 0	20.5 ± 0.1	0.4 ± 0.03	20.6 ± 0.1	0.4 ± 0.1				
Day 1	18.0 ± 0.2	2.6 ± 0.2	16.8 ± 0.3	3.6 ± 0.1	12.8 ± 0.13	7.2 ± 0.12	10.0 ± 0.2	9.6 ± 0.1
Day 2	16.4 ± 0.4	4.0 ± 0.3	14.1 ± 0.5	6.0 ± 0.3	7.5 ± 0.2	10.7 ± 0.2	2.5 ± 0.4	15.0 ± 0.4
Day 3	15.0 ± 0.3	5.1 ± 0.2	11.6 ± 0.9	7.7 ± 0.6	3.5 ± 0.5	12.5 ± 0.2	0.2 ± 0.04	17.4 ± 0.3
Day 4	13.8 ± 0.1	5.9 ± 0.3	9.5 ± 1.4	9.1 ± 0.8	0.3 ± 0.1	13.4 ± 0.1	0.2 ± 0.1	19.1 ± 0.3
Beet Greens	***100 g***		***150 g***		***100 g***		***150 g***	
Day 0	20.6 ± 0.1	0.4 ± 0.1	20.6 ± 0.1	0.3 ± 0.1				
Day 1	17.8 ± 0.3	2.8 ± 0.2	16.7 ± 0.1	3.4 ± 0.1	11.4 ± 0.1	8.2 ± 0.1	8.8 ± 0.5	10.2 ± 0.4
Day 2	16.6 ± 0.5	4.5 ± 0.3	14.1 ± 0.1	5.5 ± 0.1	5.6 ± 0.3	11.7 ± 0.2	1.7 ± 0.3	14.8 ± 0.6
Day 3	14.2 ± 0.7	5.6 ± 0.5	11.7 ± 0.1	7.3 ± 0.1	2.3 ± 0.7	13.0 ± 0.4	0.1 ± 0.1	15.3 ± 0.4
Day 4	12.8 ± 0.9	6.4 ± 0.4	9.6 ± 0.5	8.4 ± 0.3	0.3 ± 0.2	14.9 ± 0.3	0.1 ± 0.04	16.8 ± 0.5
Brussels Sprouts	***200 g***		***300 g***		***200 g***		***300 g***	
Day 0	20.2 ± 0.1	1.4 ± 0.1	20.1 ± 0.1	1.8 ± 0.05				
Day 1	10.5 ± 0.3	7.5 ± 0.2	7.1 ± 0.3	9.6 ± 0.15	0.1 ± 0.1	17.8 ± 0.7	0.1 ± 0.1	20.3 ± 0.4
Day 2	3.8 ± 0.2	11.5 ± 0.4	0.1 ± 0.1	15.5 ± 0.2	0.2 ± 0.1	21.5 ± 0.9	0.1 ± 0.02	26.4 ± 0.5
Day 3	0.2 ± 0.1	14.7 ± 0.5	0.1 ± 0.1	19.3 ± 0.5	0.1 ± 0.04	23.4 ± 1.2	0.1 ± 0.1	29.9 ± 0.3
Day 4	0.2 ± 0.1	16.6 ± 0.1	0.1 ± 0.04	22.5 ± 0.6	0.2 ± 0.1	24.2 ± 0.9	0.1 ± 0.03	32.4 ± 0.6
Broccoli Stalk	***200 g***		***300 g***		***200 g***		***300 g***	
Day 0	20.5 ± 0.2	2.0 ± 0.1	20.3 ± 0.1	1.7 ± 0.1				
Day 1	11.5 ± 0.4	6.9 ± 0.3	7.9 ± 0.3	8.1 ± 0.2	0.2 ± 0.1	17.4 ± 0.5	0.1 ± 0.1	20.4 ± 1.5
Day 2	5.6 ± 0.6	9.9 ± 0.2	0.2 ± 0.1	13.1 ± 0.2	0.2 ± 0.1	19.9 ± 0.2	0.1 ± 0.02	24.2 ± 1.2
Day 3	0.5 ± 0.01	12.1 ± 0.1	0.2 ± 0.04	15.9 ± 0.2	0.2 ± 0.1	20.1 ± 0.4	0.1 ± 0.04	28.9 ± 0.4
Day 4	0.2 ± 0.1	13.6 ± 0.2	0.1 ± 0.02	19.1 ± 0.4	0.1 ± 0.04	21.9 ± 0.6	0.1 ± 0.1	30.1 ± 0.8

Table 1. Headspace gas analysis for various types of produce and sample sizes when stored at 4 and 12°C in a 7 in X 8.5 in polyethylene bag.

RESULTS TO DATE

Preliminary experiments have been done to confirm that the proposed inoculation procedure yields targeted levels and does not negatively impact the produce. Early results also indicate that *L. innocua* can grow from an initial inoculum load of 2.9 log CFU/g to 5.9 log CFU/g on kale when held at 35°C for 24 h. To try to mimic industry standard headspace conditions in the packaged produce, a study is being done to determine the appropriate sample size for the selected packaging material (Table 1). Once ideal packaging conditions are determined, full challenge studies will be conducted. Preliminary work is underway to confirm that proposed DNA extraction procedures for microbiome testing will provide enough DNA for analysis.

BENEFITS TO THE INDUSTRY

This research will impact the produce industry by providing data that will show if and under what conditions beet greens, Brussels sprouts, kale, and broccoli stalk will support the growth of *L. monocytogenes*. Producers will be able to use this information to determine risk management practices associated with temperature abuse, to validate or revise current storage and distribution practices, and to help inform consumers of proper handling to maintain product safety. The microbiome part of the project will benefit industry by increasing the knowledge of if and how background microflora influence *L. monocytogenes* growth/no growth. While not an immediate impact of this research, it is hypothesized that this information could be used to develop novel intervention strategies in the future.

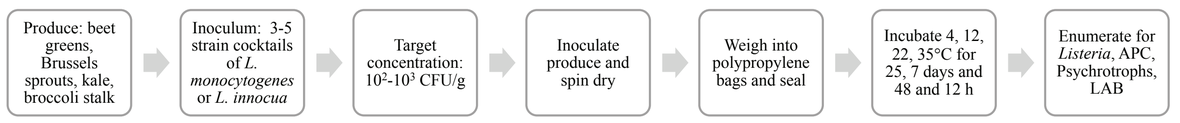

Figure 1. *Listeria* challenge study protocol

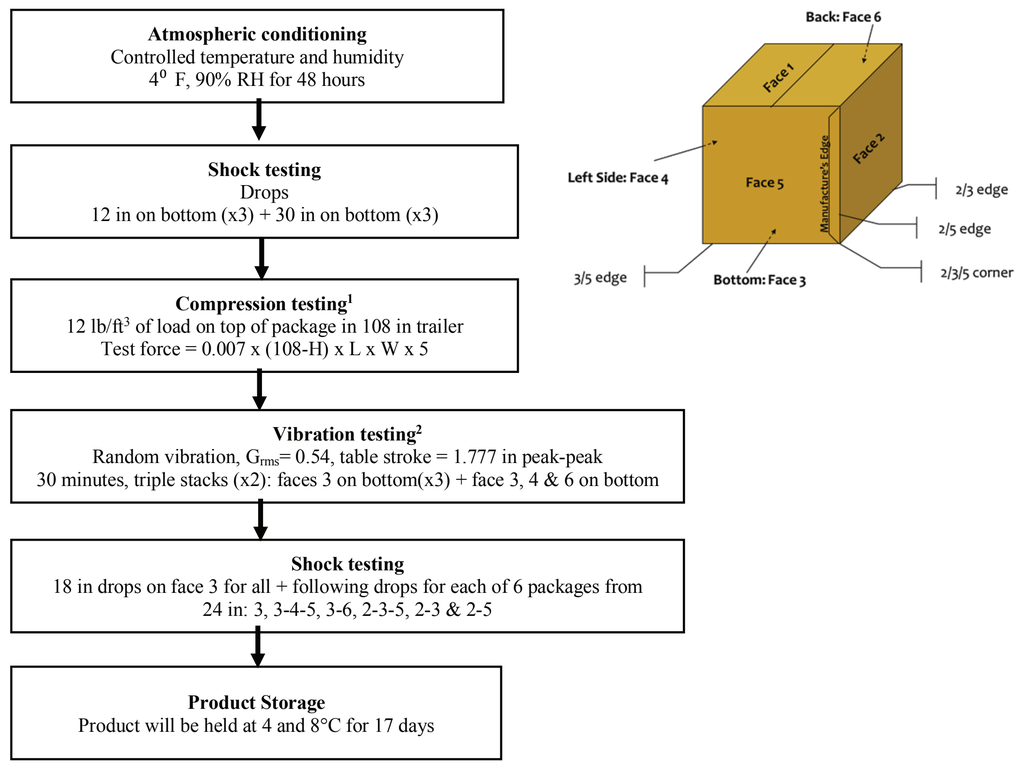

Figure 2. Proposed storage and distribution simulated study conditions with case pectoral for physical abuse testing reference. [1]H = height, L = length & W = width of package, 0.007 lb/in³ = average density of freight, 5 = compensating factor to account for effects not tested, such as stacking pattern, long-duration loading, etc. [2]The root mean square acceleration (G_{rms}) is the square root of the area under the acceleration spectral density curve in the frequency domain.

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