Assessing Romaine lettuce "Forward Processing" for potential impacts on EHEC growth, antimicrobial susceptibility, and infectivity

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Summary

"Forward processing" is a practice of transporting raw commodities to distant facilities for processing and regional marketing. This project aimed to comprehensively assess forward processing for effects on the microbial safety and product quality of Romaine lettuce – the raw commodity and packaged products. Romaine lettuce harvested from the same lots and destined to both forward and source facilities was tagged for temperature, humidity, and barometer recording. Romaine lettuce from these facilities was sampled for microbial and quality assessment. We identified key operational parameters for forward processing, and compared the microbial load, microbiome, and product quality of Romaine lettuce subjected to forward and source processing. The effects of forward processing on EHEC survival, growth, or cultivability are being assessed in laboratory simulations using pathogenic EHEC strains.

Objectives

1. Comprehensive assessment of the forward processing practice under routine operation conditions for product integrity and microbiological quality.
2. Assessment of microbiome dynamics on Romaine lettuce from harvest to retail for products being forward and source processed.
3. Comparative assessment of *E. coli* O157:H7 outbreak strains and laboratory strains, and the impacts of different practices on EHEC outbreak strains, on cell physiology that may affect their growth potential, susceptibility to antimicrobial treatment, or virulence.
4. Improvement of forward processing management by applying findings from comprehensive assessment and simulation.

Methods

- Multi-logger tracking to establish key forward processing operational parameters.
- Microbial enumeration of key indicator microorganisms and product quality assessment for lettuce samples from source and forward facilities with common lot origination.
- High throughput sequencing based microbiome analyses to determine microbial community dynamics during transportation and product storage.
- Laboratory simulation of forward and source processing conditions with pathogen inoculation to determine the effect of forward processing on survival and growth of EHEC Romaine outbreak strains.

Methods (continued)

- Metatranscriptomic analyses to determine potential effects on EHEC gene expression by forward processing conditions and by indigenous microbiota.
- Monitoring development of VBNC and persister cells to determine the effect of forward processing conditions on cell physiology.

Results to Date

- Key operational parameters for source and forward processing transportation, including time, temperature, humidity, and barometer dynamics were established (**Fig. 1**).
- Populations of total bacteria, coliform, yeast/mold on whole head lettuce before processing and on fresh-cut lettuce after storage from forward facilities were higher than that from source facilities. Visual quality of forward processed products seemed to decrease faster, in line with time since harvest (**Fig. 2**).
- The composition and shifting patterns of lettuce microbiota were impacted by production seasons, processing type (forward vs. source), and storage time (**Fig. 3**).
- The biofilm-forming capacity of lettuce EHEC outbreak strains was significantly higher than that of the reference strain (EDL933) and the spinach outbreak strain (FS4157) (**Fig. 4**).
- Preliminary data suggests that the outbreak strain survived better on lettuce than strain EDL933 under the simulated forward processing condition.

Benefits to the Industry

Data derived from this study can provide knowledge on how various conditions during "forward processing" production would affect the physiology of the pathogenic EHEC strains as well as other microorganisms on the raw commodity and packaged products. Findings will provide important information that can be used for improving the forward processing practice and reducing pathogen contamination risks for fresh produce, especially Romaine lettuce.

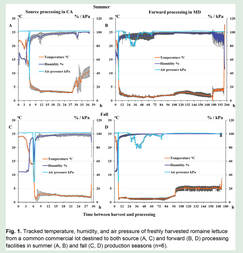

Fig. 1. Tracked temperature, humidity, and air pressure of freshly harvested romaine lettuce from a common commercial lot destined to both source (A, C) and forward (B, D) processing facilities in summer (A, B) and fall (C, D) production seasons (n=6).

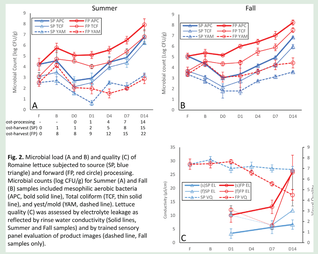

Fig. 2. Microbial load (A and B) and quality (C) of Romaine lettuce subjected to source (SP, blue triangle) and forward (FP, red circle) processing. Microbial counts (log CFU/g) for Summer (A) and Fall (B) samples included mesophilic aerobic bacteria (APC, bold solid line), Total coliform (TCF, thin solid line), and yest/mold (YAM, dashed line). Lettuce quality (C) was assessed by electrolyte leakage as reflected by rinse water conductivity (Solid lines, Summer and Fall samples) and by trained sensory panel evaluation of product images (dashed line, Fall samples only).

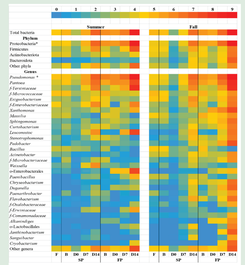

Fig. 3. Microbiota composition on forward (FP) and source (SP) processed lettuce samples as determined by 16 S repliCon sequencing. Samples were collected at harvest from field (F), before processing (B), and post-processing on days 0 (D0), 7 (D7), and 14 (D14) during Summer and Fall production seasons. Major bacterial taxa (> 4 log 16S rRNA amplicons g^{-1} in at least one type of samples) at phylum and genus levels were listed. The bacterial taxa identified at the family and order levels indicate unknown genera belonging to given taxa.

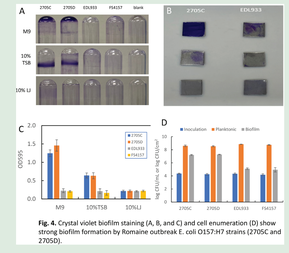

Fig. 4. Crystal violet biofilm staining (A, B, and C) and cell enumeration (D) show strong biofilm formation by Romaine outbreak E. coli O157:H7 strains (270SC and 270SD).