Quantifying risk associated with changes in EHEC physiology during post-harvest pre-processing stages of leafy green production

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Summary

The goal of this project is to determine if the time between harvest and end use of romaine lettuce impacts *E. coli* O157:H7 pathogenicity and detectability, resulting in increased health risk. Laboratory-scale experiments with inoculated lettuce undergoing simulated harvest and cooling will be used to measure changes in *E. coli* O157:H7 stress tolerance and virulence. Input from industry partners, including temperature data from commercial romaine harvesting and cooling, and details on supply chain logistics, will be combined with the laboratory-scale experimental data and used to model risk associated with specific harvest and handling practices. The resulting quantitative tool will be publicly available and allow for growers and producers to determine any practices that should be implemented to reduce the potential for O157 transmission on romaine lettuce.

Objectives

Our overall goal of the project is to characterize physiological changes in the population of *E. coli* O157:H7 cells on leafy greens during pre-processing handling and refrigerated storage that may impede detectability and increase stress resistance and/or virulence. The specific research objectives of the project are:

1. Determine the impact of pre-processing time delays on the physiological state of *E. coli* O157:H7 on romaine lettuce.
2. Evaluate the impact of physiological state on detectability of *E. coli* O157:H7 on romaine lettuce.
3. Quantify changes in *E. coli* O157:H7 risk over pre-processing cut-to-cool and refrigerated transport times.

Methods

Collection and analysis of temperature data

Temperature data used in this study were collected over a period of 3 years and 3 months (January 2016 to April 2019). Temperature probes were used to measure the bin temperature of romaine lettuce immediately after harvest and before and after cooling. A total of 5,615 and 4,623 entries were collected from the Salinas and Yuma regions, respectively, over the period. The data were anonymized and analyzed in R to identify outliers and any differences in the distribution by region.

Systematic review

Systematic review of literature was undertaken in Scopus and PubMed databases following the PRISMA guidelines. The key words comprised *Escherichia coli*, microbial kinetics, romaine lettuce, and cold storage.

Results to Date

Harvest temperatures and cut to cool times. The average temperature of the lettuce at harvest was 56.4 ± 8.1 °F whereas after cooling it was 35.1 ± 4.3 °F. A larger proportion of lettuce harvested in Salinas had significantly higher temperature than that harvested in the Yuma area ($p < 0.001$, Mode's Median test), while at the end of cooling, the produce had comparatively similar temperatures (**Figure 1, Table 1**). The distribution of temperature of romaine lettuce at harvest was a skewed distribution.

The median cut-to-cool time was significantly shorter for romaine lettuce harvested in Salinas than in Yuma ($p < 0.001$, Mode's Median test). The distribution of cut-to-cool times was a skewed distribution (**Figure 2**). Upon receipt at the cooling center, the difference in the median time taken to cool the lettuce in Salinas and Yuma was only 1 minute (**Table 2**).

Systematic review. In the systematic review, 80 articles were selected for extraction of microbial kinetics data, including temperature, duration of transportation and time (**Figure 3**). These data will be used to build the models for the quantitative microbial risk assessment.

Benefits to the Industry

The data analyzed so far will inform the time/temperature parameters used in the laboratory experiments to assess changes in *E. coli* O157:H7 physiology during pre-processing handling. The data collected from the systematic review will be used along with input from industry professionals to develop the quantitative microbial risk assessment models. This quantitative tool will be available to the public to allow for growers and producers to determine any practices that should be implemented to reduce the potential for *E. coli* O157 transmission on romaine lettuce. The study will detail the post-harvest pre-processing handling and timing practices that lower the risk of contamination as adaptable practices within the produce processing industry to reduce the potential for *E. coli* O157 transmission on romaine lettuce.

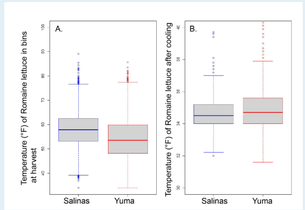

Figure 1. Temperature of romaine lettuce in bins at harvest (A) and after cooling (B). Boxplots represent 5,615 and 4,623 datapoints collected from the Salinas and Yuma growing regions, respectively.

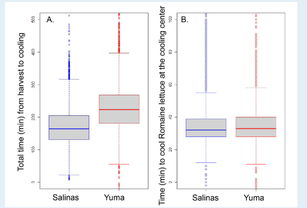

Figure 2. Cut-to-cool time for romaine lettuce in bins (A) and time to cool romaine lettuce at the cooling center (B). Boxplots represent 5,615 and 4,623 datapoints collected from the Salinas and Yuma growing regions, respectively.

Location	Average temp (°F) of bin at harvest		Average temp (°F) at end of cooling	
	Median	Mean ± SD	Median	Mean ± SD
Salinas	57.9[a]	57.8 ± 7.4	34.5[b]	34.9 ± 5.0
Yuma	53.6[b]	54.2 ± 8.2	34.7[a]	35.2 ± 3.0
P-value	<0.001		<0.001	
df	1		1	
χ^2	418.08		70.9	

Within column, values with different superscript letters are statistically different.

Table 1. Average temperature of the bin at harvest and at the end of cooling of romaine lettuce

Location	Time from harvest to end of cooling (min)	Time to cool produce at cooling center (min)
Salinas	164[a]	32[b]
Yuma	223[b]	33[a]
P-value	<0.001	<0.001
df	1	1
χ^2	418.08	26.4

Within column, values with different superscript letters are statistically different.

Table 2. Cooling times for romaine lettuce in the two growing regions

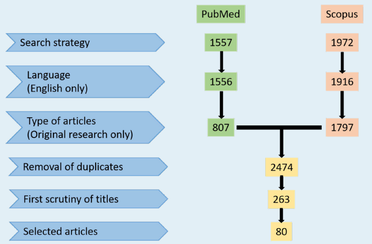

Figure 3. Criteria for selection of articles in the systematic review of literature.