Survival of *Escherichia coli* O157:H7 on Lettuce Harvested from Fields Irrigated by Different Irrigation Systems and Stored under Different Conditions

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Abstract

The objective of this study was to investigate the survival of Escherichia coli *O157:H7 on iceberg and romaine lettuces harvested from fields irrigated using drip, furrow and sprinkler irrigation systems and stored under environmental conditions simulating the growing regions. Lettuce samples were inoculated with* E. coli *O157:H7 and stored under 3 different conditions of temperature and relative humidity (RH) combinations for 10 days. Samples were taken at 6 time points during storage to enumerate the surviving* E. coli *O157:H7. Neither lettuce types nor irrigation systems had a significant effect on* E. coli *survival. The population increased 2-3 logs and 1-2 logs when samples were stored at 26℃, 40% RH and 10℃, 60% RH, respectively. There was no change in* E. coli *O157:H7 population when stored at 4℃, 60% RH. The population on mature lettuce was 0.5 log higher than that on baby lettuce after 5-day storage at 26℃, 40% RH.*

Keywords: *Escherichia coli* O157:H7, iceberg lettuce, irrigation systems, leafy greens, romaine lettuce

1. Introduction

Consumption of leafy greens has been increasing in recent years due to health concerns from consumers. However, the increased consumption of fresh produce that is subjected to human handling and processing may increase the risks of foodborne outbreaks associated with *Escherichia coli* O157:H7 contamination (Cooley et al., 2007; Doyle & Erickson, 2008; Lynch, Tauxe & Hedberg, 2009).

In the past years, several outbreaks of *E. coli* O157:H7 have been associated with lettuce consumption (Centers for Disease Control and Prevention, 2012; Delaquis, Bach & Dinu 2007; Harris et al., 2003). Distribution of leafy greens at 5°C or below can effectively prevent the growth of *E. coli* O157:H7 (McEvoy, Luo, Conway, Zhou, & Feng, 2009). However, temperature abuse may occur during transportation and storage (Khalil & Frank, 2010). The effect of temperature on *E. coli* O157:H7 survival has been studied by some researchers. Abdul-Raouf, Beuchat and Ammar (1993) reported a decline in *E. coli* O157:H7 populations on iceberg lettuce at 5°C, an increase of 2 log CFU/g at 12°C and 3.5 log at 21°C after 14 and 7 days of storage, respectively. Another study (Li, Brackett, Chen, & Beuchat, 2001) showed that *E. coli* O157:H7 population increased by 2 to 3 log CFU/g on iceberg lettuce within 2 days, then continued to increase at a slower rate through 7 days of storage at 15°C. Francis and O'Beirne (2001) observed no change in *E. coli* O157:H7 populations in shredded iceberg lettuce stored at 4 °C, while an increase of 1.0-1.5 log CFU/g was seen during a 12-day storage period at 8°C. Besides the temperature factor, produce intrinsic characteristics (specimen, type and variety) may also affect the survival and growth of *E. coli* O157:H7 in salads. A previous study (Francis & O'Beirne, 2001) reported that populations of *E. coli* O157:H7 in coleslaw salad increased during the initial days of storage at 8°C, and subsequently declined on extended storage, while on packaged Swedes, populations of *E. coli* O157:H7 increased by 1 log during a 12-day storage. The antimicrobial effects of some plant compounds in coleslaw and the competition from the indigenous microbial population were presumed to be the reasons for the inactivation of *E. coli* O157:H7. Cano and Arnao (2005) reported that the phenolic content of romaine lettuce, which has shown antimicrobial activity, was higher than that of iceberg and baby head lettuce.

Previous studies evaluated the effects of different irrigation methods on the transmission of *E. coli* O157:H7 to lettuce plants. Solomon, Potenski and Matthews (2002) reported that the number of green ice lettuce heads contaminated with *E. coli* O157:H7 by spray irrigation was significantly higher than that contaminated by surface irrigation. Another study (Song, Stine, Choi, & Gerba, 2006) showed that greater contamination of produce, including lettuce, occurred in furrow irrigation plots than in drip irrigation plots. Three different irrigation systems (overhead sprinkler, subsurface drip and surface furrow) were used in a recent study conducted under common commercial conditions in the lettuce field (Fonseca, Fallon, Sanchez, & Nolte, 2011). The study confirmed the enhanced risk of non-pathogenic *E. coli* contamination when using sprinkler irrigation. Sprinkler irrigation was also shown to exhibit larger amounts of background microflora, with the possible reason that the direct contact of water with the leaf surface changed the bacterial kinetics, the phyllosphere ecology and related plant physiology (Andrews & Harris, 2000; van der Wal, Tecon, Kreft, Mooij & Leveau 2013). Furrow-irrigated plants showed highest overall visual quality and lowest decay rate, which was associated with lower water potential and background microflora at harvest (Fonseca et al., 2011). It is not known if the irrigation system used in the field will have an impact on the survival of pathogens contaminating during post-harvest handling. To our knowledge no study has addressed this. The objectives of this study were to evaluate the effects of storage temperature (4, 10, 26°C), storage time (0, 1, 2, 5, 7, 10 days), the type of lettuce (iceberg and romaine), lettuce age (young and mature leaves), and irrigation systems (drip, furrow and sprinkler) on the survival of *E. coli* O157:H7 inoculated on lettuce post-harvest.

2. Materials and Methods

2.1. Bacterial Culture and Media

Three strains of *E.coli* O157:H7 (F4546, 960218 and SEA 13B88) were used in this study. The strains F4546 and 960218 are clinical isolates associated with alfalfa sprout outbreak, and the strain SEA 13B88 is an isolate from unpasteurized apple juice. Stock cultures of the microorganisms were maintained in cryovials (Microbank^{TM}, Austin, TX, USA) at –80 °C and activated by transferring 100 µL into tryptic soy broth with 0.6% yeast extract (TSBYE; Difco, Sparks, MD, USA). The bacterial cultures were maintained in TSBYE at 4 °C with biweekly transfers. For experimental use, an overnight culture of each strain was grown in TSBYE at 37 °C for 18-24 h. A cocktail culture was made by mixing equal volumes of each overnight culture. All dilutions were made in 0.1% peptone water (Difco, Sparks, MD, USA). Enumerations for *E. coli* O157:H7 were done by plating on sorbitol MacConkey agar (SMAC; Difco, Sparks, MD, USA).

2.2. Lettuce Samples

Lettuce was obtained from the research plots of Yuma Agricultural Center in Yuma, Arizona, USA. Lettuce plants were harvested from fields irrigated using 3 different irrigation systems: overhead sprinkler, subsurface drip and surface furrow, and shipped to Tucson, Arizona, USA. Two types of lettuce were used in this study: romaine and iceberg lettuce. For each type of lettuce, young and mature plants were tested.

2.3. Survival of *E. coli* O157:H7 on Lettuce Samples Stored Under Three Different Conditions

Leaves of romaine or iceberg lettuce were rinsed thoroughly in de-ionized water to remove the surface soil. The bottom portion of each leaf was trimmed off and the top portion was used to get a 10 g sample. Leaf samples were placed under UV light in a biohood to reduce the background microflora. Each side of the leaf was exposed to UV light for 30 min. A cocktail overnight culture of 3 strains of *E.coli* O157:H7 (F4546, 960218 and SEA 13B88) was diluted with buffered peptone water (BPW; Difco, Sparks, MD, USA) to obtain ca. 10^8 CFU/mL, from which 1 mL was inoculated onto the leaf's abaxial (lower) surface, and spread evenly using a sterile pipette. Each inoculated leaf was placed into a WhirlPak bag (Nasco, Fort Atkinson, WI, USA). The bags with leaves were stored in incubators set at varied combinations of temperature and relative humidity conditions (26°C, 40% RH; 10°C, 60% RH and 4°C, 60% RH) for up to 10 days. Samples were taken at 0, 1, 2, 5, 7 and 10 days. During each sampling, 90 ml of BPW was added into each bag. The samples were mixed using a stomacher (Lab-blender 400; Seward, London, UK) at normal speed for 1 min. Serial dilutions were made and aliquots plated on SMAC agar. Enumerations were done after 18-24 h incubation at 37°C.

2.4. Experimental Design and Statistical Analysis

The experiment was a complete 3×6×4×3 factorial design of temperature (4, 10, or 26°C), storage time (0, 1, 2, 5, 7 or 10 days), lettuce type and age (young romaine, mature romaine, young iceberg, or mature iceberg) and irrigation system (sprinkler, drip or furrow). Each experiment was repeated 3 times. The resulting Log CFU/g values (LCFU) were analyzed to determine the effect of treatment using SAS software (SAS Institute Inc., Cary, NC, USA). Analysis of covariance for the resulting Log CFU/g values was used to perform a homogeneity of regressions analysis to test for the effect of the treatments.

3. Results and Discussion

The survival of *E. coli* O157:H7 under different conditions is shown in Figures 1-4. When the leaves were stored at 26°C and 40% RH, *E. coli* O157:H7 population increased to ca. 9 Log CFU/g at day 2, and continued to grow slowly in the following days (days 5, 7 and 10). By day 10, *E. coli* O157:H7 population increased by 2-3 logs on mature lettuce, and about 2 logs on young lettuce. Similar results were observed in previous studies. When *E. coli* O157:H7 was inoculated on iceberg lettuce and held at 25°C for 5 days, there was a 2 log population increase at day 2 and 2.3 log increase at day 5 (Doering, Harrison, Morrow, Hurst & Kerr, 2009). Theofel and Harris (2009) found that on romaine lettuce, *E. coli* O157:H7 population increased by ca. 1.5 log CFU/g, 24 h after the inoculation when stored at 20°C. The increase in *E. coli* population found by Theofel and Harris (2009) was slightly lower than that in our present study and this could be because of the higher storage temperature (26°C) used in our study. In the present study, at 10°C and 60% RH storage condition, *E. coli* O157:H7 population reached ca. 8 log CFU/g at day 2 and slowly increased thereafter. At Day 10, there was 1.5-2 log increase in *E. coli* O157:H7 population on both young and mature lettuce. A previous study (Li et al., 2001) showed that *E. coli* O157:H7 population increased by 2.3 to 3.2 log CFU/g within 2 days, then continued to increase at a slower rate through the 7 days of storage at 15°C. In another study (Luo, He and Mcevoy, 2010) on *E. coli* O157:H7 inoculated romaine and iceberg lettuce, the authors found that there was more than 2.0 log CFU/g increase in *E. coli* O157:H7 populations on lettuce when held at 12°C for 3 days, followed by additional growth during the remainder of the storage period. Compared to our results, higher increases in *E. coli* O157:H7 population were observed in their studies (Li et al., 2001; Luo et al., 2010), since higher storage temperatures were used in these studies.

The present study showed that there was no change in *E. coli* O157:H7 population on any of the types of lettuce (romaine, iceberg, young or mature) when stored at 4°C, 60% RH. Similar results were observed in other studies. Luo et al. (2010) found that storage at 5°C allowed *E. coli* O157:H7 to survive on lettuce, but limited its growth. Doering et al. (2009) found that *E. coli* O157:H7 population on lettuce slightly decreased (<0.5 log) on day 10 when stored at 4°C. Our results showed similar survival patterns on both romaine and iceberg lettuce.

When samples were stored at 26°C, *E. coli* O157:H7 showed ca. 0.5 log CFU/g higher population on mature lettuce than on young lettuce. This result was consistent with a previous study on romaine lettuce (Brandl & Amundson, 2008), in which *E. coli* O157:H7 inoculated young and mature romaine lettuce were stored at 28°C for 3 days, and the pathogen population was ca. 0.5 log higher on the mature lettuce than on the young lettuce. This may be due to the different nutrient contents in the young and mature plants.

There were no major differences between the surviving *E. coli* O157:H7 populations among lettuce grown using various irrigation systems. The background microflora population present in these lettuce samples were investigated by Fonseca et al. (2011) who showed that the lettuce grown using different irrigation systems had varied background microflora. The levels of background microflora in lettuce after harvest ranged between 7-8 logs, 6-7 logs and 4-7 logs at days 8, 16 and 24, respectively, after storage at refrigeration temperature (Fonseca et al., 2011). Thus, our results showed that the differences in background microflora may not affect the survival and/or growth of *E. coli* O157:H7.

Statistical analysis of our data showed that neither the lettuce type nor the irrigation system type had a significant ($p>0.05$) effect on the survival of *E. coli* O157:H7 on lettuce during post-harvest storage, while temperature/RH ($p<0.05$) and storage time played an important role on the survival and/or growth of *E. coli* O157:H7 on lettuce. Many microbial growth predictive models showed temperature as one of the most essential environmental factors influencing the survival of foodborne pathogens on fresh produce during processing, storage, and distribution. Ding et al. (2012) investigated the combined effect of temperature and RH on the growth of *E. coli* O157:H7 on cabbage, and used the Baranyi model to predict the growth risk of the pathogen. In their study, the temperature and RH had a great influence on the growth of *E. coli* O157:H7. The pathogen showed better growth at higher temperatures and RHs, while some inactivation was observed at 15 and 25°C during 4-day storage. In another study, using Baranyi primary growth model, the growth of *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* on iceberg lettuce under constant and fluctuating temperatures was examined to estimate the microbial safety of the produce during distribution from the farm to the table (Koseki & Isobe, 2005). Their results showed that the growth rate of *E. coli* O157:H7 at 25°C was 14 times higher than that at 10°C. Wang and Oh (2012) used the response surface methodology (RSM) to model the growth of *E. coli* O157:H7 on spinach as a function of temperature and relative humidity. Their model demonstrated that both temperature and RH presented positive effects on growth rate of *E. coli* O157:H7 on spinach; however, the effects of temperature were more significant than that of RH on the growth rate. In the present study, different temperature and RH levels were chosen to simulate the field conditions at certain times during the growing season in the southwestern US (California and Arizona) and the high temperature with low RH (26°C 40% RH) can also be used to mimic the condition when lettuce could be exposed to temperature abuse during transportation and storage.

4. Conclusions

Our result showed that if *E. coli* O157:H7 contamination occurs in different steps during post-harvest, the pathogen can survive under refrigerated storage conditions on lettuce. If temperature abuse occurs during transportation and/or storage, the pathogen can grow to dangerous levels on lettuce. This study also showed that, irrespective of the irrigation system used in the field, *E. coli* O157:H7 can survive/grow to similar levels in lettuce, when the contamination occurs post-harvest. The survival was similar on romaine and iceberg lettuce and the population difference was minimal between baby and mature lettuce. The data obtained from this research can be used for science based risk assessment of *E. coli* O157:H7 in lettuce production.

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Figure 1: Survival of *E. coli* O157:H7 (Log CFU/g) on Mature Romaine Lettuce (Grown under Three Irrigation Systems) at Varying Combinations of Temperature and Relative Humidity Storage Conditions

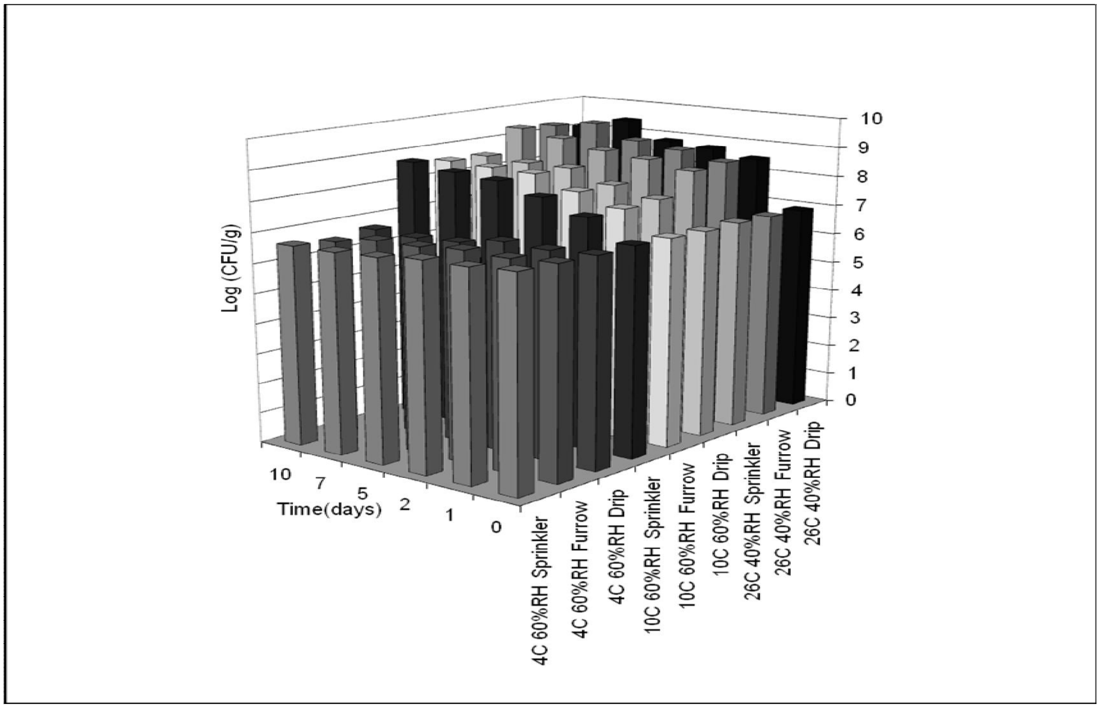

Figure 2: Survival of *E. coli* O157:H7 (Log CFU/g) on Mature Iceberg Lettuce (Grown under Three Irrigation Systems) at Varying Combinations of Temperature and Relative Humidity Storage Conditions

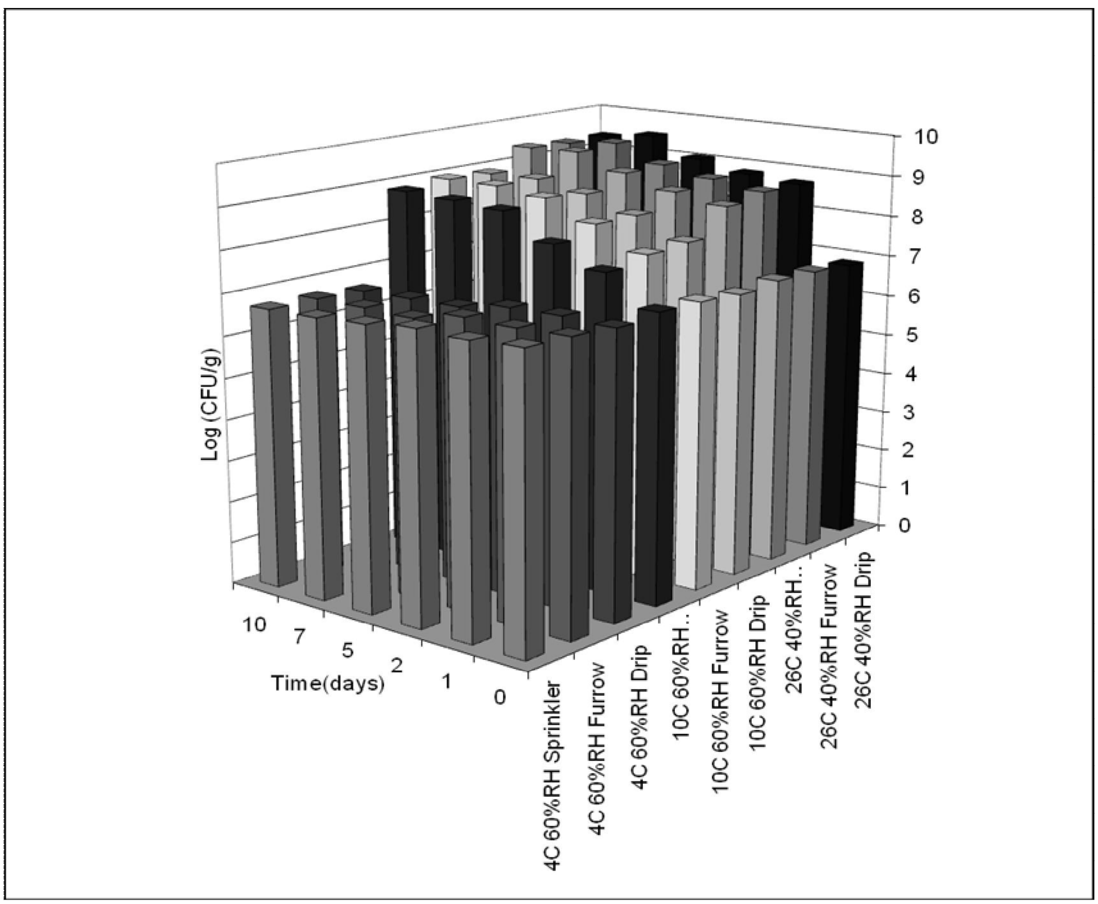

Figure 3: Survival of *E. coli* O157:H7 (Log CFU/g) on Young Romaine Lettuce (Grown under Three Irrigation Systems) at Varying Combinations of Temperature and Relative Humidity Storage Conditions

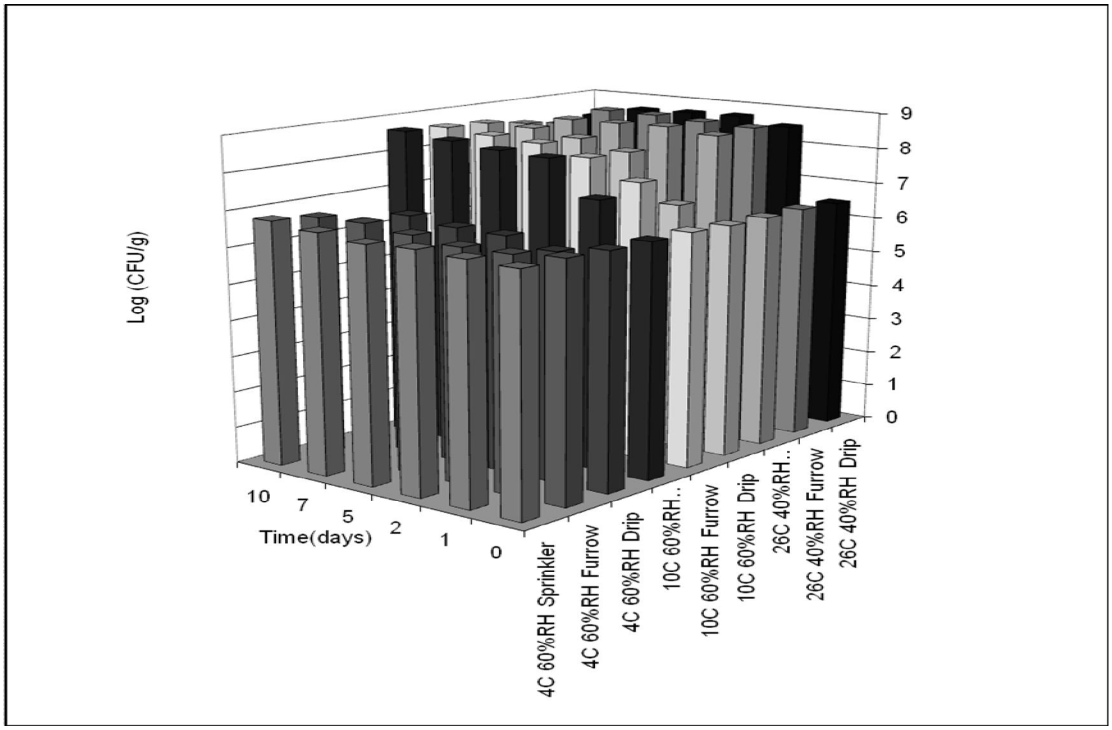

Figure 4: Survival of *E. coli* O157:H7 (Log CFU/g) on Young Iceberg Lettuce (Grown under Three Irrigation Systems) at Varying Combinations of Temperature and Relative Humidity Storage Conditions