Fate of *Escherichia coli* O157:H7 in the Presence of Indigenous Microorganisms on Commercially Packaged Baby Spinach, as Impacted by Storage Temperature and Time†

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ABSTRACT

This study investigated the effect of storage temperature and time on the survival and growth of *Escherichia coli* O157:H7, the growth of indigenous microorganisms, and the changes in product quality of packaged baby spinach. Commercial packages of spinach within 2 days of processing were cut open at one end, sprayed with fine mists of *E. coli* O157:H7 inoculum, resealed, and then stored at 1, 5, 8, and 12°C for 12 days until their labeled best-if-used-by dates. Microbial enumeration and product quality evaluation were conducted on day(s) 0, 3, 6, 9, and 12 postinoculation. Spinach held at 12°C supported significant (*P* < 0.001) *E. coli* O157:H7 growth, with a 1.0-log CFU/g increase within 3 days postinoculation, which was followed by additional growth during continued storage. *E. coli* O157:H7 grew slowly when held at 8°C, with a significant (*P* < 0.01) level of growth reached after 6 days of storage. However, on products held at 1 and 5°C, *E. coli* O157:H7 populations declined significantly (*P* < 0.01 and *P* < 0.001, respectively) within 3 days of storage. Aerobic mesophilic bacteria, psychrotrophic bacteria, and yeast and mold populations increased significantly at all storage temperatures, with more growth on products held at elevated temperatures. Product quality scores remained high within the first 6 days of storage, with a sharp decline noted on samples held at 12°C on day 9. Results suggest that *E. coli* O157:H7 can grow significantly on commercially packaged spinach held at 8°C or above before significant product quality deterioration occurs.

The fresh-cut industry has been growing rapidly, fueled by strong consumer demand for convenient, fresh and nutritious food. In North America, the annual sales of fresh-cut produce have reached approximately $15 billion and account for nearly 15% of all produce sales (43). The increased consumption of fresh-cut products is accompanied by an increased number of associated foodborne illnesses due to increased consumption of fresh-cut produce (43). Between 1996 and 2006, there were more than 20 foodborne illness outbreaks implicating fresh and fresh-cut leafy green vegetables, notably lettuce and spinach (6, 17, 31). The 2006 *Escherichia coli* O157:H7 outbreak associated with the consumption of prepackaged baby spinach in the United States had significant economic impact (9) on the produce industry, and resulted in 205 confirmed infections, 103 hospitalizations, 31 cases of hemolytic uremic syndrome, and three deaths (8). In 2003, another spinach-related outbreak sickened 16 and killed 2 in a retirement facility (7). Although the incidence of foodborne illness outbreaks is still relatively low in relation to the quantity consumed, fresh and fresh-cut produce have emerged as significant vehicles for enteric pathogens (6, 40). There is an urgent need for the development of effective measures that can significantly reduce the public health risk due to production (6, 12).

Produce grows in the natural environment and is exposed to many potential sources of human pathogen contamination en route from farm to table (10, 20). Although fresh-cut products are marketed as “prewashed” and “ready-to-eat,” the only measure that is widely relied on for pathogen reduction is a chlorinated water wash (4, 28, 38). However, extensive research has shown that this wash step achieves no more than a 1- to 2-log CFU/g reduction on microbial loads (5, 16, 37, 38). Therefore, in the absence of effective pathogen-killing steps during the preparation of packaged fresh-cut products, preventing pathogen contamination and reducing the potential for pathogen proliferation on produce during distribution are critical steps to ensure fresh-cut produce safety.

Temperature control is the single most important factor for maintaining quality and shelf life of fresh-cut fruits and vegetables (18, 22). Many studies have shown that reducing the storage temperature can significantly reduce the growth of spoilage microorganisms and physiological deterioration of plant tissues (19, 41). Therefore, fresh-cut produce processors often recommend that fresh-cut products be stored at 1 to 3°C to maintain food quality. However, in reality, temperature abuse occurs frequently during fresh-cut product distribution and retail display. In the United States, 20% of domestic and commercial refrigerators were found to operate at >10°C (21), and in Greece, large variability

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was found in cold chains, with almost 10% of the distribution centers having an average temperature >10°C (42).

The general effect of temperature on microbial growth on food products is well documented, but research into the effect of storage temperature on pathogen survival and growth on packaged fresh-cut products is scarce (12, 13). Limited studies have shown that populations of viable E. coli O157:H7 declined on shredded lettuce stored at 5°C, and increased at 12 and 21°C (1). Francis and O’Beirne (14) reported more than a 1-log CFU/g increase of E. coli O157:H7 when packaged lettuce was held at 8°C for 12 days. Li et al. (24) reported that E. coli O157:H7 populations declined on samples stored at 5°C for 18 days, but increased significantly at 15°C within 2 days. However, those studies were conducted with produce prepared under laboratory conditions, which differed markedly from those employed during commercial processing.

Fresh-cut products are known to harbor diverse indigenous microorganisms, which may coexist with human pathogens, if present. These indigenous microorganisms may be well adapted to the produce packaging environment and therefore, the survival and growth of E. coli O157:H7 may be affected by strong competition with the fast-growing epiphytic bacteria (11). However, the effect of such interactions on the fate of E. coli O157:H7 on commercially packaged fresh-cut leafy vegetables is unclear. Since optimal growth temperatures vary significantly among different microorganisms, temperature changes can have profoundly diverse effects on their survival and growth. Furthermore, fresh-cut produce is highly perishable, and product quality deterioration occurs rapidly at elevated temperatures. Therefore, it is questionable whether E. coli O157:H7 would survive and grow on fresh-cut produce in the presence of large populations of native microflora, and whether product quality deterioration would precede significant growth of E. coli O157:H7 at elevated storage temperature or vice versa. A series of studies were undertaken in our laboratory to examine the effect of temperature-abuse patterns and duration on the fate of E. coli O157:H7 on fresh-cut leafy green vegetables. The specific objectives of the experiments presented in this article were to investigate the effect of storage temperature and time on the survival and growth of E. coli O157:H7 in the presence of indigenous microflora in commercially packaged baby spinach, and to correlate microbial growth with changes in product quality.

**MATERIALS AND METHODS**

**Bacterial strains and growth conditions.** Nalidixic acid-resistant derivatives of E. coli O157:H7, strains F6460 and RM4407, were used in this study. Strain F6460, originally isolated from patients during a 1999 lettuce outbreak linked to a Nebraska restaurant, was obtained from Timothy Barrett at the Centers for Disease Control (Atlanta, GA) and Thomas Safranek at Nebraska Health and Human Services System (Lincoln, NE) (32, 45). Strain RM4407 was a clinical isolate associated with a 2003 spinach outbreak, and was provided by Drs. Patricia Millner and Robert Mandrell at the U.S. Department of Agriculture. The stock culture was maintained at −80°C in Luria-Bertani broth (Difco, Becton Dickinson, Sparks, MD) containing 25% (vol/vol) glycerol. The E. coli O157:H7 cells were grown overnight in Luria-Bertani broth supplemented with nalidixic acid (25 mg/liter) at 37°C, and were then subjected to cold and nutrient stress at 5°C for 2 days. The culture was then diluted with sterile phosphate-buffered saline (PBS) to give an initial inoculation level of ~3.5 log CFU/g for experimental trial 1 and a range of ~2.2 to 3.4 log CFU/g for trial 2.

**Inoculation of packaged spinach with E. coli O157:H7.** Within 2 days after processing, commercially packaged (in microperforated film bags) baby spinach was obtained from the warehouse of a national distribution center. The product was transported to the Produce Quality and Safety Laboratory (Beltsville, MD), under refrigerated conditions and used immediately on arrival. At the time of inoculation, each bag was cut open at one end, and a fixed amount (6 g) of spinach leaves was removed in order to compensate for the reduction in package size caused by resealing. Spinach leaves in each package were then sprayed (in a biosafety cabinet) with the diluted inoculum preparation by using an atomizer. A total of four sprays were administered per package, with a total of 0.5 ml of inoculum preparation per bag (160 g spinach). This method, previously developed, ensured even distribution of inoculum on the spinach samples, with minimal liquid introduced to the products. After spraying, the packages were sealed hermetically with an impulse sealer (model PFS-F450, Kingstar Group, Wenzhou Zhejiang, China), and shaken gently to more evenly distribute the inoculum on the spinach leaves. The samples were stored at 1, 5, 8, and 12°C until the expiration of the marketed shelf life (12 days postinoculation).

**Package atmosphere and product quality evaluation.** On each sampling day, four bags of spinach from each treatment were removed from storage. Gas samples of the package headspace were withdrawn with a gas-tight syringe and passed through a 0.22-µm-pore-size cellulose filter for sterilization. The concentrations of O2 and CO2 of the gas samples were analyzed with an infrared gas analyzer (models S-3A/I and CD-3A, Ametek, Pittsburgh, PA).

The quality of the packaged spinach was evaluated for overall freshness of appearance by a panel of six trained personnel using a 9-point hedonic scale, where 9 = like extremely, 7 = like moderately, 5 = neither like nor dislike, 3 = dislike moderately, and 1 = dislike extremely (26, 27, 33). The samples were coded with three-digit numbers to mask the treatment identity in an effort to minimize the test subjectivity and to ensure test accuracy (35).

After visual evaluation, 15 g of spinach leaves was removed from each package and submerged in 300 ml of deionized water for 30 min at 20°C. The electrolyte content of the solution was measured with a conductivity meter (model 135A, Orion Research, Inc., Beverly, MA). Total electrolytes of the samples were determined after heating at 121°C for 1 h and cooling at 25°C (30). Electrolyte leakage was expressed as a percentage of total electrolytes.

**Microbiological analyses.** At each sampling time, 15 g of spinach from each package was placed into a sterile stomacher bag. The samples were macerated in sterile PBS in a stomacher blender (Biomaster 400, Seward, Ltd., London, UK) for 2 min at 230 rpm. The supernatant for each sample was filtered through sterile glass wool and serially diluted with sterile PBS. The filtrate and its appropriate dilutions were logarithmically spread onto selected
culture media with a Whitley Automatic spiral plater (Wasp 2, Don Whitley Scientific, Ltd., West Yorkshire, UK). Samples for enumeration of *E. coli* O157:H7 were plated on sorbitol MacConkey agar supplemented with 25 mg/liter nalidixic acid and 0.1% sodium pyruvate and incubated at 37°C for 18 to 24 h (16, 45). Populations of total aerobic mesophilic bacteria and psychrotrophic bacteria were determined on tryptic soy agar (Difco, Becton Dickinson) and incubated at 28 and 5°C for 2 days and 7 to 10 days, respectively (3). Yeast and mold counts were obtained by plating the samples on potato dextrose agar (Difco, Becton Dickinson) supplemented with chloramphenicol (250 mg/liter) and incubated at 25°C for 3 days (28). Microbial colonies were counted with a Protos colony counter (model 50000, Synoptics, Ltd., Cambridge, UK) and reported as log CFU per gram of tissue. Five representative *E. coli* O157:H7 colonies per sample were further confirmed via serological tests with the rapid RIM *E. coli* O157:H7 latex agglutination assay (Remel, Inc., Lenexa, KS).

**Experimental design and statistical analysis.** In preliminary studies, we determined the baseline growth pattern of native microflora on commercially packaged spinach products without inoculation, and evaluated the growth of *E. coli* O157:H7 lettuce outbreak strain (F6460) inoculated onto spinach. This was then followed by the two experimental trials presented in this article. One trial used spinach leaves inoculated with the *E. coli* O157:H7 strain RM4407 (trial 1), and a second one used spinach leaves inoculated with either RM4407 or F6460 at low or medium inoculation levels (trial 2). Both experiments were conducted with a factorial design, with temperature and time as the main factors for trial 1, and pathogen strain, inoculation level, temperature, and time as the main factors for trial 2. The experimental unit was a bag, and there were four bags per treatment per sampling date. The log (CFU per gram) data were analyzed according to a general linear model, using the PROC MIXED procedure of SAS software (SAS Institute, Inc., Cary, NC). Normality and variance homogeneity of the linear model were checked for the log-transformed data. A variance grouping technique was used to address variance heterogeneity for means comparisons. When effects were statistically significant, mean comparisons were done with Sidak test--adjusted *P* values to maintain an experiment-wise error of ≤0.05.

**RESULTS**

**Changes in package atmosphere and product quality.** There was a rapid increase in CO$_2$ (Fig. 1A) and a decrease in O$_2$ (Fig. 1B) within the first 3 days after sealing in sample bags held at all temperatures (Fig. 1). After an initial equilibration period, the package atmospheres remained relatively stable until the end of the storage period. Samples stored at 12°C had the highest CO$_2$ at equilibrium, and these were followed by those stored at 8, 5, and 1°C. The level of O$_2$ at equilibrium followed the reverse trend observed for CO$_2$, with the lowest O$_2$ in packages stored at 12°C, which was then followed by those stored at 8, 5, and 1°C. The similarity between the equilibrium O$_2$ and CO$_2$ levels of these packages with those of unopened (uninoculated) control bags (data not shown) suggests that the techniques used to inoculate the samples did not alter the package atmospheres of the commercial products.

All of the products maintained good visual appearance for 6 days postinoculation, regardless of storage temperature (Fig. 2A). However, a considerable decline in visual quality was recorded on samples held at 12°C for 9 days, although the quality was still considered as “like moderately,” with an average score of 7.0 on the 9-point hedonic scale. Visual quality of samples held at 12°C declined further from days 9 to 12, reaching an average rating of 5.6. The changes in visual quality of spinach held at 8°C followed similar trends, although the quality decline on samples was much less pronounced at 8°C than it was at 12°C. Samples held at 1°C, which were followed by those samples held at 5°C, received high-quality scores throughout the storage period, with an average score of 8.0 recorded 12 days postinoculation. Similar results were obtained on uninoculated samples (data not shown). The score of the visual quality from this trial was higher than that obtained from our previous experiment, probably because of the high quality of spinach leaves at the start of this trial.

Within the first 6 days postinoculation, there was a gradual decrease in tissue electrolyte leakage (Fig. 2B) on samples stored at all temperatures, attributable to the recovery from minor tissue injuries sustained during the initial handling. Similar trends were reported on packaged fresh-cut cilantro and lettuce (28, 30). However, a significant (*P* < 0.0001) increase in tissue electrolyte leakage was observed on samples stored at 12°C, coinciding with the significant decline in visual quality between days 6 and 12, while samples stored at all other temperatures

**FIGURE 1. Head space partial pressures of O$_2$ (A) and CO$_2$ (B) of spinach packaged in microperforated bags and stored at 1, 5, 8, and 12°C for up to 12 days. Values are the means of four replications.**

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Changes in *E. coli* O157:H7 and other background microorganism populations. Storage temperature and time had a significant (*P* < 0.0001 and *P* = 0.0098, respectively) impact on *E. coli* O157:H7 growth on spinach. Populations of *E. coli* O157:H7 increased significantly (*P* < 0.0001) over time when held at 12°C, with a 1.0-log CFU/g increase within 3 days, which was followed by an additional significant (*P* < 0.05) increase observed on days 6 and 9 of storage, respectively (Fig. 3A). The growth of *E. coli* O157:H7 was more gradual at 8°C, and a statistically significant (*P* = 0.0052) level was not observed until the sixth day of storage. On the other hand, *E. coli* O157:H7 populations on samples stored at 5 and 1°C declined over time, and a significant (*P* < 0.002) reduction was noted within 3 days. Overall, there were significant differences in *E. coli* O157:H7 population growth on spinach held at different temperatures. Growth was significantly (*P* < 0.0001) higher at 12 than at 8°C, and at 8 than at 5 and 1°C. No significant (*P* > 0.05) difference was found between 5 and 1°C. This result is in agreement with our earlier study on commercially packaged lettuce-containing salad in which *E. coli* O157:H7 (strain F6460) grew by more than 2.0 log CFU/g within 3 days when held at 12°C, and the population declined at 5°C (29). It was further confirmed by a follow-up experiment with *E. coli* O157:H7 strains F6460 and RM4407 at two different inoculation levels (2.2 and 3.4 log CFU/g).

Aerobic mesophilic bacteria counts on the day of inoculation averaged 3.4 log CFU/g (Fig. 3B). These data agree with the findings of Babic et al. (3) and Nguyen-the and Carlin (34). Over time, aerobic mesophilic bacteria increased significantly (*P* = 0.0002) on spinach held at all storage temperatures, with a 1- to 2-log CFU/g increase within the first 3 days, which was followed by an additional increase during the remaining storage life. There was generally a more rapid growth on samples stored at higher temperatures, with a 2.0-log CFU/g increase on samples stored at 12°C, and a 1.0-log CFU/g increase on samples stored at 1°C after 3 days in storage. At the end of the 12-day storage period, aerobic mesophilic bacteria reached 7.8, 7.3, 6.4, and 5.5 log CFU/g, at 12, 8, 5, and 1°C, respectively. Valentin-Bon et al. (44) surveyed 45 bags of commercially packaged spinach and reported that the total aerobic bacterial counts ranged from 4.0 to 8.3 log CFU/g. Our results of the growth from 3.4 to 8.1 log CFU/g from day 0 to the end of storage at different temperatures may explain the large differences in mesophilic bacterial counts found at market (15, 44).

Psychrotrophic bacterial populations averaged 3.2 log CFU/g on day 0 and increased significantly (*P* = 0.026) during storage (Fig. 3C). Similar to aerobic mesophilic bacteria, psychrotrophic bacterial populations increased more appreciably on samples held at 12°C than did those held at 1°C. Similar trends in temperature response were observed by Babic and Watada (4) on spinach samples stored at 5 and 10°C in various package atmospheres.

Yeast and mold populations increased gradually during storage from an initial density of 2.9 log CFU/g (Fig. 3D). Compared with aerobic mesophilic and psychrotrophic bacteria, the growth of yeasts and molds during storage was substantially slower and less responsive to the increase in temperature. However, the growth rate of yeasts and molds observed on spinach was higher than that reported for packaged lettuce, due to the increased O2 content in the spinach packages, resulting from the use of perforated film. Unlike packaged lettuce, which requires low O2 to prevent browning, baby spinach is usually packaged in microperforated films, because of its high respiration rate and susceptibility to CO2 injury (2).

Modeling the effect of temperature on growth of *E. coli* O157:H7 and native microflora. The populations of *E. coli* O157:H7, aerobic mesophilic bacteria, psychrotrophic bacteria, and yeasts and molds as a function of storage temperature at each sampling time were fitted to the linear model *y* = *ax* + *b*, where *y* denotes the population (in log CFU per gram) of each microbial group, *a* represents the slope of the curve, *x* is storage temperature, and *b* is a
FIGURE 3. Changes in populations of E. coli O157:H7 strain RM4407 (A), aerobic mesophilic bacteria (B), psychrotrophic bacteria (C), and yeast and mold (D) on spinach packaged in microperforated bags and stored at 1, 5, 8, and 12°C for up to 12 days. Values are the means of four replications.

constant (the intercept). Figure 4A through 4D shows the slopes for E. coli O157:H7, aerobic mesophilic bacteria, psychrotrophic bacteria, and yeast and mold, which ranged from 0.20 to 0.38, 0.11 to 0.28, 0.11 to 0.25, and 0.15 to 0.20, respectively. Although the slopes varied slightly during each sampling date, the slope for E. coli O157:H7 growth as a function of storage temperature was consistently larger than were the slopes for all other native microflora.

FIGURE 4. Regression of populations of E. coli O157:H7 strain RM4407, aerobic mesophilic bacteria, yeast and mold, and psychrotrophic bacteria as a function of storage temperature. Linear regression was calculated based on the equation \( y = ax + b \), where \( y \) represents the population of each microbial group, \( a \) represents the slope, \( x \) represents storage temperature, and \( b \) represents the intercept. Values are the means of four replications.
This suggests that storage temperature had a much more pronounced effect on the growth of E. coli O157:H7 than on the growth of native microflora. E. coli O157:H7 grew rapidly on spinach at 12°C, which was followed by significantly slower growth rate at 8°C, and significant die-offs at 5°C or below, as shown in Figure 3A. Unlike E. coli O157:H7, at low temperatures (1 and 5°C), the populations of all groups of native microorganisms increased, although more slowly than they did at higher temperatures, and no die-offs occurred.

The effect of E. coli O157:H7 strains and the initial inoculation level. The behavior of E. coli O157:H7, native microorganisms, and their interactions in fresh and fresh-cut produce is known to be influenced by raw-material growing conditions, postharvest handling practices, and fresh-cut produce processing conditions. Studies have also shown that the fate of E. coli O157:H7 on produce may vary by strain and by initial inoculation level. In order to evaluate the effect of pathogen strain and initial inoculation level on E. coli O157:H7 growth, an additional experiment was conducted with two different strains, F6460 and RM4407, at two inoculation levels, 2.2 to 3.4 log CFU/g. Both strains of E. coli O157:H7 grew significantly on baby spinach when held at 12°C within 3 days, as shown in Figure 5A (strain RM4407) and 5B (strain F6460). No significant (P > 0.05) difference was noticed between the growth rates of these two strains. Similarly, both strains at the relatively low (2.2 to 2.5 log CFU/g) and medium (3.1 to 3.5 log CFU/g) inoculation levels increased in population rapidly when held at 12°C for 3 days. No significant (P > 0.05) difference in the growth rate was observed between the two inoculation levels.

DISCUSSION

Various studies have shown that E. coli O157:H7 can grow on lettuce and other leafy green vegetables held at elevated temperatures (1, 14, 23, 24). However, all of those studies used produce prepared and packaged under laboratory conditions that did not emulate real life. To our knowledge, there are no studies that have examined the relationships between E. coli O157:H7 growth, background microflora, and quality deterioration of packaged baby spinach. It is presently not known whether E. coli O157:H7 can grow on commercially processed salad products in the presence of large populations of native microorganisms, or whether quality would deteriorate to a level that would render products unacceptable before significant E. coli O157:H7 growth could occur.

In this study, we obtained commercially packaged spinach products early in their shelf lives. The packages were carefully cut open, product inoculated with E. coli O157:H7, and the packages resealed. A fixed amount (6 g) of spinach leaves was removed before inoculation to compensate for the reduction in package size during resealing. The same product weight–to–total respiring surface area of the original product was maintained, facilitating the monitoring of product quality and microbial growth under package atmosphere conditions similar to commercial samples. Since E. coli O157:H7 was inoculated directly onto the commercially processed spinach products, the growth of E. coli O157:H7 on spinach could be monitored in the presence of natural microflora.

As shown in Figure 3, E. coli O157:H7 exhibits significant growth in the presence of large background populations of native microorganisms on commercially packaged baby spinach held at 8°C or above. This is an important finding in light of current speculation about the role of competition with native microorganisms on the fate of human pathogens in fresh-cut produce. Conflicting reports exist in the literature about the competitive or commensal relationship between human pathogens and indigenous microorganisms. Schuenzel and Harrison (39) and Liao and Fett (25) conducted an extensive screening of bacteria on fresh-cut produce, and were able to identify a number of native microorganisms—Pseudomonas fluorescens, Aeromonas hydrophila—that exhibited antagonistic effect on E. coli O157:H7. Cooley et al. (11) examined the interaction between E. coli O157:H7 and epiphytic bacteria in lettuce seedlings and reported that coinoculation with Enterobacter asburiae reduced survival while coinoculation with Wausteria paucula enhanced survival, suggesting that species-specific competitive or commensal relationships likely occur on fresh produce.
Baby spinach leaves at the time of inoculation were early in their shelf lives (2 days postprocessing), and were kept under strict temperature control prior to arriving at our research facility. Although some level of microbial growth was expected during this 2-day period, the populations of native microorganisms remained at 2.8 to 3.4 log CFU/g, a level that is similar to the day 0 value on baby spinach samples prepared in our on-site, fresh-cut, pilot-scale facility (data not shown). The populations of all native microflora increased over time during storage with aerobic mesophilic, psychrotrophic, and yeast and mold populations reaching 8.06, 7.75, and 6.02 log CFU/g, respectively, while the population of \textit{E. coli} O157:H7 reached to 5.7 log CFU/g. This suggests a general commensal relationship between \textit{E. coli} O157:H7 and native microflora on baby spinach.

It should be noted that the competition between \textit{E. coli} O157:H7 and native microflora is expected to be dose-dependent. Levels of \textit{E. coli} O157:H7 that may exist in the environment or on fresh produce is unknown, but they are assumed very low. Researchers are challenged to apply inoculum levels low enough to represent realistic conditions, yet sufficiently high enough for accurate quantification. During this experiment, although the authors attempted to use the inoculation levels (2.2 log CFU/g) at the low end allowed by the culture method for quantification, the inoculation level was still higher than what may be found in real life. Therefore, additional studies at even lower \textit{E. coli} O157:H7 inoculation levels, e.g., 1.0 log CFU/g or below, using other methods such as most-probable-number method, are warranted.

All microorganisms tested were affected by storage temperature, with a slower growth rate at lower temperatures. However, the growth of \textit{E. coli} O157:H7 was more responsive to changes in temperatures than that of any of the native microorganisms tested, as evidenced by a larger slope of the growth trend as a function of storage temperature (Fig. 4). Since pathogen strains may behave differently when exposed to different environmental conditions, additional studies with an extended range of \textit{E. coli} O157:H7 strains may be needed in future experiments.

Holding products at higher temperatures accelerated product quality deterioration in general. However, the visual quality of spinach leaves was fully acceptable after 6 days at all temperatures (Fig. 2A), despite a significant growth of \textit{E. coli} O157:H7 occurring within 3 days at 12°C and within 6 days at 8°C. These results suggest that significant growth of \textit{E. coli} O157:H7 can occur on spinach prior to product quality becoming unacceptable.

Fresh-cut products are marketed as ready-to-eat, yet without an effective microbial-killing step during their preparation. Preventing pathogen contamination is a critical step in maintaining product safety. In recognizing the importance of contamination prevention, the U.S. produce industry has taken various active steps in identifying microbial hazards in the farm environment, as well as developing and implementing best agricultural practices to improve produce safety. However, until this goal is accomplished, it is important to take every step possible to limit the potential for pathogen proliferation in the supply chain.

In summary, this study demonstrated that \textit{E. coli} O157:H7, strains F6460 and RM 4407, can grow significantly on commercially packaged fresh-cut spinach in the presence of large populations of aerobic mesophilic and psychrotrophic bacteria when held at 8°C or above. Although nonpathogenic background microorganisms also grow faster at elevated temperatures than at lower temperature, the growth of \textit{E. coli} O157:H7 benefits more from increased storage temperature than does the growth of the other background microorganisms tested. Furthermore, although holding products at abusive temperatures also accelerates product quality deterioration, significant growth of \textit{E. coli} O157:H7 can occur even though these products are still visually acceptable to the consumer. Additionally, there is a significant die-off of \textit{E. coli} O157:H7 when the products are stored at 1 and 5°C. All these data suggest that cold-chain maintenance is critical for reducing the food safety risks, as \textit{E. coli} O157:H7 grows at a rapid, temperature-dependant rate, despite the presence of large populations of native microorganisms.

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