Research Paper

Effects of Package Atmosphere and Storage Conditions on Minimizing Risk of Escherichia coli O157:H7 in Packaged Fresh Baby Spinach

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ABSTRACT

Packaged fresh spinach has been associated with outbreaks of illness caused by Escherichia coli O157:H7. The purpose of this study was to assess the behavior of E. coli O157:H7 in packaged baby spinach in response to storage conditions of temperature and package atmosphere and including effects of inoculation level, spinach leaf damage (cut leaves), internalized or leaf surface contamination, exposure to hypochlorite sanitizer, and package size. Behavior of E. coli O157:H7 inoculated at 2 and 4 log CFU/g on spinach packaged in polymer bags composed of a two-layer laminate (polypropylene and polyethylene) and stored under atmospheres of 20% O2–3% CO2 and 0% O2–15% CO2 (aerobic and anaerobic, respectively) was assessed at 5, 7, 12, and 15°C for up to 14 days. Growth kinetics were calculated using DMFit software. Temperature decreases progressively diminished growth or survival of the pathogen, and an aerobic package atmosphere resulted in longer lag times (4 to 6 days) and lower population levels (0.2 to 1.4 log CFU/g) compared with the anaerobic atmosphere at 15°C. Internalized contamination, leaf cuts, or exposure to 100 ppm of hypochlorite did not result in changes in pathogen behavior compared with controls; however, a growth minimization trend consisting of longer lag times and lower population levels was repeatedly observed in the aerobic compared with the anaerobic package atmospheres. In contrast, growth of indigenous mesophiles and Enterobacteriaceae was unaffected by package atmosphere. Spinach stored at 5 to 7°C in two sizes (5 and 16 oz) of polyethylene terephthalate clamshell packages with ambient air atmospheres was more likely to progress to lower-oxygen conditions in 16-oz compared with 5-oz packages after 7 days of storage (P < 0.05). Practices to maintain aerobic conditions within the package, as well as storage of the package at low temperature, are ways to limit growth of E. coli O157:H7 in packaged spinach.

HIGHLIGHTS

- Cold aerobic conditions limited survival of E. coli O157:H7 in packaged spinach.
- Low-oxygen atmosphere increased pathogen risk in temperature-abused packages.
- Internalization, leaf cuts, and hypochlorite stress did not increase pathogen risk.
- Large spinach packages trended toward lower-oxygen conditions more than small packages.
- Maintaining cold aerobic conditions can limit pathogen risk in packaged spinach.

Key words: Escherichia coli O157:H7; Leafy greens; Modified atmosphere packaging; Spinach

Fresh-cut produce is an increasingly important commodity for the retail food market (39). Designed to reduce preparation work by the consumer and packaged to retain product quality and improved shelf life, fresh-cut produce satisfies consumer preferences for healthy, ready-to-eat convenience foods. Nevertheless, because it may not be cooked before consumption, fresh-cut produce may represent a risk to health if contaminated with microbial pathogens.

The microbiological risks associated with fresh produce have long been recognized (18, 25), and fresh produce has been reported to be a significant source of foodborne illness outbreaks annually (6, 11). Leafy vegetables such as lettuces and spinach have contributed to the public health burden (46). From 1998 to 2008, leafy vegetables contaminated with pathogens accounted for 262 outbreaks of illness in the United States, most of them (141) attributed to norovirus Shiga toxin–producing Escherichia coli (STEC) and Salmonella were the causative agents in 22 and 11 of the outbreaks, respectively (24). While leafy salads have a greater association with norovirus outbreaks than do leafy...
vegetables, the opposite was true for the STEC outbreaks (26). Between 1998 and 2016, at least six STEC infection outbreaks were associated with consumption of spinach (8). In particular, two multistate outbreaks—in 2006 (54) and in 2012 (7)—were associated with packaged fresh spinach products.

Modified atmosphere packaging (MAP) has been widely used, in combination with refrigeration, to maintain product quality and to extend the shelf life of fresh produce. Leafy vegetables such as spinach continue to respire after harvest, with consumption of O2 and release of CO2. A desired gas concentration inside a package (usually reduced O2 and elevated CO2 levels), depending on the product, evolves and is maintained by an intricate interplay between the respiration of the produce and the gas permeability of the packaging material (10, 17, 57). Conditions of packaging and storage that help to reduce respiration are generally recommended to extend product shelf life. For spinach, the use of low-oxygen package atmospheres (0.8 to 3% O2, 8 to 10% CO2) at storage temperatures of 0 to 5°C have been recommended (17, 31, 44).

Package atmosphere and storage conditions have long been known to influence the microbiology of packaged fresh produce (1, 3, 21, 55). The dynamics of the entire microbial community present in the product can be influenced by packaging conditions (14, 34, 50, 51). If microbial pathogens are present, their behaviors also may be affected (20, 27, 36, 44). The behavior of STEC in packaged leafy vegetables such as lettuce and spinach has been investigated in many studies, although a review of the data noted variability and inconsistencies in the reported trends in survival and growth (13). Various experimental conditions and parameters may lead to different results, including choice of microbial strains, inoculation methods and levels, product type and variety, storage temperature, and packaging type and atmosphere (9, 15, 29, 33, 35, 37, 48). Many studies focused on STEC strains in packaged lettuce (13); however, a few have reported survival trends of the pathogen in packaged spinach. Lee and Baek (33) studied the survival of E. coli O157:H7 on spinach packaged in air, N2, CO2, and vacuum at 7°C. They reported that the pathogen’s population increased in the aerobic packages but was static or showed minimal decreases in the N2, CO2, and vacuum packages. Luo et al. (37) also studied E. coli O157:H7 survival in spinach packaged in microperforated film with no adjustment of the package gas composition and observed a significant increase in populations at 12°C storage, slower but significant growth at 8°C, and significant population decreases when packages were held at 1 and 5°C. Brown et al. (5) tested E. coli O157:H7 for its ability to grow in spinach packaged in air (traditional), high-oxygen (80% O2, 20% CO2), or low-oxygen (80% N2, 20% CO2) atmospheres and stored in a retail display case for 9 days at 4°C. The air and high-oxygen atmospheres demonstrated minimal effects on E. coli O157:H7 populations, i.e., 0.26 and 0.15 log CFU lower population levels, respectively, compared with the low-oxygen atmosphere. Poineniondo et al. (47) reported a significant decrease in E. coli O157:H7 level when spinach was packaged and stored in low oxygen (80% N2-20% CO2) at 5°C. Although some of these studies included effects of other factors on STEC populations, e.g., washing with antimicrobials and use of biocontrol agents before packaging, the effects of package atmosphere taken alone could be regarded as variable across the studies.

The purpose of this study was to assess the behavior of E. coli O157:H7 in packaged spinach in response to storage conditions, such as temperature, time, packaging type, and atmosphere. It included not only the published low-oxygen package recommendations for spinach but also the aerobic atmospheres that were revealed in a survey of local retail packages. The effects of inoculation level, spinach leaf damage, internalized or leaf surface-inoculated pathogen contamination, and exposure to hypochlorite were also studied.

**MATERIALS AND METHODS**

**E. coli O157:H7 and culture conditions.** All microbiological media were obtained from BD (Franklin Lakes, NJ). All experiments were conducted using E. coli O157:H7 strain ISEHGFP, which expresses green fluorescent protein (GFP) (43). Use of this fluorescent marker, along with the rifampin resistance encoded within the GFP cassette, allowed the strain to be selectively differentiated and enumerated among the natural microbial populations of the spinach. Insertion of the GFP marker has been reported to affect certain metabolic activities, although reports differ on whether growth rate is affected (2, 45). Oscar (45) reported a reduced growth rate and maximum population density, but only a small effect on lag time, in three GFP-expressing serovars of Salmonella. Allison and Sattenstall (2), however, reported no effect on growth rate or lag time in E. coli or Pseudomonas putida. For this study, growth kinetics of strain ISEHGFP at 35°C were compared with those of 15 other strains of E. coli O157:H7 derived from clinical and food isolations, including isolates from the U.S. outbreak of infections associated with consumption of packaged spinach in 2006 (54). When measured over an 18-h period using the Bioscreen-C Automated Growth Curve Analysis System (Growth Curves USA, Piscataway, NJ) and analyzed using DMFit software (http://www.combase.cc), the GFP-expressing strain E. coli O157:H7 ISEHGFP demonstrated a similar average growth rate (0.23 optical density [OD] unit change per h) and maximum population level (1.26 OD unit) but showed a longer average lag time (6.5 h) compared with that of other strains (5.6 h) in brain heart infusion (BHI) broth. E. coli O157:H7 ISEHGFP was cultured in BHI broth at 35°C for 24 h, harvested by centrifugation (6,000 × g for 10 min at 4°C), and washed twice in Butterfield’s phosphate buffer (BBP). The washed cells were resuspended in BBP for inoculation of spinach. To determine the inoculum concentration, serial 10-fold dilutions were prepared in BBP and plated on BHI agar supplemented with 100 μg/mL rifampin. After incubation at 35°C for 18 to 24 h, fluorescent colonies were enumerated using a UV light at 280 nm.

**Package design and atmosphere.** A direct sampling headspace gas analyzer (Gaspace Advance Micro, Systech Illinois, Johnsburg, IL) was used to measure O2 and CO2 compositions inside packages. A preliminary study was conducted to determine the package atmospheres in five brands of packaged spinach in polymer bags (5 to 9 oz) obtained from local groceries. The survey of spinach products demonstrated that the O2 composition was 15 to 20% and the CO2 composition was 3 to 7%, in contrast to the
published low-oxygen recommendations for spinach (17, 31, 44). The gas compositions inside the packages remained stable when the products were stored at temperatures ranging between 4 and 10°C for up to 10 days. In this study, two levels of initial gas compositions were selected: 20% O₂–3% CO₂ (aerobic) to mimic the commercial packaging and 0% O₂–15% CO₂ (anaerobic) for comparison.

The information collected from the retail packages was used to guide the size of the packages and the weight of spinach used for experiments in this study. A stock roll of polypropylene and polyethylene laminate film with a thickness of 75 ± 3 μm was obtained from Amcor Flexibles (Mundelein, IL). The film had perforations (500 μm in length, 160 perforations per m²) regularly spaced over its surface in a longitudinal direction to control the transfer of gases. The films were cut and sealed to form three-sided pouches, which were then filled with spinach (100 ± 1.0 g) and heat sealed. The final dimension of a pouch was 225 by 250 mm.

Preliminary experiments to achieve a target atmosphere of 15 to 20% O₂ were performed by storing 100 g of spinach in the perforated packages at various temperatures for up to 14 days and by measuring packaging parameters as follows. The equilibrium volume of the package measured by conventional residual air test (53) was 1.3 L on average. The package had a silicone sampling port through which a mixture of atmospheric gases (N₂, CO₂, and O₂) was delivered using a 2-L syringe. After packages were vacuum sealed, a preformulated gas mixture was added to a final volume of 1.3 L to achieve the desired atmosphere. A twist drill with a diameter of 200 μm (Titex Plus USA, Greenfield, MA) was used to make an additional number of perforations in the film that were determined in preliminary experiments to be appropriate for each temperature to maintain the correct atmospheric composition. Depending on the storage temperature, the number of perforations was varied to achieve and maintain the 20% O₂–3% CO₂ package atmospheres during storage at 5 or 12°C for up to 14 days. Nonperforated film made of the same polymeric materials (Amcor Flexibles) was used to obtain the anaerobic package atmosphere (0% O₂–15% CO₂). The O₂ and CO₂ compositions in the permeable packages were monitored to ensure their stability for the duration of each storage study.

For experiments involving clamshell-type containers, atmospheres within the clamshells were measured using the direct sampling analyzer by inserting a needle probe into a silicone sampling port on the side of the container. For retail product testing, different brands of baby spinach marketed in 5- and 16-oz clamshell packages (n = 17 packages of each size), with varying times of product expiration dating, were obtained from local grocery stores, and package atmospheres were measured on the day of purchase. For experimental work, clamshell packages were prepared in the laboratory as follows: 142 or 454 g of baby spinach was placed into 5- or 16-oz rectangular clamshell containers (160 by 200 by 80 mm high and 190 by 290 by 120 mm high, respectively) composed of polyethylene terephthalate (PET; Polar Pak, Brampton, ON, Canada). The containers were sealed with lids of PET and shrink wrapped with polyvinyl chloride tamper-evident bands. The initial headspace in each was confirmed as normal air composition (20.9% O₂–0.3% CO₂). The packages were then placed at 5 to 7°C, and the headspace gas was measured after 7 days of storage. Four independent trials of the PET clamshell storage experiment were conducted using different batches of bulk baby spinach.

**Inoculation and packaging of spinach.** Washed baby spinach was received in bulk from a commercial production facility after overnight refrigerated shipment and stored at 5 to 7°C before use in experiments. Baby spinach leaves were apportioned in 100-g quantities per package, unless otherwise noted. Packaging film type (perforated or nonperforated) was chosen depending on the package atmosphere to be maintained.

Washed cells (10 μL of appropriate dilutions of *E. coli* O157:H7 ISEHGFP to achieve the target contamination levels (10³ or 10⁴ CFU/g) were spot inoculated onto each of five spinach leaves. Simulating a contamination as might occur during spinach washing, the inoculated leaves were immediately added without drying to a 100-g quantity of leaves in each package. The packages were then heat sealed and manually shaken to distribute the inoculated leaves within the package. Packages containing unoinoculated spinach were prepared by adding 10 μL of BPB onto each of five leaves per package. A small hole at the corner of the package containing 100 g of spinach, including the five inoculated or unoinoculated leaves, was created and resealed under vacuum. The package atmospheres were adjusted to either 20% O₂–3% CO₂ or 0% O₂–15% CO₂ as described earlier.

For internalized inoculation of leaves, *E. coli* O157:H7 was injected into the leaf petioles for uptake into leaf tissue by capillary action, as follows. Spinach leaves with intact petioles were selected, and the leaf petioles were trimmed with a scalpel to prepare a fresh cut at the petiole end. For internalization of the inoculum, washed cells (2 μL) were injected into the petiole of each of five leaves using a 25-μL glass Hamilton syringe. For these experiments comparing surface versus internalized contamination, the surface inoculum volume applied to the leaf was adjusted to 2 μL instead of 10 μL.

**Effect of spinach leaf cutting.** To assess the effect of leaf damage by cutting on pathogen behavior, spinach leaves were cut into pieces of approximately 2 to 4 cm. Each of five cut leaves was spot inoculated with 10 μL of washed cells to result in 10⁴ CFU/g and then added to each package containing 100 g of cut leaves. Uninoculated controls were prepared by addition of 10 μL of BPB onto each of five cut leaves.

**Effect of sanitizer exposure.** To assess the effect of sanitizer stress on pathogen behavior, each of five spinach leaves was spot inoculated with 10 μL of washed cells to result in approximately 10⁴ CFU/g after sanitizer treatment. (In preliminary experiments, the hypochlorite treatment of *E. coli* O157:H7–inoculated leaves resulted in an approximately 0.5- to 1.0-log reduction of the population; therefore, to ensure equivalent initial populations for the storage experiment, the hypochlorite-treated leaves were inoculated at a population approximately 0.5 to 1.0 log greater than that of the control leaves.) After inoculation, the leaves were dried in a biosafety cabinet for 20 min and then exposed to sanitizer by dipping each leaf for 1 to 2 s into a 100-ppm hypochlorite solution prepared in deionized water, followed by immersion into deionized water for another 1 to 2 s. The rinsed leaves were gently shaken to remove water and then added to 100 g of spinach leaves in each package. Dipping of inoculated leaves into water was used to test unstressed control cells. The chlorine concentration was verified using a Hach pocket colorimeter II Cl₂ (Loveland, CO).

**Microbiological analysis.** Each spinach sample was prepared for microbiological analysis by adding 100 mL of 0.1% BPB to the package. After homogenization in a stomacher blender at medium speed for 1 min, 100 μL of the homogenate was removed from the package and serially diluted in BPB for plating in duplicate on appropriate agar media.
Populations of the inoculated E. coli O157:H7 were assessed by counting fluorescent colonies that grew on BHI agar supplemented with 100 μg/mL rifampin after incubation of the plates at 35°C for 24 h. Various indigenous microbial populations were measured as follows (52). Aerobic mesophiles and aerobic psychrotrophs were determined on plate count agar plates after incubation at 35°C for 48 h and at 7°C for 10 days, respectively. Enterobacteriaceae were assessed by plating on violet red bile agar overlay plates and counting of purple-red colonies (≥0.5 mm in diameter) after incubation at 35°C for 18 to 24 h.

Experimental design. Study variables included two E. coli O157:H7 inoculation levels (10^2 and 10^4 CFU/g), four storage temperatures (15, 12, 7, and 5°C), two package atmospheres (aerobic of 20% O_2–3% CO_2 and anaerobic of 0% O_2–15% CO_2, respectively) at different temperatures (5, 7, 12, and 15°C) was determined. The growth kinetics, including lag time, growth rate, and change in population level calculated using DMFit software, are summarized in Table 1, and the survival curves are displayed in Figure 1. Not all modeled growth kinetics fit the data well, as noted by several low R^2 values in Table 1.

In general, E. coli O157:H7 exhibited the highest growth rates and the highest population changes during storage at the product abuse temperature of 15°C (Table 1 and Fig. 1). Only the 15°C growth curves demonstrated apparent lag phases under all inoculation-level and package atmosphere conditions (Fig. 1). E. coli O157:H7 maintained a stable population or showed a moderate increase at 12°C. At the lower temperatures (5 and 7°C), the population levels of the pathogen decreased.

Packaging conditions affected the behavior of the pathogen, and these behavioral effects were most obvious at temperature-abuse conditions. At 15°C, the lag time was longer by 4 to 6 days and the population change was lower by 0.2 to 1.4 log CFU/g in the aerobic compared with the anaerobic packages. Lower population increases also

RESULT

Effects of temperature, inoculation level, and package atmosphere on E. coli O157:H7. The behavior of E. coli O157:H7 inoculated onto spinach leaves at two levels (2 and 4 log CFU/g) and stored under aerobic and anaerobic package conditions (20% O_2–3% CO_2 and 0% O_2–15% CO_2, respectively) at different temperatures (5, 7, 12, and 15°C) was determined. The growth kinetics, including lag time, growth rate, and change in population level calculated using DMFit software, are summarized in Table 1, and the survival curves are displayed in Figure 1. Not all modeled growth kinetics fit the data well, as noted by several low R^2 values in Table 1.

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Packaging conditions affected the behavior of the pathogen, and these behavioral effects were most obvious at temperature-abuse conditions. At 15°C, the lag time was longer by 4 to 6 days and the population change was lower by 0.2 to 1.4 log CFU/g in the aerobic compared with the anaerobic packages. Lower population increases also
occurred in aerobic compared with anaerobic packaging at 15°C. Therefore, based on this study, higher-oxygen packaging may represent lower risks than the low-oxygen packaging of spinach recommended in the published literature (17, 31, 44) if temperature abuse occurs.

Effects on indigenous microbiota. The growth of indigenous microorganisms was also examined in both low- and high-oxygen package atmospheres, and the data for the changes in populations of mesophiles, psychrotrophs, and Enterobacteriaceae at 12°C are depicted in Figure 2 for control (uninoculated) spinach. Psychrotrophs reached the maximum population of 8.5 log CFU/g at 12°C in high-oxygen package conditions and demonstrated a 3.0 versus 2.1 log increase in the aerobic versus anaerobic atmospheres, respectively, whereas the mesophiles and Enterobacteriaceae showed similar growth (3.0 to 4.0 log CFU/g) in both atmosphere compositions.

Unlike the E. coli O157:H7 levels, which varied at 12°C storage, the indigenous microbial populations demonstrated increases during the 14 days of storage at the same temperature (Figs. 1 and 2, respectively). With respect to package atmosphere, indigenous populations on spinach were less influenced by differences in the atmosphere composition compared with the pathogen, for which higher oxygen had a growth-controlling influence on the mesophiles and Enterobacteriaceae, although not on psychrophilic populations.

Comparison of internalized and surface-inoculated E. coli O157:H7. Pathogens may become internalized within fresh produce at pre- and postharvest stages (16, 23). The effect of the location of contamination on the spinach was examined using E. coli O157:H7 cells that were

TABLE 1. Effect of inoculation level, temperature, and package atmosphere on growth kinetics of E. coli O157:H7 in packaged spinach during 14 days of storagea

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<tr>
<th>Inoculation level (log CFU/g)</th>
<th>Temp (°C)</th>
<th>Package atmosphere (%O2/%CO2)</th>
<th>Lag time (day)</th>
<th>Growth rate (log CFU/g/day)</th>
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a Growth kinetics determined by DMFit software (http://www.combase.cc).
b —, no lag time calculated by DMFit.
c Single trial.

FIGURE 2. Growth of indigenous microbiota in uninoculated spinach at 12°C in (A) aerobic and (B) anaerobic package atmospheres. ▲, Psychrotrophs; ■, mesophiles; ◆, Enterobacteriaceae.
inoculated onto the leaf surface or internalized into the leaf petiole, with storage at 5 or 15°C in both aerobic and anaerobic package atmospheres.

As in the initial trials depicted in Figure 1, the pathogen grew significantly at 15°C. Growth at 15°C was observed regardless of whether the pathogen contaminated the leaf on the surface or by internalization in the petiole (Fig. 3). In addition, as in Figure 1, the aerobic atmosphere promoted a longer lag time for growth (6 to 10 days) compared with anaerobic conditions (3 days).

**Effect of spinach leaf cutting on *E. coli* O157:H7.** Pathogen survival has been reported to increase when contaminated leaf surfaces have been damaged or cut (4, 22, 29). To study the influence of a type of leaf damage associated with minimal processing, i.e., cutting, pathogen behavior was monitored on whole versus cut spinach leaves in aerobic and anaerobic atmospheres during storage, and the results for 15°C are presented in Figure 4. The pathogen again showed more growth in the anaerobic compared with aerobic atmospheres when the lag-phase durations and maximum population levels were compared. During aerobic storage of 6 days, populations of the pathogen were significantly higher in cut spinach than in whole spinach, but the differences became minimal after 10 days. For the anaerobic storage, there were no significant differences in populations between whole and cut spinach leaves throughout the storage.

**Effect of sanitizer treatment on *E. coli* O157:H7.** During postharvest washing processes, pathogens may be exposed to sanitizers, and many studies have been published to document the effect of the most commonly used sanitizer in the produce industry, sodium hypochlorite, on the survival of *E. coli* O157:H7 on fresh produce and in the wash water (12, 30, 38). The effect of exposing *E. coli* O157:H7 on the spinach leaf surface to a 100-ppm sodium hypochlorite solution for 1 to 2 s was evaluated for aerobic and anaerobic package atmosphere storage at 15°C (Fig. 5). A 0.5- to 1.0-log reduction of the pathogen population resulted from the hypochlorite treatment; therefore, the population levels were adjusted during the spinach leaf inoculation to be approximately equivalent in size for the storage experiments in packages (see “Effect of sanitizer exposure”). Regardless of the sanitizer’s effect on the pathogen, the observation that the aerobic atmosphere decreased pathogen growth trends compared with the anaerobic atmosphere was again repeated.

**Effects of package size on atmosphere in PET clamshell packages.** Clamshell packaging is a popular way of marketing retail spinach, and a survey was conducted to determine the package atmosphere compositions in various commercial brands of spinach in 5- and 16-oz clamshell containers obtained from local grocers. Figure 6 shows the percentage of O₂ and CO₂ in the clamshell package plotted against the number of remaining shelf-life days before the labeled expiration date. In general, regardless of the expiration date, all 5-oz packages maintained aerobic...
atmospheres, i.e., compositions ranging from approximately 17 to 21% O₂ and 1 to 5% CO₂. The 16-oz packages, however, demonstrated a trend toward lower-oxygen atmospheres as the product approached its expiration date; i.e., within 6 days of expiration, package atmosphere compositions ranged from approximately 14 to 16% O₂ and 5 to 9% CO₂. Because the PET material used in clamshell packages provides a good gas barrier, the difference is possibly attributed to the respiration effect of the larger amount of spinach packed in the 16-oz packages. In particular, one package, with 7 days of shelf life remaining, exhibited an atmosphere in which the oxygen was measured to be lower than the carbon dioxide, i.e., approximately 7% O₂ and 12% CO₂ (Fig. 6B, circled data points).

Spinach packaged in 5- and 16-oz clamshells was also prepared in the laboratory for comparison before and after 7 days of storage at 5 to 7°C. On day 0, both sizes demonstrated similar package atmospheres reflecting the normal composition of air, i.e., approximately 20.9% O₂ and 0.3% CO₂. After storage, average gas compositions inside the 5-oz packages were 18.6% ± 1.2% O₂ and 3.4% ± 1.1% CO₂, but these were significantly different (P < 0.01) in the 16-oz packages, which averaged 9.4% ± 5.2% O₂ and 10.3% ± 4.1% CO₂ (n = 17 for each package size; Fig. 7). The results demonstrated that under the storage conditions tested, spinach packaged in the larger 16-oz clamshell containers was significantly more likely to progress to lower-oxygen conditions than spinach packaged in smaller 5-oz clamshells.

DISCUSSION

Fresh leafy vegetables such as spinach are subject to a range of conditions as they move through different stages from the field to the retail package. These various conditions include humidity and temperature fluctuations, mechanical trimming and cutting processes, sanitizer treatment, and atmosphere modifications. An understanding of how pathogens survive, if they are present, is required to determine risk and to develop appropriate risk-reduction measures.

In this study, we investigated the behavior of E. coli O157:H7 on fresh baby spinach leaves under varying postharvest conditions of leaf cutting, sanitizer exposure, packaging atmospheres, and temperatures during packaged storage. We demonstrated that aerobic atmospheres and lower storage temperatures can reduce risk by minimizing the growth of the pathogen in packaged spinach. Package
atmosphere and storage temperature are the two most reliable parameters for quality preservation in processed vegetables (49).

It has long been recognized that low temperatures can control the growth of various pathogens in fresh produce (13, 21, 42), and this study supports the general recommendation to maintain low temperatures for delivering fresh spinach to the consumer. In practice, however, products are not likely to be held under refrigerated conditions consistently throughout the postharvest chain. Data and modeling of pathogen behavior in leafy greens through dynamic temperature fluctuations have been widely published (15, 32, 40, 41, 56) and may provide more realistic approaches for risk assessment.

Packaging for fresh produce has also been known to affect pathogen survival, but the literature on package atmosphere effects has been conflicting (19). In this study, the maintenance of an aerobic atmosphere was reproducibly shown to better control the proliferation of E. coli O157:H7 when compared with an anaerobic packaging condition in all phases of experimentation in this study. Limiting the size of the spinach packages, which can influence the package atmosphere, may be considered another approach to ensure that pathogen survival is minimized.

Oxidative stress caused by the pathogen’s exposure to an aerobic atmosphere could lead to bacterial cell injury (28). If not controlled by the design of the package, respiration of the spinach within the package would lead to a decrease in O2 concentration and increase in CO2 concentration (20), which may result in relief of oxidative stress, causing microbial growth facilitation. The aerobic atmospheres found in the commercially packaged spinach from the survey are preferable for minimizing growth of E. coli O157:H7, as opposed to the low-oxygen conditions that have been recommended for extension of shelf life (17, 31, 44).

In summary, this study revealed factors affecting E. coli O157:H7 survival in packaged spinach. Maintenance of aerobic conditions within the package, as well as storage of the package at low temperature, are effective ways to limit growth of E. coli O157:H7, thus minimizing risk at this stage of the farm-to-table continuum.

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