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FOOD-BORNE PATHOGENS

Effects of vegetable type, package atmosphere and storage temperature on growth and survival of *Escherichia coli* O157:H7 and *Listeria monocytogenes*

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The survival and growth of *Escherichia coli* O157:H7 (ATCC 43888 and NCTC 12900) and *Listeria monocytogenes* (ATCC 19114 and NCTC 11994) during storage (4 and 8°C) on ready-to-use (RTU) packaged vegetables (lettuce, swedes (rutabaga), dry coleslaw mix, soybean sprouts) were studied. The vegetables were sealed within oriented polypropylene packaging film, and modified atmospheres developed in packs during storage due to produce respiration. Survival and growth patterns were dependent on vegetable type, package atmosphere, storage temperature and bacterial strain. Populations of *L. monocytogenes* and *E. coli* O157:H7 increased ($P<0.05$, by 1.5 to 2.5 log cycles, depending on strain) during a 12-day storage period on shredded lettuce (8°C). *L. monocytogenes* populations also increased (by ~1 log cycle) on packaged swedes, did not change significantly ($P>0.05$) in packages of soybean sprouts and decreased by ~1.5 log cycles ($P<0.05$) on coleslaw mix (8°C). *E. coli* O157:H7 populations on packaged coleslaw and soybean sprouts increased (by 1.5 to 2.5 log cycles) up to day 5, but declined during subsequent storage (8°C). On packaged swedes (8°C), populations of *E. coli* O157:H7 strain ATCC 43888 increased (by ~1 log cycle) during storage, whereas populations of strain 12900 increased between days 2 and 5, and declined during subsequent storage. Reducing the storage temperature from 8 to 4°C reduced the growth of *L. monocytogenes* and *E. coli* O157:H7 on packaged RTU vegetables. However, viable populations remained at the end of the storage period at 4°C. *Journal of Industrial Microbiology & Biotechnology* (2001) **27,** 111–116.

Keywords: foodborne pathogens; food safety; fresh-cut produce; modified atmosphere packaging; refrigeration

Introduction

Ready-to-use (RTU) vegetables may be intact, washed vegetables (e.g., soybean sprouts) or may consist of trimmed, peeled, sliced and washed vegetables (e.g., cut lettuce, dry coleslaw mix). These products are usually sealed within semipermeable packages and stored at refrigeration temperatures [24].

RTU vegetables harbor large and diverse populations of microorganisms. Pathogens, such as *Listeria monocytogenes* and *Escherichia coli* O157:H7 may also contaminate vegetables, and a number of outbreaks of foodborne disease have been traced to vegetables [15,24,30]. For example, foodborne outbreaks due to contamination with *E. coli* O157:H7 have been associa- ted with elettuce, alfalfa sprouts and apple juice, and an outbreak due to nterotoxigenic *E. coli* has been linked to carrots [5]. *L. monocytogenes* and *E. coli* O157:H7 survive and/or grow on a range of RTU vegetables, such as shredded lettuce [1,6,7,16,20,21,27,32], minimally processed endive [10,27], cut cabbage [9,28] and sliced cucumbers [1].

Modified atmosphere packaging (increased CO_2, decreased O_2) is widely used, in combination with refrigeration, to retard product respiration, delay physiological ageing and thereby extend the shelf life of RTU vegetables. Vegetable respiration alone will also decrease O_2 and increase CO_2 levels inside the package, thereby passively modifying the in-pack atmosphere [24]. Extending the shelf life increases the time available for pathogens, if present, to grow and overextending the shelf life may allow the development of significant populations. A number of workers have shown that modified atmosphere packaging can enhance survival and growth of *L. monocytogenes* and *E. coli* O157:H7 on vegetables [1,11, 16,21,30].

Storage temperature is probably the single most important factor affecting the growth of microorganisms in RTU vegetables. Although *L. monocytogenes* is capable of growth at low temperatures, reducing the storage temperature will extend the lag phase and reduce the rate of growth [7,10]. The mesophilic status of *E. coli* O157:H7 makes growth in RTU vegetables unlikely where temperature control is adequate (4°C or less). However, temperature abuse during storage and distribution may allow growth, and growth has been reported at 8°C on minced beef [34].

In this work, the survival and growth of *L. monocytogenes* and *E. coli* O157:H7 on packaged RTU vegetables were studied. The effects of vegetable type (lettuce, swedes, dry coleslaw mix, soybean sprouts), passively modified atmospheres, storage temperature (4 and 8°C) and bacterial strain were investigated.

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Materials and methods

Preparation of the model vegetable products

Lettuce: Heads of Irish iceberg lettuce were purchased from a local supplier. They were processed as follows: outer and damaged leaves as well as the core of the lettuce heads were removed and discarded. Inner leaves were sliced manually using a sharp knife to approximately 10-mm strips.

Swedes: Irish swedes (rutabaga), from a local supplier, were hand-peeled and diced into approximately 10-mm^2-cube pieces using a sharp knife.

Dry coleslaw and soybean sprouts: Dry coleslaw mix (80% shredded cabbage, 20% shredded carrot) and raw soybean sprouts was obtained from a local supplier.

Intact (in the case of soybean sprouts) or shredded/diced vegetable portions were washed in distilled water for 5 min with agitation by hand. Washed vegetables were left to drain on absorbent paper for 15 min.

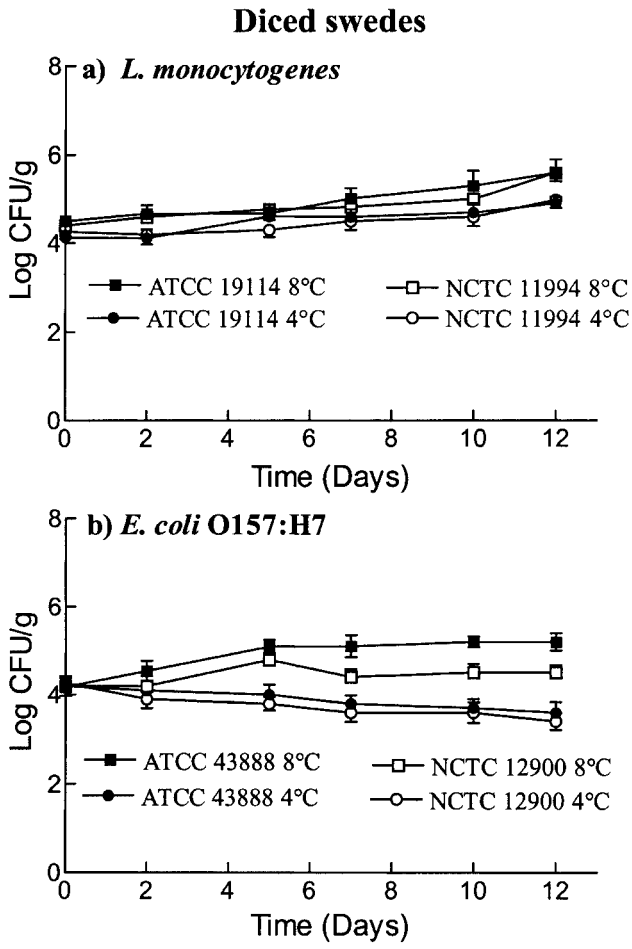

Figure 1 Survival and growth of (a) *L. monocytogenes* (strains ATCC 19114 and NCTC 11994) and (b) *E. coli* O157:H7 (strains 43888 and 12900) on diced packaged swedes (rutabagas) during storage at 8°C and 4°C. Reported populations represent the means of four values. Error bars show standard deviations.

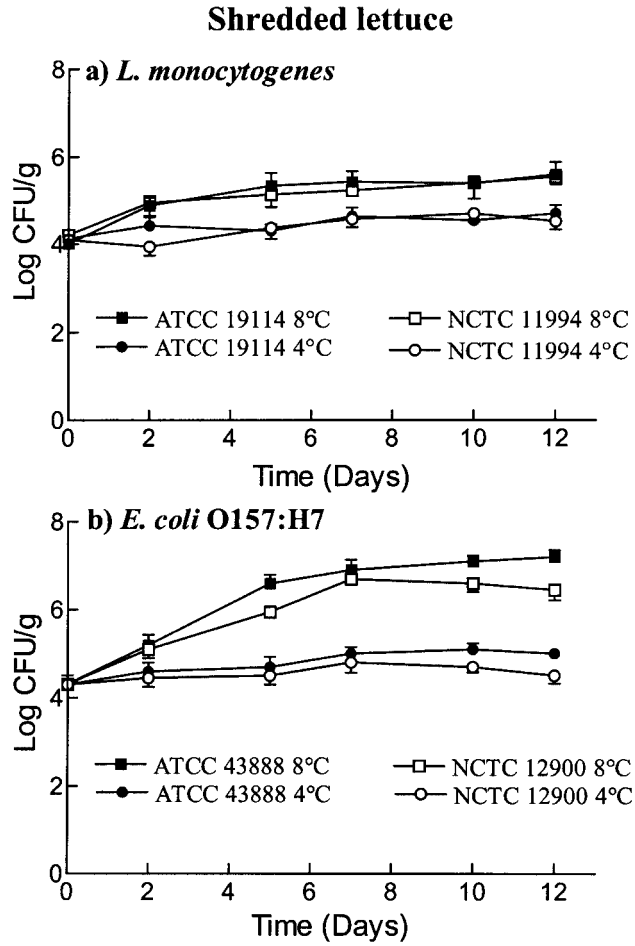

Figure 2 Survival and growth of (a) *L. monocytogenes* (strains ATCC 19114 and NCTC 11994) and (b) *E. coli* O157:H7 (strains 43888 and 12900) on shredded packaged lettuce during storage at 8°C and 4°C. Reported populations represent the means of four values. Error bars show standard deviations.

Vegetable portions (25 g) were transferred aseptically into bags (10 cm×10 cm), composed of 35-μm oriented polypropylene packaging film (Cannings Packaging, Dublin, Ireland), which were later heat-sealed. According to the manufacturer, this film had a permeability to O_2 of 1200 ml/m^2/day/atm and to CO_2 of 4000 ml/m^2/day/atm.

Strains and preparation of inocula

L. monocytogenes (strains ATCC 19114 and NCTC 11994) and two nontoxigenic *E. coli* O157:H7 strains (ATCC 43888 and NCTC 12900) were maintained at −20°C in nutrient broth (Oxoid CM 1) supplemented with 15% (v/v) glycerol. Resuscitation was achieved by thawing cultures at room temperature (17 to 22°C) followed by a loop-transfer in tryptone soya broth (10 ml TSB in screw-cap tubes, Oxoid CM 129) and static incubation at 37°C for 24 h. Cultures were centrifuged (4000×*g*, 15 min), the cells were washed twice with sterile distilled water, resuspended and diluted in sterile distilled water to desired concentrations (~5×10^5 CFU ml^{-1}) to allow for contamination of vegetables at initial levels of approximately 10^4 CFU g^{-1} of vegetable.

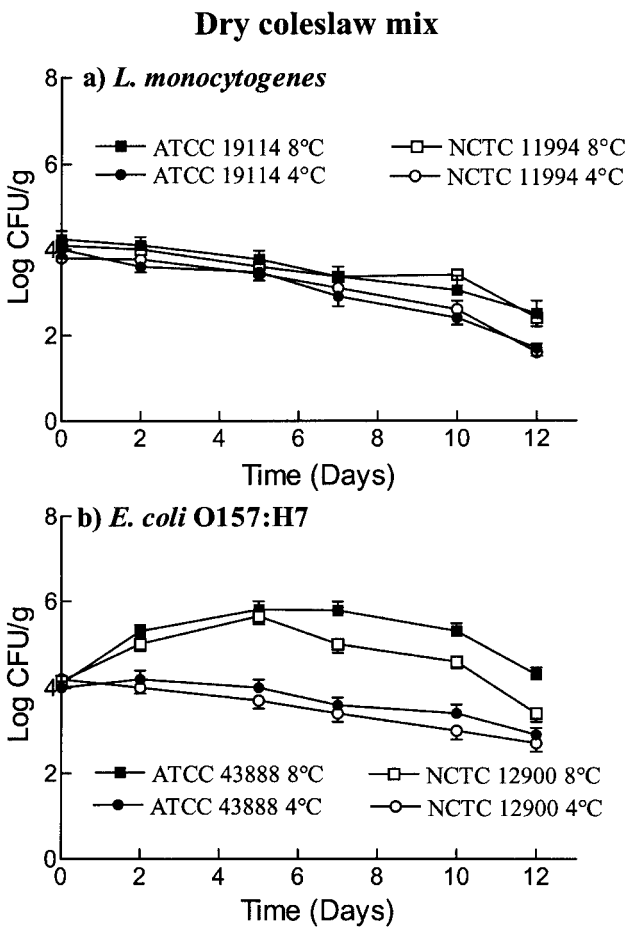

Figure 3 Survival and growth of (a) *L. monocytogenes* (strains ATCC 19114 and NCTC 11994) and (b) *E. coli* O157:H7 (strains 43888 and 12900) on packaged dry coleslaw mix during storage of 8°C and 4°C. Reported populations represent the means of four values. Error bars show standard deviations.

Inoculation of vegetables and storage

After appropriate dilution, ten 10-μl aliquots of the cell suspensions were distributed over the vegetables contained within the packages. After sealing, packs were gently shaken to assist inoculum distribution. Immediately after inoculation and sealing, samples were transferred to storage temperatures of 4 and 8°C and stored for a period of 12 days.

Microbiological analyses

Microbiological analyses were carried out on day 0 (day of inoculation) and at regular intervals throughout the storage period (days 2, 5, 7, 10 and 12). At each sampling, duplicate packs from the same experiment were analysed for *L. monocytogenes* or *E. coli* O157:H7 populations. The 25-g sample from each pack was aseptically transferred into a stomacher bag. Samples were homogenized for 2 min at high speed with 225 ml sterile peptone–water (Oxoid CM 9) using a Seward laboratory stomacher (Model 400, AGB Scientific, Ireland). Serial dilutions of each homogenized sample were made in peptone–water and were surface spread (100 μl/plate) in duplicate onto appropriate media. Numbers of *L. monocytogenes* were determined on *Listeria*-selective agar (LSA, Oxoid CM 856) after incubation for 48 h at 37°C. Colony confirmation was performed on characteristic black, aesculin-producing colonies by Gram stain, catalase, motility and biochemical tests (API-*Listeria* strips, Biomerieux). Populations of *E. coli* O157:H7 were determined on sorbitol MacConkey agar (Oxoid CM 813), supplemented with cefixime (50 μg/l) and potassium tellurite (2.5 mg/l), after incubation at 37°C for 24 h [18]. Sorbitol nonfermenting (colorless) colonies were selected for confirmatory testing with a rapid latex agglutination assay for *E. coli* O157 (Oxoid DR 620).

Analyses of the gaseous atmospheres inside the packages

On each sampling date (days 0, 2, 5, 7, 10, 12), gases within three of each package type were analysed using an O_2 and CO_2 gas analyser (PBI-Dansensor, PBI Development, Denmark, Model TIA-III LV).

Statistical analyses

All experiments were carried out in duplicate and replicated twice. At each analysis time material from duplicate packs was serially

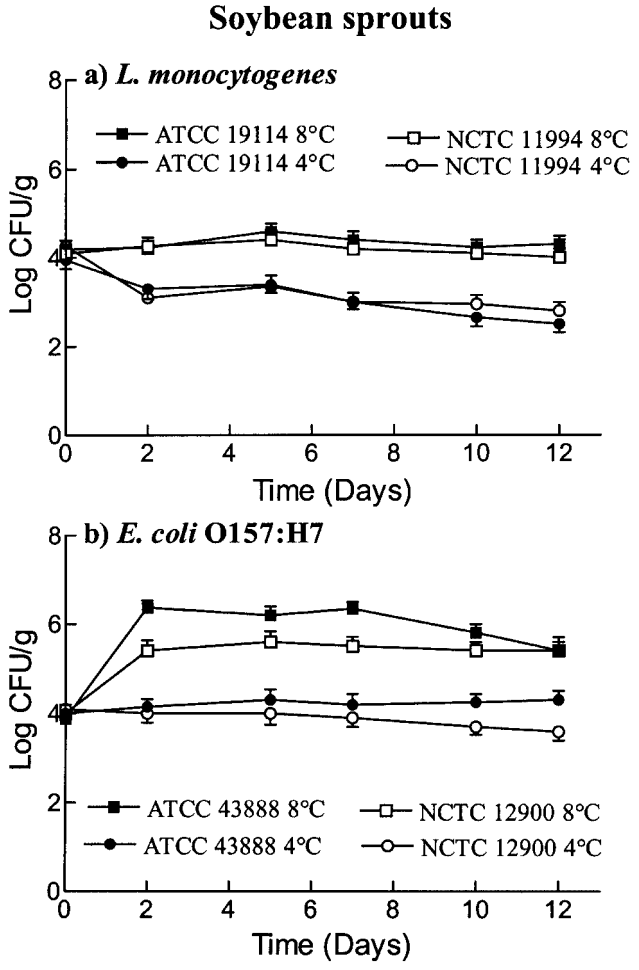

Figure 4 Survival and growth of (a) *L. monocytogenes* (strains ATCC 19114 and NCTC 11994) and (b) *E. coli* O157:H7 (strains 43888 and 12900) on packaged soybean sprouts during storage at 8°C and 4°C. Reported populations represent the means of four values. Error bars show standard deviations.

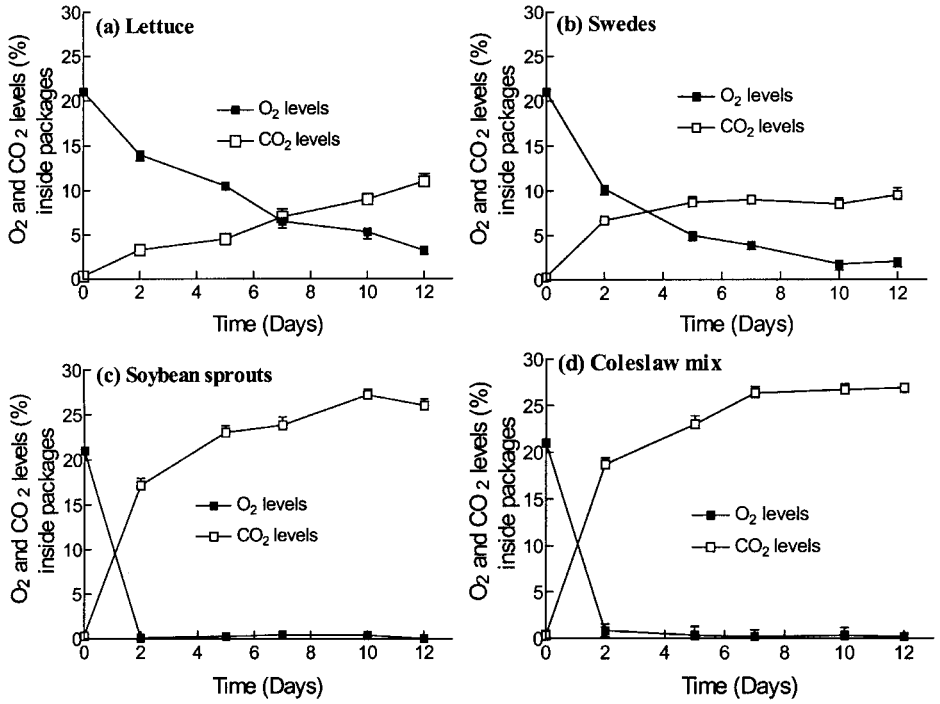

Figure 5 Changes in gas (O_2 and CO_2) levels inside packages of (a) shredded lettuce, (b) diced swedes, (c) soybean sprouts and (d) dry coleslaw mix during storage at 8°C. Values are the means of six determinations. Error bars show standard deviations.

diluted and plated in the duplicate. Reported populations therefore represent the means of four values. Means were separated using the least significant difference test at the 5% level. Figures 1–5 show the means±standard deviation. Statistical differences were determined using Student's *t*-test after analysis of variance.

Results

Figure 1 shows the survival and growth of two strains of *L. monocytogenes* and of *E. coli* O157:H7 on diced packaged swedes during storage at 4 and 8°C. *L. monocytogenes* populations gradually increased ($P<0.05$) during storage in packs of diced swedes stored at 8°C. Populations had increased by approximately 1 log cycle by day 12 of storage. The two strains of *E. coli* O157:H7 behaved differently on swedes, with strain ATCC 43888 generally surviving and growing better at 8°C than strain NCTC 12900. Numbers of *E. coli* O157:H7 strain ATCC 43888 increased (by approximately 1 log cycle) during storage on packaged swedes at 8°C, whereas populations of strain NCTC 12900 slightly increased between days 2 and 5, but decreased thereafter. *L. monocytogenes* populations did not change significantly ($P>0.05$) on swedes held at 4°C between days 0 and 10, but slightly increased between days 10 and 12, whereas *E. coli* O157:H7 populations declined (by 0.5 log cycles) during storage at 4°C.

Survival and growth of (a) *L. monocytogenes* and (b) *E. coli* O157:H7 on shredded packaged lettuce during storage at 4 and 8°C are shown in Figure 2. Populations of *L. monocytogenes* and *E. coli* O157:H7 significantly increased ($P<0.05$) during storage on lettuce held at 8°C. *L. monocytogenes* populations had increased by approximately 1.5 log cycles during the 12-day storage period, whereas final population densities of *E. coli* O157:H7 were 6.5–7.0 $\log_{10}$ CFU/g. Populations of *L. monocytogenes* and *E. coli* O157:H7 did not change significantly ($P>0.05$) on lettuce stored at 4°C.

Survival of *L. monocytogenes* and *E. coli* O157:H7 on packaged dry coleslaw mix during storage is shown in Figure 3. *L. monocytogenes* numbers in packs of coleslaw mix decreased by 1.5 log cycles during storage for 12 days at 8°C, and by approximately 2.0 log cycles when samples were held at 4°C. Populations of *E. coli* O157:H7 on coleslaw mix had increased by approximately 1.5 log cycles by day 5, but populations declined by 1 to 2 log cycles, depending on strain, with further storage at 8°C. At 4°C, populations of *E. coli* O157:H7 decreased (by 1 to 1.5 log cycles), but viable cells were still detected at the end of the storage period.

Survival and growth of *L. monocytogenes* and *E. coli* O157:H7 on packaged soybean sprouts during storage at 4 and 8°C are shown in Figure 4. *L. monocytogenes* numbers on soybean sprouts did not change significantly during storage at 8°C, but decreased by 1.5 log cycles ($P<0.05$) when samples were stored at 4°C. Populations of *E. coli* O157 strain 43888 on soybean sprouts (8°C) increased (by approximately 2.5 log cycles) between days 0 and 2, did not change significantly ($P>0.05$) between days 2 and 5, and declined during subsequent storage at 8°C. Numbers of strain 12900 also increased (by approximately 2.5 log cycles) between days 0 and 2, but did not change significantly ($P>0.05$) for the remainder of the storage period. When soybean sprouts were stored at 4°C, numbers of strain 43888 did not change significantly ($P>0.05$), whereas populations of strain 12900 gradually decreased (by approximately 0.5 log cycles) during storage.

The RTU vegetables were sealed in packages initially enclosing air. During storage, the gas atmospheres within packages were modified, mainly as a result of the respiration of the packaged vegetables. The concentrations of O_2 and CO_2

achieved within the packs varied with the packaged product (Figure 5). With lettuce, levels of CO_2 increased to 10–12% and O_2 levels fell to 3–4%. During the same period, O_2 levels fell to 2% and CO_2 levels had risen to 8–10% in packs of diced swedes. Dry coleslaw mix has a relatively high respiration rate, and higher levels of CO_2 were reached in packages of coleslaw than those attained in packs of lettuce or swedes. Inside packs of coleslaw, CO_2 levels rose to 25–27% and O_2 levels fell to 0–1% during the 12-day storage period. During the same time, packs containing soybean sprouts achieved similar gas levels to packs containing coleslaw.

Discussion

During these investigations, the two strains of *L. monocytogenes* generally behaved similarly on vegetable products and at both temperatures. *E. coli* O157:H7 survived and grew better than *L. monocytogenes* on most packaged vegetables, and strain ATCC 43888 generally survived better than strain NCTC 12900. Survival and growth patterns for *L. monocytogenes* and *E. coli* O157:H7 were dependent on product type, package atmosphere and storage temperature.

The ability of *L. monocytogenes* to survive and grow on RTU vegetables has previously been demonstrated [7,9,10,21,28,32]. The rate of growth was significantly reduced when the storage temperature was decreased [7,10]. The effect of storage temperature (5, 12, 21°C) on survival and growth of *E. coli* O157:H7 inoculated on shredded iceberg lettuce was examined [1]. Populations of viable *E. coli* O157:H7 significantly decreased on lettuce stored at 5°C and significantly increased during storage at 12 or 21°C.

Gas atmospheres within packages were modified, mainly as a result of the respiration of the packaged vegetables. Gas atmospheres within packs of lettuce and swedes (CO_2 levels of 9% to 12% and O_2 levels of 2% to 4%) were not inhibitory to growth of *L. monocytogenes* or *E. coli* O157:H7. Previous work showed that CO_2 concentrations of 5% to 10% did not inhibit growth of *L. monocytogenes*, and increasing the CO_2 concentration to 20% reduced the growth rate, but not the maximum population densities reached [4,22]. Hao and Brackett [25] concluded that growth of *E. coli* O157:H7 was not inhibited by gas mixtures containing up to 10% CO_2 at 5 or 10°C. Other workers reported that CO_2 concentrations of 30% had no inhibitory effect on growth of *E. coli* O157:H7 on shredded lettuce stored at 13 or 22°C [16].

Coleslaw was largely unsuitable for growth of *Listeria* presumably due to a combination of factors, such as the antibacterial effects of carrot on *Listeria* species [8,31], competition from the extensive indigenous microbial population (10^7 to 10^8 CFU g^{-1} on day 0, data not shown) of the cabbage/carrot mixture, and inhibitory effects from the relatively high CO_2 levels (25 to 30%) within the packs. The inhibitory effects of CO_2 are related to reduction of the intracellular and extracellular pH, and interference with cellular metabolism [14,17,19,26]. The quantity and quality of the background microflora may also affect growth of *L. monocytogenes*. Strains of lactic acid bacteria, enterobacteria and pseudomonads inhibit the growth of *L. monocytogenes* and other pathogens on minimally processed vegetables [3,12,22,23,33].

The inclusion of shredded carrots in the dry coleslaw mix may have also affected survival and growth of *E. coli* O157:H7. Abdul-Raouf *et al* [1] reported that populations of *E. coli* O157:H7 numbers on shredded carrots (12°C) increased during initial days of storage, and subsequently declined on extended storage. A known carrot phytoalexin, 6-methoxymellein, inhibits the growth of several fungi and bacteria, and may also be inhibitory to *E. coli* O157:H7 [1,29].

Soybean sprouts did not support growth of *L. monocytogenes*, due presumably to competition from the high populations of background microflora (10^8 CFU g^{-1} on day 0, data not shown), inhibition from the relatively high levels of CO_2 present within packs (25–30%), and the more limited nutrient availability of intact vegetables. *E. coli* O157 grew better on soybean sprouts than *L. monocytogenes*. Packaging vegetables under an atmosphere containing 3% O_2 and 97% N_2 had no apparent effect on growth of populations of *E. coli* O157:H7, in comparison to vegetables that were sealed in packs initially enclosing air [1]. In addition, CO_2 had no inhibitory effect on growth of *E. coli* O157:H7 on shredded lettuce stored at 13 or 22°C, and growth increased in an atmosphere of $O_2/CO_2/N_2$: 5/30/65, compared to growth in air [16]. *E. coli* O157:H7 was more competitive against spoilage microorganisms than *Salmonella* [21], and due to its high acid tolerance [2,13], is hypothetically more resistant to the fermentation end-products of lactic acid populations.

Conclusions

Survival and growth patterns for *L. monocytogenes* and *E. coli* O157:H7 were dependent on product type, package atmosphere, storage temperature and bacterial strain. *E. coli* O157:H7 generally survived and grew better than *L. monocytogenes* on packaged vegetables. Storage at 4°C enabled survival of both pathogens on all products throughout the storage period. The work underlines the importance of strict temperature control from processing to consumption. Refrigerated temperatures must be maintained during transportation, distribution, storage or handling in supermarkets and by consumers. It is essential that contamination of produce be minimized through the use of good agricultural and strict hygiene practices, and that HACCP programs specific for the pathogen of concern be applied at all stages of production.

Acknowledgements

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