

Survival and Growth of *Escherichia coli* O157:H7 on Salad Vegetables

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The influence of modified-atmosphere packaging, storage temperature, and time on survival and growth of *Escherichia coli* O157:H7 inoculated onto shredded lettuce, sliced cucumber, and shredded carrot was determined. Growth of psychrotrophic and mesophilic microorganisms and changes in pH and sensory qualities of vegetables, as judged by subjective evaluation, were also monitored. Packaging under an atmosphere containing 3% oxygen and 97% nitrogen had no apparent effect on populations of *E. coli* O157:H7, psychrotrophs, or mesophiles. Populations of viable *E. coli* O157:H7 declined on vegetables stored at 5°C and increased on vegetables stored at 12 and 21°C for up to 14 days. The most rapid increases in populations of *E. coli* O157:H7 occurred on lettuce and cucumbers stored at 21°C. These results suggest that an unknown factor(s) associated with carrots may inhibit the growth of *E. coli* O157:H7. The reduction in pH of vegetables was correlated with initial increases in populations of *E. coli* O157:H7 and naturally occurring microfloras. Eventual decreases in *E. coli* O157:H7 in some samples, e.g., those stored at 21°C, are attributed to the toxic effect of accumulated acids. Changes in visual appearance of vegetables were not influenced substantially by growth of *E. coli* O157:H7. The ability of *E. coli* O157:H7 to growth on raw salad vegetables subjected to processing and storage conditions simulating those routinely used in commercial practice has been demonstrated.

Human gastrointestinal illness caused by enteropathogenic, enteroinvasive, and enterotoxigenic *Escherichia coli* has been recognized for several decades (18). It was not until 1975 that enterohemorrhagic *E. coli*, which includes *E. coli* O157:H7 and *E. coli* O26:H11, was isolated from a woman with bloody diarrhea, and it was not until 1982 that *E. coli* O157:H7 was associated with two food-related outbreaks of hemorrhagic colitis in the United States (27). Both outbreaks were linked epidemiologically to eating ground-beef sandwiches. Additional outbreaks that have subsequently been documented in several countries are summarized by Doyle (13) and Padhye and Doyle (26). These outbreaks have implicated foods other than beef as vehicles of *E. coli* O157:H7. Raw milk was documented as a vehicle of transmission in two cases of hemorrhagic colitis and hemolytic uremic syndrome (21). Cheese and turkey roll sandwiches (29) and ham and turkey sandwiches (10) have been identified as vehicles of *E. coli* O157:H7 in outbreaks in Canada and England, respectively. McGowan et al. (22) isolated *E. coli* O157:H7 from a countryside reservoir, providing evidence that the organism can be waterborne.

Dairy cattle have been identified as a reservoir for *E. coli* O157:H7 (11, 21, 24). Dairy calves apparently harbor the organism most frequently (25). *E. coli* has been reported to colonize the cecae of chickens (2). Considering that *E. coli* O157:H7 has been isolated from retail samples of pork, lamb, and chicken meats, it is possible that swine, sheep, and poultry are also carriers.

Opportunities for cross-contamination of meats and other types of foods with *E. coli* O157:H7 during processing, handling, and marketing are substantial. Contamination of raw salad vegetables with *E. coli* O157:H7 would be partic-

ularly likely to occur during the assembling of ready-to-eat meals which also include beef or other potential carriers of the organism and in preparation kitchens of food service establishments. The possibility of *E. coli* O157:H7 being present on raw vegetables originating from agronomic systems employing irrigation with contaminated water should also not be disregarded. Forty-nine percent of the participants at a medical conference who ate salads containing raw vegetables were infected with enteropathogenic *E. coli* (23).

Although modified-atmosphere packaging is known to extend the shelf life of raw vegetables, such processing treatments are also known to increase the possibility of pathogenic bacteria reaching large populations (5, 6, 8). *Escherichia coli* O157:H7 is capable of growing at 8°C (30), a temperature below which ready-to-eat meals and lightly processed salad vegetables may be exposed for several hours during marketing and on restaurant buffet counters. However, the survival and growth characteristics of *E. coli* O157:H7 on raw vegetables have not been investigated. This article reports the results of studies designed to determine the effects of modified-atmosphere packaging, storage temperature, and time on the behavior of the organism on shredded lettuce, sliced cucumber, and shredded carrot.

MATERIALS AND METHODS

Vegetables investigated. Iceberg lettuce was purchased from a retail grocer in Griffin, Ga. The outer leaves and core were removed and discarded. The remaining leaves were then shredded into pieces 2 to 5 mm wide. Sliced cucumber (5-mm disks) and shredded carrot were purchased from a wholesale produce company in Forest Park, Ga. These vegetables were processed in accordance with specifications established by a large fast-food chain restaurant. All vege-

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tables were kept at 2 to 5°C between the time of purchase and initiation of experiments, which never exceeded 4 h.

Preparation of inocula. Five strains of *E. coli* O157:H7 were included in the study. Strain 301C (a chicken isolate) was kindly provided by Donald E. Conner, Auburn University. Strains 204P (a pork isolate), 505B (a beef isolate), A9218-CL (a human isolate) and 45753-35 (a human isolate) were also investigated. Stock cultures were maintained at 5°C on tryptic soy agar (Difco, Detroit, Mich.) and activated in tryptic soy broth (pH 7.0) at 35°C. Cultures were transferred by loop inocula twice at 24-h intervals to 10 ml of tryptic soy broth in screw-cap tubes (16 by 150 mm). A third 24-h transfer was made to 100 ml of tryptic soy broth in 250-ml Erlenmeyer flasks. These cultures (24 h, 35°C) were combined in equal volumes to serve as a five-strain mixture for inoculating test vegetables.

Procedure for inoculating vegetables. Two populations of *E. coli* O157:H7 inoculum on vegetables, in addition to uninoculated controls, were studied. A large-population suspension (7.5×10^5 to 1.2×10^6 CFU/ml) of *E. coli* O157:H7 was prepared by adding 10 ml of the undiluted five-strain mixture to 10 liters of sterile deionized water; a small-population suspension (1.0×10^3 to 9.7×10^3 CFU/ml) was prepared by adding 10 ml of the diluted (10^{-3} in 0.1 M potassium phosphate buffer, pH 7.0) five-strain mixture to 10 liters of sterile deionized water.

Approximately 4 kg of each vegetable was separately placed in a perforated container, submerged in sterile deionized water, gently washed for 1 min, and drained. Vegetables were then submerged in large- or small-population inocula or in sterile deionized water (control) for 1 min. Again, vegetables were gently mixed during the submersion period. The drained vegetables were then packaged.

Packaging procedure and storage conditions. Uninoculated (control) and inoculated lettuce samples (140 to 150 g) were placed in polyolefin L-Bags (O_2 transmission rate, 3,000 $cm^3/m^2/24$ h at 23°C and 0% relative humidity; CO_2 transmission rate, 9,800 $cm^3/m^2/24$ h at 23°C and 0% relative humidity; water transmission rate, 0.65 ml/100 in^2 [ca. 645 $cm^2/24$ h at 38°C and 100% relative humidity] (Cryovac Inc., Duncan, S.C.); cucumbers and carrots were placed in polyolefin PD-961 Bags (O_2 transmission rate, 7,000 $cm^3/m^2/24$ h at 23°C and 0% relative humidity; CO_2 transmission rate, 20,500 $cm^3/m^2/24$ h at 23°C and 0% relative humidity; water transmission rate, 1 g/100 $in^2/24$ h at 38°C and 100% relative humidity) (Cryovac). The film used to fabricate these bags was formulated to enhance the shelf life of raw vegetables. Equal numbers of bags were sealed under ambient atmospheric gas conditions and under an initially modified atmosphere containing 3% oxygen and 97% nitrogen. The procedure for modifying the atmosphere consisted of evacuating and flushing bags containing the vegetables five times before sealing the bags. Packages of vegetables were incubated at 5 or 12°C for 0, 3, 7, 10, and 14 days or at 21°C for 0, 3, and 7 days before the vegetables were subjected to microbiological and subjective sensory analyses.

Procedures for enumeration of microorganisms. Populations of *E. coli* O157:H7 in 24-h cultures, five-strain mixtures, and inoculum suspensions (large and small populations) were enumerated by surface plating duplicate samples (0.1 ml) which were serially (1:10) diluted in 0.1% peptone (pH 7.0) on sorbitol MacConkey agar (SMA) (pH 7.1; Unipath Oxoid US, Columbia, Md.) supplemented with 4-methylumbelliferyl- β -D-glucuronide (MUG) (0.2 g/liter). The addition of MUG to SMA would not be necessary to enumerate *E. coli* O157:H7 in inoculum suspensions but did

facilitate detection of O157:H7 strains inoculated onto vegetables. The fact that *E. coli* O157:H7 is sorbitol negative and MUG fluorescence negative aided in differentiating test strains from non-O157:H7 *E. coli* strains which might have been present in vegetable samples. MUG reagent was added to heat-sterilized (121°C for 15 min) molten (47 to 50°C) SMA. The modified SMA was deposited (18 to 20 ml) into 90-cm-diameter petri plates and allowed to dry at room temperature before being used to enumerate *E. coli* O157:H7. Plates on which samples had been spread with a sterile bent glass rod were incubated at 35°C for 20 to 22 h before colonies of *E. coli* O157:H7 were counted.

Duplicate 50-g samples of vegetables subjected to various storage times and temperatures were analyzed for populations of *E. coli* O157:H7, mesophilic aerobic microorganisms, and psychrotrophic microorganisms. Samples were combined with 200 ml of sterile 0.1% peptone in a sterile polyethylene bag and pummeled with a stomacher for 2 min. Wash fluid was serially diluted and surface plated (0.1 ml) in duplicate on modified SMA and quadruplicate on plate count agar (Difco, Detroit, Mich.). The modified SMA plates were incubated at 35°C for 20 to 22 h before presumptive colonies of *E. coli* O157:H7 were counted. One-half of the plate count agar plates inoculated with all dilutions of samples were incubated at 30°C for 48 h. Microorganisms forming colonies on these plates are reported as aerobic mesophiles. The other half of the inoculated plate count agar plates were incubated at 7°C for 10 to 12 days before enumeration of psychrotrophic microorganisms.

Samples in which *E. coli* O157:H7 was anticipated to be present at populations of less than 10/g were subjected to enrichment in modified Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) (14). Vegetables (25 g) were combined with 225 ml of broth in 500-ml Erlenmeyer flasks and incubated at 37°C for 18 h on a rotary shaker (150 rpm). Cultures were then streaked on modified SMA and incubated at 35°C for 20 to 24 h before being examined for presumptive *E. coli* O157:H7 colonies. Samples yielding colonies eventually confirmed by biochemical and serological tests to be *E. coli* O157:H7 were recorded as positive.

Randomly selected presumptive *E. coli* O157:H7 colonies were confirmed with an API-20E miniaturized diagnostic kit (Analytab, Div. Sherwood Medical, Plainview, N.Y.), the *E. coli* O157:H7 latex agglutination assay (Unipath Oxoid US), and the Bacto *E. coli* H antiserum H7 assay (Difco).

pH measurement. The pH of the primary diluent in which vegetable samples were pummeled was measured at each time of microbiological analysis with a Digital 112 pH meter (Corning Co., Corning, N.Y.).

Statistical analysis. Data were subjected to the Statistical Analysis System (SAS Institute, Cary, N.C.) for analysis of variance and Duncan's multiple range tests. Each value presented represents the mean of eight values (duplicate values from duplicate samples analyzed from two independent trials).

RESULTS

Shredded lettuce. Populations of *E. coli* O157:H7 on shredded lettuce stored at 5°C significantly ($P \leq 0.05$) decreased during the 14-day storage period, whereas the organism grew on lettuce stored at 12 and 21°C (Table 1). Significant increases were detected within 7 and 3 days when lettuce initially containing 2.2×10^5 CFU/g was kept at 12 and 21°C, respectively. An initially small population (2.4×10^2 CFU/g) also increased significantly within 3 days at 21°C. *E. coli*

TABLE 1. Populations of *E. coli* O157:H7 on shredded lettuce

Storage temp (°C)	Initial package atmosphere	Inoculum ^a	log ₁₀ (CFU/g) after indicated storage time (days) ^b				
			0	3	7	10	14
5	Air	Control	0	0	0	0	0
		Small	b 2.38	+	+	+	+
		Large	a 5.34 a	5.06 b	5.38 a	4.91 bc	4.23 c
	Modified ^c	Control	0	0	0	0	0
		Small	b 2.38	+	+	+	0
		Large	a 5.34 a	4.97 b	4.88 b	4.88 b	3.10 c
12	Air	Control	0	0	0	0	0
		Small	b 2.38	+	+	+	+
		Large	a 5.34 d	6.85 d	7.29 c	7.48 b	7.52 a
	Modified	Control	0	0	0	0	0
		Small	b 2.38	+	+	+	+
		Large	a 5.34 d	7.04 cd	7.66 bc	7.74 ab	7.95 a
21	Air	Control	0	0	0	0	0
		Small	b 2.38 c	b 6.66 b	b 8.45 a		
		Large	a 5.34 c	a 8.47 b	a 8.83 a		
	Modified	Control	0	0	0	0	0
		Small	b 2.38 c	b 6.70 b	b 8.78 a		
		Large	a 5.34 c	a 8.46 b	a 8.68 a		

^a Small inoculum of *E. coli* O157:H7 = 10^{2.38} CFU/g; large inoculum = 10^{5.34} CFU/g.

^b Means of eight values. Values in the same row which are not followed by the same letter are significantly different ($P \leq 0.05$). Values in the same column for each storage temperature which are not preceded by the same letter are significantly different. A value of 0 indicates that no *E. coli* O157:H7 was detected by enrichment. A value of + indicates that the sample was positive (≥ 1 CFU/25 g of lettuce) by enrichment.

^c Composition of modified atmosphere: 3% O₂ and 97% N₂.

O157:H7 was detected on all inoculated lettuce samples under all combinations of experimental parameters, with one exception, viz., small-inoculum samples packaged under a modified atmosphere and stored at 5°C for 14 days. Nevertheless, the composition of gas initially present in packages did not, overall, have an influence on growth and survival patterns of *E. coli* on shredded lettuce subjected to the storage time and temperature treatments investigated in this study. For any given storage temperature on any given day of analysis, considering each inoculum population sepa-

rately, the composition of the gaseous atmosphere within packages did not influence the population of *E. coli* O157:H7.

Populations of psychrotrophs on shredded lettuce stored at 5 and 12°C are summarized in Table 2. Significant increases were detected within 3 or 7 days and were followed by additional significant increases as storage time progressed. The population of psychrotrophs naturally present on lettuce was large enough to mask any effect that *E. coli* O157:H7 might have had on counts throughout the 14-day

TABLE 2. Populations of psychrotrophic and mesophilic microorganisms on shredded lettuce

Storage temp (°C)	Initial package atmosphere	Inoculum ^a	log ₁₀ (CFU/g) after indicated storage time (days) ^b				
			0	3	7	10	14
5	Air	Control	4.89 d	6.85 d	ab 7.80 c	bc 7.97 b	b 8.11 a
		Small	4.68 b	6.88 b	ab 7.88 a	abc 8.01 a	b 8.01 a
		Large	5.09 c	6.62 c	b 7.13 b	c 7.83 b	b 8.00 a
	Modified ^c	Control	4.89 c	7.02 c	a 7.98 b	a 8.22 a	a 8.32 a
		Small	4.68 d	6.68 c	c 6.02 c	ab 8.16 b	a 8.31 a
		Large	5.09 b	6.72 b	ab 7.82 b	a 8.22 a	ab 8.20 a
12	Air	Control	4.89 b	bc 8.09 b	ab 8.64 b	c 8.78 b	a 9.23 a
		Small	4.68 d	bc 7.89 d	ab 8.62 c	c 8.76 b	b 8.94 a
		Large	5.09 c	c 7.77 c	b 8.59 b	c 8.73 b	b 8.90 a
	Modified	Control	4.89 c	a 8.32 c	a 8.78 b	a 9.04 a	ab 9.12 a
		Small	4.68 d	a 8.33 c	ab 8.74 b	b 8.83 b	b 8.98 a
		Large	5.09 d	ab 8.16 d	a 8.78 c	b 8.93 b	ab 9.08 a
21	Air	Control	5.04 b	9.00 a	ab 9.21 a		
		Small	4.92 c	9.14 b	a 9.48 a		
		Large	5.49 b	9.08 a	ab 9.27 a		
	Modified	Control	5.04 c	9.00 b	ab 9.28 a		
		Small	4.92 c	9.14 a	b 8.84 b		
		Large	5.49 b	8.99 b	ab 9.34 a		

^a Small inoculum of *E. coli* O157:H7 = 10^{2.38} CFU/g; large inoculum = 10^{5.34} CFU/g.

^b Means of eight values. Values in the same row which are not followed by the same letter are significantly different ($P \leq 0.05$). Values in the same column for each storage temperature which are not preceded by the same letter are significantly different.

^c Composition of modified atmosphere: 3% O₂ and 97% N₂.

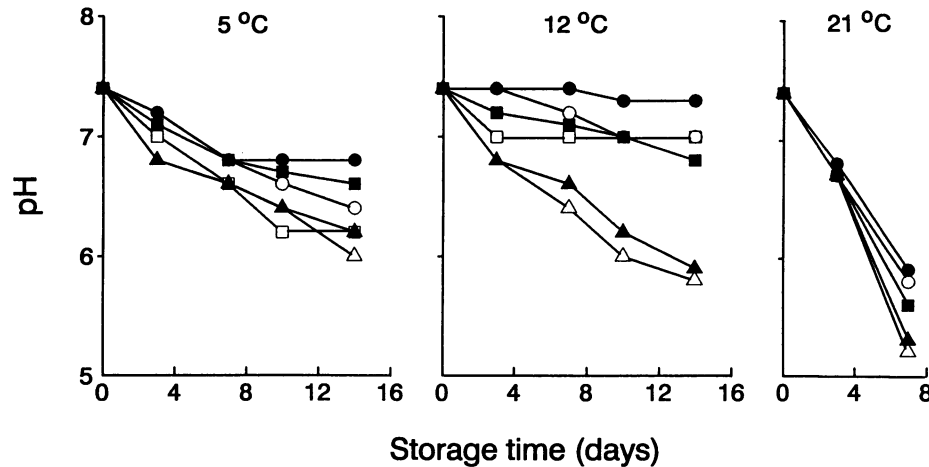


FIG. 1. Changes in pH of shredded lettuce during storage at 5, 12, and 21°C for up to 14 days. Open symbols represent packaging under an ambient atmosphere, and closed symbols represent packaging under a modified atmosphere (3% O₂, 97% N₂). Symbols: ○ and ●, control; □ and ■, small initial population of *E. coli* O157:H7; △ and ▲, large initial population of *E. coli* O157:H7.

storage period. The modified atmosphere may have favored the growth of psychrotrophs at 12°C, but a strong case for this effect cannot be made.

Populations of mesophilic aerobic microorganisms on lettuce stored at 21°C are also listed in Table 2. Significant increases at 3 and again at 7 days are not surprising. The initial atmospheric gas composition in packages had no apparent effect on growth of mesophiles.

The pH values of lettuce subjected to various test parameters are illustrated in Fig. 1. The most rapid rate of decrease in pH occurred in lettuce stored at 21°C. Reductions in pH of lettuce stored at 5°C and of lettuce inoculated with a large population of *E. coli* O157:H7 and then stored at 12°C were less marked but clearly evident. The pHs of uninoculated lettuce and lettuce inoculated with small populations of *E. coli* O157:H7 changed little throughout storage at 12°C. The initial composition of atmospheric gas in packages had no apparent effect on changes in pH.

Subjective evaluations of appearance of lettuce were made throughout the 14-day test period. Deterioration of appearance was judged as a loss of typical color, loss of turgidity, development of sliminess, and weeping of tissue fluid. Deterioration occurred less rapidly as the storage temperature was decreased from 21 to 12 and, finally, to 5°C. Changes in appearance of lettuce were also delayed in packages treated with the modified atmosphere, regardless of the storage temperature.

Sliced cucumber. As observed in studies of lettuce, populations of *E. coli* O157:H7 significantly ($P \leq 0.05$) declined during storage of sliced cucumber at 5°C and increased at 12 and 21°C (Table 3). Significant increases after 3 days of storage at 21°C were followed by significant increases at 7 days. Survival may have been favored at 5°C when cucumbers were packaged under ambient atmospheric conditions in comparison with the modified atmosphere. When enrichment procedures were used, no *E. coli* O157:H7 was detected on sliced cucumber initially containing a small inoculum (1.8×10^2 CFU/g) after 7 and 10 days of storage at 5°C; cucumbers with the same initial population of *E. coli* O157:H7 were negative for the organism after 10 days at 12°C. The initial atmospheric gas composition within pack-

ages did not influence survival and growth of *E. coli* O157:H7 on sliced cucumber.

The population of psychrotrophs on sliced cucumber stored at 5°C increased significantly throughout the 10-day storage period (Table 4). Populations on cucumbers stored at 12°C increased significantly during the first 3 days and then remained constant. Mesophilic aerobic populations on cucumbers stored at 21°C also increased significantly during the first 3 days of storage.

Changes in pH of sliced cucumber are shown in Fig. 2. The rate of decrease in pH increased as the storage temperature increased. The level of inoculum and type of packaging had no effect on changes in pH.

The appearance of sliced cucumber stored at 5°C remained good throughout the 10-day test. Deterioration, as subjectively judged by loss of typical color, development of translucence, loss of turgidity, weeping of tissue fluid, and development of a slimy appearance, was observed after storage at 12°C for 7 days and 21°C for 3 days. The level of inoculum and packaging conditions had no effect on changes in appearance of sliced cucumber.

Shredded carrot. Populations of *E. coli* O157:H7 decreased significantly on shredded carrot stored at 5°C but, with the exception of samples initially containing a small inoculum and packaged under the modified atmosphere, survived throughout the 14-day incubation period (Table 5). Unlike *E. coli* O157:H7 on lettuce and cucumbers, initially small populations of this organism on shredded carrots stored at 12°C declined within the first 3 days of storage and did not increase throughout the study. Populations in large-inoculum samples stored at 12°C and small- and large-inoculum samples stored at 21°C increased during the first 3 days but leveled off or declined significantly thereafter. Atmospheric gas composition did not affect the behavior of *E. coli* O157:H7. Regardless of the combination of test parameters, the maximum populations of *E. coli* O157:H7 observed on shredded carrot were substantially smaller than the populations observed on lettuce or cucumber subjected to the same set of parameters.

Populations of psychrotrophs on shredded carrot stored at 5 and 12°C and mesophiles on shredded carrot stored at 21°C

TABLE 3. Populations of *E. coli* O157:H7 on sliced cucumbers

Storage temp (°C)	Initial package atmosphere	Inoculum ^a	log ₁₀ (CFU/g) after indicated storage time (days) ^b			
			0	3	7	10
5	Air	Control	0	0	0	0
		Small	b 2.26	+	0	0
		Large	a 5.10 a	a 4.60 b	a 4.27 c	a 3.43 d
	Modified ^c	Control	0	0	0	0
		Small	b 2.26	+	0	0
		Large	a 5.05 a	b 4.45 b	b 3.84 c	b 2.27 d
12	Air	Control	0	0	0	0
		Small	b 2.26	+	+	+
		Large	a 5.05 c	6.08 a	5.84 b	a 5.74 b
	Modified	Control	0	0	0	0
		Small	b 2.26	+	+	0
		Large	a 5.05 c	5.19 c	5.85 a	b 5.54 b
21	Air	Control	0	0	0	0
		Small	b 2.26 b	c 4.64 a	b 3.08 b	
		Large	a 5.05 b	a 7.26 a	a 4.64 b	
	Modified	Control	0	0	0	0
		Small	b 2.26 b	c 4.14 a	b 2.64 b	
		Large	a 5.05 b	b 7.03 a	a 4.62 b	

^a Small inoculum of *E. coli* O157:H7 = 10^{2.26} CFU/g; large inoculum = 10^{5.05} CFU/g.

^b Means of eight values. Values in the same row which are not followed by the same letter are significantly different ($P \leq 0.05$). Values in the same column for each storage temperature which are not preceded by the same letter are significantly different ($P \leq 0.05$). A value of 0 indicates that no *E. coli* O157:H7 was detected by enrichment. A value of + indicates that the sample was positive (≥ 1 CFU/25 g of cucumber) by enrichment.

^c Composition of modified atmosphere: 3% O₂ and 97% N₂.

significantly increased with storage times (Table 6). The largest psychrotroph populations were detected on carrots stored at 12°C. Neither the population of *E. coli* O157:H7 in the inoculum nor the composition of atmospheric gas initially present in packages influenced the rate of growth of psychrotrophs or mesophiles on carrots.

Changes in pH of shredded carrots are shown in Fig. 3. The rate of decrease was not as dramatic as that observed for sliced cucumber but was more marked than that observed for shredded lettuce. As noted for cucumbers, the rate of

decrease in pH of carrots increased as the storage temperature increased. Compared with uninoculated carrots stored at 12 and 21°C, carrots inoculated with *E. coli* O157:H7 and stored at the same temperatures appear to have undergone a more rapid decrease in pH.

The appearance of shredded carrot stored at 5°C for 14 days was unchanged, while only slight deterioration was noted at 12°C. Carrots stored at 21°C showed visible deterioration within 3 days. Mold growth was evident after 7 days. Neither the population of the *E. coli* O157:H7 inoculum nor

TABLE 4. Populations of psychrotrophic and mesophilic microorganisms on sliced cucumbers

Storage temp (°C)	Initial package atmosphere	Inoculum ^a	log ₁₀ (CFU/g) after indicated storage time (days) ^b			
			0	3	7	10
5	Air	Control	5.30 c	7.43 c	8.74 b	8.93 a
		Small	5.65 c	7.74 c	8.79 b	9.00 a
		Large	5.67 c	7.47 c	8.76 b	8.96 a
	Modified ^c	Control	5.30 c	7.49 c	8.59 b	9.00 a
		Small	5.65 c	7.52 c	8.66 b	8.99 a
		Large	5.67 c	7.61 c	8.74 b	8.92 a
12	Air	Control	5.30 b	8.71 a	ab 8.75 a	8.82 a
		Small	5.65 b	8.70 a	ab 8.78 a	8.78 a
		Large	5.67 b	8.85 a	a 8.83 a	8.77 a
	Modified	Control	5.30 b	8.70 a	b 8.64 a	8.78 a
		Small	5.65 b	8.70 a	b 8.62 a	8.66 a
		Large	5.67 b	8.70 a	ab 8.73 a	8.65 a
21	Air	Control	5.43 b	b 8.73 a	a 8.59 a	
		Small	5.80 b	b 8.66 a	a 8.57 a	
		Large	5.80 c	a 9.00 a	b 8.43 b	
	Modified	Control	5.43 b	b 8.60 a	b 8.41 a	
		Small	5.80 b	b 8.62 a	b 8.29 a	
		Large	5.80 c	b 8.65 a	b 8.32 b	

^a Small inoculum of *E. coli* O157:H7 = 10^{2.26} CFU/g; large inoculum = 10^{5.05} CFU/g.

^b Means of eight values. Values in the same row which are not followed by the same letter are significantly different ($P \leq 0.05$). Values in the same column for each storage temperature which are not preceded by the same letter are significantly different ($P \leq 0.05$).

^c Composition of modified atmosphere: 3% O₂ and 97% N₂.

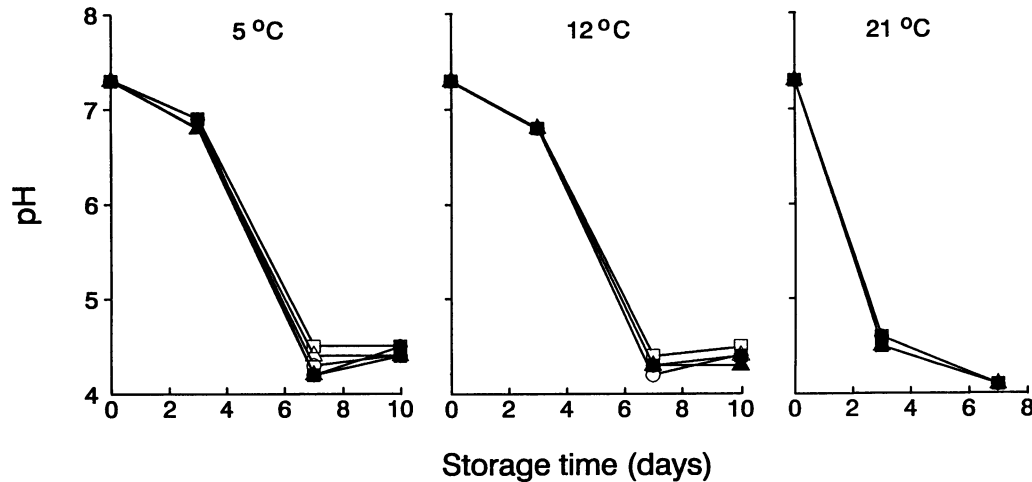


FIG. 2. Changes in pH of sliced cucumbers during storage at 5, 12, and 21°C for up to 10 days. Open symbols represent packaging under an ambient atmosphere, and closed symbols represent packaging under a modified atmosphere (3% O₂, 97% N₂). Symbols: ○ and ●, control; □ and ■, small initial population of *E. coli* O157:H7; △ and ▲, large initial population of *E. coli* O157:H7.

the initial atmospheric gas composition within packages had an effect on changes in the appearance of shredded carrot during the 14-day storage period.

DISCUSSION

Observations of the ability of pathogenic bacteria to grow on refrigerated, minimally processed salad vegetables are not new. *Listeria monocytogenes* can grow on shredded lettuce stored at 5°C (7) and on asparagus, broccoli, and cauliflower stored at 4°C (3). Callister and Agger (9) and Berrang et al. (3) reported that *Aeromonas hydrophila* can grow on raw vegetables stored at 5 and 4°C, respectively. Results from our studies of shredded lettuce, sliced cucumber, and shredded carrot show that *E. coli* O157:H7 can be

included as one of several pathogenic bacteria capable of growing on salad vegetables stored at refrigeration temperature, i.e., 12°C.

The behavior of *E. coli* O157:H7 on shredded carrot is of particular interest. We have observed that raw carrot juice is lethal to *L. monocytogenes* (6). Although the effect is not as marked, *E. coli* O157:H7 also appears to be inhibited or killed upon exposure to carrot tissue fluid, particularly at 5 and 12°C. 6-Methoxymellein, a known carrot phytoalexin, inhibits the growth of several fungi and bacteria (19) and may also be inhibitory or toxic to *E. coli* O157:H7.

As observed for *L. monocytogenes* and *A. hydrophila* (3, 4, 6, 7), the composition of the atmosphere initially present in vegetable packages had little or no effect on survival or

TABLE 5. Populations of *E. coli* O157:H7 on shredded carrots

Storage temp (°C)	Initial package atmosphere	Inoculum ^a	log ₁₀ (CFU/g) after indicated storage time (days) ^b				
			0	3	7	10	14
5	Air	Control	0	0	0	0	0
		Small	b 2.46	+	+	+	+
		Large	a 5.31 a	4.55 b	+	+	+
	Modified ^c	Control	0	0	0	0	0
		Small	b 2.46	+	+	+	0
		Large	a 5.31 a	4.13 b	+	+	+
12	Air	Control	0	0	0	0	0
		Small	b 2.46	+	+	+	+
		Large	a 5.31 d	7.10 a	6.78 b	6.61 bc	6.30 cd
	Modified	Control	0	0	0	0	0
		Small	b 2.46	+	+	+	+
		Large	a 5.31 b	6.71 a	6.70 a	6.68 a	6.61 a
21	Air	Control	0	0	0	0	0
		Small	b 2.46 b	b 6.06 a	c 3.84 b		
		Large	a 5.31 b	a 7.36 a	b 6.03 b		
	Modified	Control	0	0	0	0	0
		Small	b 2.46 b	b 5.73 a	c 4.21 b		
		Large	a 5.31 b	b 6.45 a	a 6.48 a		

^a Small inoculum of *E. coli* O157:H7 = 10^{2.46} CFU/g; large inoculum = 10^{5.31} CFU/g.

^b Means of eight values. Values in the same row which are not followed by the same letter are significantly different ($P \leq 0.05$). Values in the same column within each storage temperature which are not preceded by the same letter are significantly different. A value of 0 indicates that no *E. coli* O157:H7 was detected by enrichment. A value of + indicates that the sample was positive (≥ 1 CFU/25 g of carrot) by enrichment.

^c Composition of modified atmosphere: 3% O₂ and 97% N₂.

TABLE 6. Populations of psychrotrophic and mesophilic microorganisms on shredded carrots

Storage temp (°C)	Initial package atmosphere	Inoculum ^a	log ₁₀ (CFU/g) after indicated storage time (days) ^b				
			0	3	7	10	14
5	Air	Control	b 4.87 c	a 6.49 c	ab 8.21 b	b 8.40 b	a 8.67 a
		Small	b 4.87 c	a 6.55 c	a 8.44 b	b 8.37 b	a 8.82 a
		Large	a 5.03 c	ab 6.41 c	ab 8.38 b	ab 8.57 b	a 8.84 a
	Modified ^c	Control	b 4.87 d	bc 6.25 d	b 8.16 c	b 8.53 b	a 8.84 a
		Small	b 4.87 c	c 6.20 c	ab 8.31 c	a 8.71 b	a 8.89 a
		Large	a 5.03 c	c 6.21 c	ab 8.37 b	ab 8.59 b	a 8.80 a
12	Air	Control	b 4.87 d	c 8.16 d	bc 8.62 c	b 8.90 b	ab 9.21 a
		Small	b 4.87 c	abc 8.41 c	a 8.98 b	a 9.08 ab	abc 9.17 a
		Large	a 5.03 c	ba 8.47 c	abc 8.82 b	b 8.96 ab	c 9.06 a
	Modified	Control	b 4.87 d	bc 8.26 cd	c 8.48 c	b 8.93 b	a 9.26 a
		Small	b 4.87 d	a 8.50 c	ab 8.95 b	b 8.95 b	ab 9.19 a
		Large	a 5.03 c	abc 8.33 c	ab 8.92 b	b 8.88 b	bc 9.12 a
21	Air	Control	b 4.96 c	a 9.16 a	bc 8.70 b		
		Small	b 5.01 c	b 8.87 a	d 8.39 b		
		Large	a 5.40 c	b 8.92 a	bcd 8.62 b		
	Modified	Control	b 4.96 c	a 9.07 a	a 8.87 b		
		Small	b 5.01 c	b 8.79 a	cd 8.56 b		
		Large	a 5.40 b	b 8.76 a	ab 8.76 a		

^a Small inoculum of *E. coli* O157:H7 = 10²⁻⁴⁶ CFU/g; large inoculum = 10⁵⁻³¹ CFU/g.

^b Means of eight values. Values in the same row which are not followed by the same letter are significantly different (*P* ≤ 0.05). Values in the same column within each storage temperature which are not preceded by the same letter are significantly different.

^c Composition of modified atmosphere: 3% O₂ and 97% N₂.

growth of *E. coli* O157:H7. Under the treatment conditions investigated in this study, the potential beneficial effects of flushing vegetables with a modified atmosphere is not diminished by an enhanced effect on the rate of growth of *E. coli* O157:H7.

Growth of *E. coli* O157:H7 in Trypticase soy broth adjusted to pHs 4.5 and 5.0 with HCl and lactic acid, respectively, has been reported (16). Test broths were incubated at 37°C. Enteropathogenic *E. coli* can grow in brain heart infusion broth (pH 5.6) at 10°C (15). Results from our studies of sliced cucumber and shredded carrot stored at 12 and 21°C indicate that a reduction in pH to less than 5.0 may have had an adverse effect on the viability of *E. coli* O157:H7. The decrease in pH can be attributed in part to the fermentative capability of the organism. The cellular fluids of shredded

and sliced vegetables examined in this study contain substantial amounts of simple sugars and other nutrients that can be fermented and utilized by *E. coli* O157:H7. While these metabolic activities may ultimately be self-limiting, large populations of the organism can still be reached before death occurs.

The documented presence of salmonellae (28), *Shigella sonnei* (12), *L. monocytogenes* (17), and *A. hydrophila* (9, 20) on salad vegetables, coupled with the inefficiency of washing and sanitizing procedures administered for the purpose of removing microfloras (1), indicates the need for increased attention to microbiological safety implications associated with their consumption. The ability of *E. coli* O157:H7 to grow at 12 and 21°C on three types of salad vegetables has been demonstrated. Packaging, storage, and

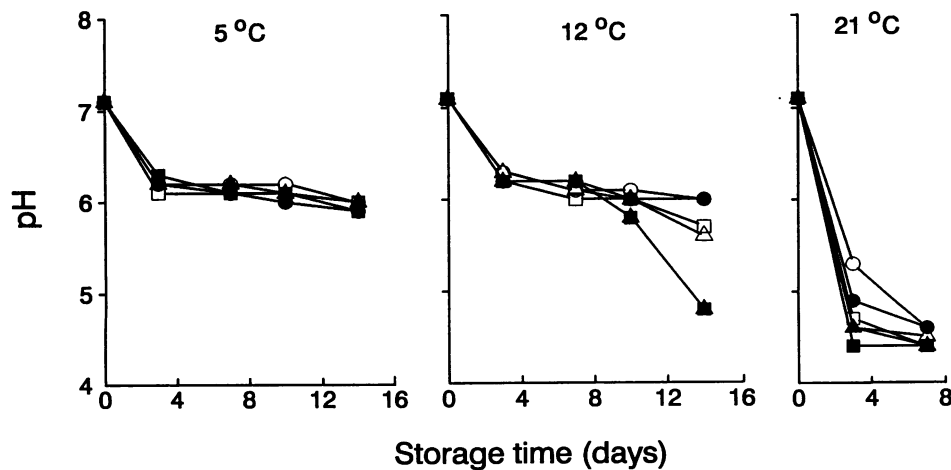


FIG. 3. Changes in pH of shredded carrots during storage at 5, 12, and 21°C for up to 14 days. Open symbols represent packaging under an ambient atmosphere, and closed symbols represent packaging under a modified atmosphere (3% O₂, 97% N₂). Symbols: ○ and ●, control; □ and ■, small initial population of *E. coli* O157:H7; △ and ▲, large initial population of *E. coli* O157:H7.

handling conditions not unlike those used in commercial practice will not prevent growth of the organism on these vegetables. Precautions should be taken to prevent contamination of salad vegetables with *E. coli* O157:H7 during production, harvesting, processing, marketing, and preparation in restaurants and homes.

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