*Journal of Food Protection, Vol. 64, No. 9, 2001, Pages 1334–1340*


# Survival of *Escherichia coli* O157:H7 on Strawberry Fruit and Reduction of the Pathogen Population by Chemical Agents

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MS 00-472: Received 29 December 2000/Accepted 10 April 2001

## ABSTRACT

Survival of *Escherichia coli* O157:H7 was studied on strawberry, a fruit that is not usually washed during production, harvest, or postharvest handling. Two strains of the bacteria were tested separately on the fruit surface or injected into the fruit. Both strains of *E. coli* O157:H7 survived externally and internally at 23°C for 24 h and at 10, 5, and −20°C for 3 days. The largest reduction in bacterial population occurred at −20°C and on the fruit surface during refrigeration. In all experiments, the bacteria inside the fruit either survived as well as or better than bacteria on the surface, and ATCC 43895 frequently exhibited greater survival than did ATCC 35150. Two strains of *E. coli* also survived at 23°C on the surface and particularly inside strawberry fruit. Chemical agents in aqueous solution comprising NaOCl (100 and 200 ppm), Tween 80 (100 and 200 ppm), acetic acid (2 and 5%), $Na_3PO_4$ (2 and 5%), and $H_2O_2$ (1 and 3%) were studied for their effects on reduction of surface-inoculated ($10^8$ CFU/ml) *E. coli* O157:H7 populations on strawberry fruit. Dipping the inoculated fruit in water alone reduced the pathogen population about 0.8 log unit. None of the compounds with the exception of $H_2O_2$ exhibited more than a 2-log CFU/g reduction of the bacteria on the fruit surface. Three percent $H_2O_2$, the most effective chemical treatment, reduced the bacterial population on strawberries by about 2.2 log CFU/g.

*Escherichia coli* O157:H7 has become recognized as a widespread foodborne pathogen *(11, 30, 33, 35)* that may cause serious illness and death. The earliest reports of *E. coli* O157:H7 outbreaks were associated with meat, primarily ground beef *(28),* and it was found that cattle were primary carriers for the pathogen *(6, 23).* Subsequently, other carriers were reported, including such diverse sources as deer *(26),* birds *(34),* and fruit flies *(19),* and it was shown that plant-derived foods also presented a disease threat from *E. coli* O157:H7 contamination. The largest reported *E. coli* O157:H7 outbreak, which affected about 6,000 people in Japan, was traced to contaminated radish sprouts *(37).* Other *E. coli* O157:H7-associated disease outbreaks were attributed to fresh produce and their products, including lettuce *(2, 21),* alfalfa sprouts *(9),* and unpasteurized apple cider *(4, 8, 32).* The latter association with apple products was especially notable because it demonstrated that *E. coli* O157:H7 was tolerant to low pH conditions existing in the products.

Recent studies have shown that the organism can survive for prolonged periods on freshly peeled Hamlin orange *(24),* watermelon, and cantaloupe *(12).* A study of broccoli, cucumber, and green pepper found that *E. coli* O157:H7 could survive on these produce items held at 4°C and maintain initial levels or grow at 15°C *(27). E. coli* O157:H7 also grew in wounds on apples *(20),* cut cucumbers *(1),* and shredded lettuce *(13).* Studies on radish sprouts suggest that *E. coli* O157:H7 contamination could be from contaminated hydroponic water *(14, 17).*

Based on the above findings, we were interested in the ability of *E. coli* O157:H7 to survive on strawberry fruit. Commercial strawberries in the United States are most frequently produced in large plantings grown on black plastic using irrigation with plastic tubing beneath the mulch. The fruit is not washed during the entire production, harvest, packing process, transportation, or marketing due to its high susceptibility to fungal (gray mold) deterioration, which is promoted by the presence of water. Thus, if the fruit were contaminated during production by, for example, wildlife excrement, irrigation water, or unsanitary handling practices during harvest or packing, it is conceivable that strawberries could pose a risk for infection following purchase by the consumer.

To date, there have been no reported incidents of contamination of strawberries with *E. coli* O157:H7. However, two multistate outbreaks of hepatitis A virus have been traced to frozen strawberries in the United States *(10, 16, 22).* There was a serious outbreak of foodborne illness recently in the United States on raspberries *(15),* another important berry crop. The latter involved a protozoan, *Cyclospora,* and was traced to fruit imported from Guatemala. The source of contamination was thought to be water used to produce the crop. Like strawberries, raspberries are not washed at harvest or packing due to possible promotion of fungal decay associated with water and a moist environment.

In the present study, we have investigated the capacity of two strains of *E. coli* O157:H7 to survive on strawberries at room temperature (23°C), two refrigeration temperatures (5 and 10°C), and frozen (−20°C). In addition, the capacity of five commonly used disinfecting or cleansing agents to

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reduce *E. coli* O157:H7 populations on inoculated fruit was investigated.

## MATERIALS AND METHODS

**Inoculum preparation.** *E. coli* O157:H7 strains ATCC 43895 and ATCC 35150 and *E. coli* ATCC 15597 and ATCC 43896 were obtained from the American Type Culture Collection, Rockville, Md. Bacteria were maintained on tryptic soy agar (Difco Laboratories, Detroit, Mich.) slants at 4°C with biweekly transfers to tryptic soy agar slants. Bacterial cultures for inoculation were prepared by three daily subcultures in tryptic soy broth (Difco) at 35°C before experiments. Final broth cultures were grown to approximately $10^9$ CFU/ml. The broth culture was diluted with phosphate buffer (pH 7.2) to yield a final inoculum.

**Survival of bacterial strains on strawberries.** Strawberries were purchased from a local supermarket, stored refrigerated at 5°C, and placed at room temperature about 4 h before use. Twenty-five microliters of a $10^7$ CFU/ml suspension of bacteria was placed as an external droplet on the side of each fruit midway between the cap and the apex (five fruit per sample). Additionally, 25 μl of the inoculum was injected into the cortex of a separate fruit (five fruit per sample). This was accomplished by inserting the syringe needle at a point midway between the cap and apex and moving the needle about two-thirds of the distance across the fruit and then injecting the inoculum. Food coloring was used in preliminary experiments to demonstrate that inoculum remained in the cortex after injection. There were two strains of *E. coli* O157:H7 used in each experiment; in addition, the room temperature studies included two strains of *E. coli*. Uninoculated control fruit were also included in the experiment. Treated and control fruit were immediately placed on a wire test tube rack, and the rack was inserted into a polyethylene bag, which formed an enclosure that did not touch the fruit. The storage conditions used in the survival studies were as follows: 23°C for 24 h; 10°C for 3 days; 5°C for 3 days; and −20°C for 3 days. The durations of the storage periods at 23°C and at refrigeration temperatures were chosen to avoid development of mold on the supermarket-purchased fruit. There were three replicates of each treatment per experiment, and each experiment was performed three times, with the exception of that at 10°C, which was performed twice.

**Chemical treatments for reducing *E. coli* O157:H7 on strawberries.** Strawberries (three fruit per sample) with their calyx removed were inoculated with *E. coli* O157:H7 strains ATCC 43895 and ATCC 35150 by submerging the fruit in bacterial suspension (approximately $10^8$ CFU/ml) for 5 s. The fruit were then removed and placed on a wire screen to air dry for 30 min. This yielded fruit inoculation levels of 6.48 ± 0.19 and 6.45 ± 0.20 log CFU/g for ATCC 43895 and ATCC 35150, respectively. The inoculated fruit were dipped for 1 min in aqueous solutions of the following compounds to evaluate effectiveness in removing the bacteria: 100 ppm (65 ppm free chlorine) and 200 ppm (130 ppm free chlorine) NaOCl made from commercial bleach containing 6% NaOCl; 100 and 200 ppm (wt/vol) Tween 80 (Fisher Scientific, Fair Lawn, N.J.); 2 and 5% (vol/vol) acetic acid from glacial acetic acid (Fisher Scientific); 2 and 5% (wt/vol) $Na_3PO_4$ from $Na_3PO_4 \cdot 12H_2O$ (Fisher Scientific); and 1 and 3% (vol/vol) hydrogen peroxide made from 30% hydrogen peroxide (VWR, West Chester, Pa.). Free chlorine in sodium hypochlorite solutions was determined with a chlorine test kit (Hach Co., Ames, Iowa) that has been approved by the U.S. Environmental Protection Agency. After submerging the fruit in the above solutions, they were immediately rinsed for 1 min with distilled water to remove the chemical residue. To determine the effectiveness of water alone in removing *E. coli* O157:H7 from fruit, a group of inoculated fruit was dipped twice in distilled water (1-min duration) instead of the chemical solution treatments described above. There were three replicates of each treatment per experiment, and each experiment was performed three times.

**Determination of bacterial populations.** The fruit were mixed a minimum of 1 min for survival studies and chemical treatment studies using a stomacher (model 400; Techmar Co., Cincinnati, Ohio). Subsequently, 25 g of strawberry homogenate and 225 ml 0.1% peptone water were combined in a stomacher bag and stomached for 1 min. Serial dilutions were made with phosphate buffer (pH 7.2). Subsequently, 0.1 ml of the solutions was spread on agar plates in duplicate. For the freezing temperature experiment, 1 ml of the strawberry homogenate with peptone water was also spread on three agar plates in duplicate. Sorbitol MacConkey agar and Levine eosin methylene blue agar were used to recover *E. coli* O157:H7 and *E. coli,* respectively. The plates were then incubated at 37°C for 24 h. Colonies were counted using a Darkfield Quebec colony counter (model 3325; American Optical, Buffalo, N.Y.). Where necessary, *E. coli* O157:H7 colonies were confirmed using the Bacto *E. coli* O antiserum O157 agglutination test (Difco).

**Statistical analysis.** Bacterial population changes from replications in each experiment and the replicate experiments were analyzed statistically using the GLM procedure in Statistical Analysis Systems (SAS Institute, Cary, N.C.).

## RESULTS AND DISCUSSION

**Survival of *E. coli* O157:H7 on strawberry fruit: room temperature.** No *E. coli* was recovered from any uninoculated fruit. *E. coli* O157:H7 survived both internally and externally on strawberry fruit after inoculation and storage at 23°C for 24 h (Table 1). With both bacterial strains, the recovered CFUs were similar for bacteria placed on the fruit surface and those injected into the strawberry fruit. The population of strain ATCC 43895, recovered from the 23°C treatment, was greater than the initial inoculated level, which represented the only observation of a population increase in any storage experiment with inoculated strawberry fruit. Recovery of strain ATCC 43895 was approximately 1 log CFU/g greater than that for ATCC 35150 both on the surface and inside the fruit. The survivability of *E. coli* O157:H7 in acid conditions such as exist inside fruit (pH 3.5 to 3.7) could be attributed to the known acid tolerance of this bacterium.

For comparison, we evaluated two strains of *E. coli* to determine if *E. coli* O157:H7 exhibited exceptional compatibility with the acidic and other host characteristics, such as moisture and nutrient availability, displayed by strawberry fruit. These experiments showed that *E. coli* strains ATCC 15597 and ATCC 43896 survived inside and on the surface of the fruit (Table 1). There was a significant reduction of the CFUs of the two strains of nonpathogenic *E. coli* on the surface of the fruit when compared to the populations inside the fruit at 23°C. The population of *E.*

TABLE 1. E. coli *O157:H7 and* E. coli *populations after placing a droplet (25 μl) on or injecting it into strawberry fruit and storage at 23°C for 24 h*

| Bacterial strain | Population recovered (log CFU/g)[a] | | Population reduction (log CFU/g) | |
|---|---|---|---|---|
| | Surface inoculated | Inoculum injected | Surface inoculated | Inoculum injected |
| O157:H7 ATCC 43895 | 4.58 ± 0.51[b] | 4.87 ± 0.56 | (0.24 ± 0.47) DE[c,d] | (0.52 ± 0.57) E |
| O157:H7 ATCC 35150 | 3.62 ± 1.04 | 3.78 ± 0.37 | 0.66 ± 1.03 BC | 0.51 ± 0.39 BC |
| ATCC 15597 | 2.20 ± 0.32 | 4.20 ± 0.57 | 2.31 ± 0.28 A | 0.31 ± 0.50 CD |
| ATCC 43896 | 3.25 ± 0.78 | 4.17 ± 0.33 | 1.05 ± 0.82 B | 0.13 ± 0.24 CD |

[a] Initial inoculation level of 4.36 ± 0.13 log CFU/g (mean ± SD). Variations were from inoculum quantities and sample fruit weight.
[b] Data are means ± standard deviation for three samples per experiment and three experiments.
[c] Data within parentheses indicate population increase.
[d] Data followed by different letters are significantly different by least significant difference at $P < 0.05$.

*coli* strain ATCC 15597 was greater than 1 log CFU/g lower than that of ATCC strain 43896 on the fruit surface.

The decrease in surface population of bacteria on the fruit might be due to limitation of nutrients for the bacteria, drying of the inoculum, or competitive microflora on the fruit. *E. coli* strain ATCC 15597 appeared to be much less suited for survival on the surface of the fruit than the other bacterial strains; however, its population remained relatively high inside the fruit. Overall, the results indicate that the strawberry is a suitable host for *E. coli* O157:H7 and *E. coli,* but there are differences among isolates. A similar study revealed that *E. coli* O157:H7 grew on the rind of cantaloupe and watermelon stored under high relative humidity at 25°C for 14 to 22 days *(12).* Another study showed that *E. coli* O157:H7 survived 2 to 3 days in apple cider at 25°C *(38).* Richert et al. *(27)* found that *E. coli* O157:H7 populations did not change on inoculated broccoli, cucumber, and green pepper stored at 15°C for 7 days.

Bacterial populations inside the fruit were similar to, or exceeded, those on the surface. The survivability of the bacteria was frequently quite variable externally but relatively stable internally. The fruit is more likely to be contaminated on the surface. However, bacterial survival within the berry might be of concern, as well, if berries with wounds were contaminated during handling. In addition, the internal survivability could be relevant for fruit salads in which strawberry halves are frequently added. *Salmonella* Montevideo can be taken up by tomato tissue under conditions similar to those used in packing and handling, and the bacteria populations increased significantly on cut tissue after storage at 20 and 30°C *(39).*

**Refrigeration temperatures.** The results of refrigerated storage of inoculated strawberries stored at 5 and 10°C for 3 days are presented in Table 2. Storage of supermarket-purchased fruit for periods longer than 3 days resulted in sporadic incidence of gray mold development. Riordan et al. *(29)* found that *E. coli* O157:H7 populations increased in the presence of one pathogenic mold at room temperature but decreased in the presence of another fungal pathogen in wounds on apple surfaces. Under both refrigeration storage temperatures in the present study, the two tested strains of *E. coli* O157:H7 were recovered from the inside and the surface of the fruit. However, there was a significant reduction in the populations of both strains of bacteria on the surface of the fruit when compared to populations inside the strawberry fruit. The difference was also manifested at both refrigeration temperatures studied. This result contrasted with that from 23°C storage studies on *E. coli* O157:H7 as described above—i.e., the bacteria survived about equally well both inside and outside the fruit at room temperature. However, the duration of the room temperature and refrigeration experiments was different, 1 and 3 days, respectively. The decrease in bacterial populations on the strawberry fruit surfaces during refrigerated storage may have been due to drying of the bacteria, lack of sufficient nutrients, or growth of competing microorganisms.

A similar study by Richert et al. *(27)* on whole broc-



TABLE 2. E. coli *O157:H7 populations after placing a droplet (25 μl) on or injecting it into strawberry fruit and storage at 5 or 10°C for 3 days*

| Storage temperature (°C) | Bacterial strain | Population recovered (log CFU/g)[a] | | Population reduction (log CFU/g) | |
|---|---|---|---|---|---|
| | | Surface inoculated | Inoculum injected | Surface inoculated | Inoculum injected |
| 10 | ATCC 43895 | 2.24 ± 0.34[b] | 3.58 ± 0.12 | 2.08 ± 0.26 AB[c] | 0.74 ± 0.10 D |
| | ATCC 35150 | 2.11 ± 0.19 | 3.16 ± 0.17 | 2.25 ± 0.24 A | 1.20 ± 0.15 C |
| 5 | ATCC 43895 | 2.93 ± 0.48 | 3.71 ± 0.15 | 1.30 ± 0.49 C | 0.52 ± 0.14 D |
| | ATCC 35150 | 2.42 ± 0.40 | 3.48 ± 0.21 | 1.77 ± 0.41 B | 0.69 ± 0.21 D |

[a] Initial inoculation level of 4.26 ± 0.09 log CFU/g (mean ± SD). Variations were from inoculum quantities and sample fruit weight.
[b] Data are means ± standard deviation for three samples per experiment and three experiments for 5°C and two experiments for 10°C.
[c] Data followed by different letters are significantly different by least significant difference at $P < 0.05$.

TABLE 3. E. coli *O157:H7 populations after placing a droplet (25 μl) on or injecting it into strawberry fruit and storage at −20°C for 3 days*

| | Population recovered (log CFU/g)[a] | | Population reduction (log CFU/g) | |
|---|---|---|---|---|
| Bacterial strain | Surface inoculated | Inoculum injected | Surface inoculated | Inoculum injected |
| ATCC 43895 | 1.47 ± 0.77[b] | 1.99 ± 0.68 | 2.93 ± 0.84 AB[c] | 2.41 ± 0.67 BC |
| ATCC 35150 | ≤1.00[d] | 2.06 ± 0.51 | 3.26 ± 0.06 A | 2.21 ± 0.51 C |

[a] Initial inoculation level of 4.33 ± 0.12 log CFU/g (mean ± SD). Variations were from inoculum quantities and sample fruit weight.
[b] Data are means ± standard deviation for three samples per experiment and three experiments.
[c] Data followed by different letters are significantly different by least significant difference at $P < 0.05$.
[d] Population recovered was below the level of detection for the enumeration method.

coli, cucumber, and green pepper stored at 4°C showed that *E. coli* O157:H7 populations decreased by 1 log unit after 3 days when the initial inoculum level was $10^6$ CFU/ml. *E. coli* O157:H7 died rapidly on the rind surface of cantaloupe and watermelon stored at 5°C *(12)*.

**Freezing temperature.** As a further investigation into the survival of *E. coli* O157:H7 on strawberries, experiments were done on the two test strains using frozen storage at −20°C. Under these conditions, both strains were recovered from inside the fruit, although the populations decreased approximately 1 to 2 logs when compared to the populations obtained from inoculated refrigerated fruit at 5 and 10°C (Table 3). The results with bacteria on the surface of the fruit yielded further decreases in *E. coli* O157:H7 populations, with strain ATCC 35150 decreasing more than 3 logs compared to the initial level. These results showed that the organism could survive on the surface of the frozen fruit, although the populations declined markedly. There was no statistically significant difference between the two strains of *E. coli* O157:H7 regarding their survivability on strawberry fruit at −20°C. A recent study in resuscitation of *E. coli* O157:H7 on various foods including strawberries showed that the pathogen could be recovered after frozen storage at an inoculation level of approximately 10 CFUs per fruit *(14)*.

Collectively, the results from all storage conditions showed that when there was a difference in the recovery of the two *E. coli* O157:H7 strains from strawberry fruit, ATCC 43895 could be recovered to a greater extent than ATCC 35150. It is conceivable that *E. coli* O157:H7 strains exist that have greater capacity for survival on the fruit than those tested here. In addition, in most comparisons, except in those with *E. coli* O157:H7 at 23°C, bacteria survived better inside the fruit than on its surface, even though the internal pH was low (3.5 to 3.7).

Refrigeration and freezing reduced the levels of the pathogen, particularly on the surfaces, but the population of the organism in these studies could still present a threat since the infectious dose for *E. coli* O157:H7 has been found to be as low as 2 to 2,000 cells *(6)*. del Rosario and Beuchat *(12)* have shown that *E. coli* O157:H7 strains can survive on the surface of watermelons and cantaloupes in cattle fecal material and later be spread into the edible parts of these melons by cutting and food preparation methods. Apples become contaminated on the surface and, to a much lesser extent, internally when submerged in inoculum *(7)*. It was thought that the inoculum entered the apple fruit cavity through openings in the blossom end. A subsequent study showed that the internalization of *E. coli* O157:H7 on apples occurred at wounded surfaces *(18)*. The results with strawberry fruit from the present studies indicate that if surface *E. coli* O157:H7 contamination entered the fruit during cutting for food preparation or through a wound, the organism has the capacity to survive and create a potential food safety hazard.

**Effects of chemical agents on *E. coli* O157:H7 inoculated on strawberry fruit.** After establishing that *E. coli* O157:H7 could survive on strawberries after inoculation, the question arose as to how effective various commonly employed chemical agents would be in reducing *E. coli* O157:H7 populations on contaminated fruit. For the purpose of these experiments, strawberries were inoculated by submersion in an *E. coli* O157:H7 suspension, air dried for 30 min, and submerged in the chemical agent. The quantities of the various compounds studied were based on the amounts used on other foods, especially produce, to reduce microbial populations, as reported in a recent review by Beuchat *(5)*. Inoculation levels on fruit were 6.48 ± 0.19 and 6.45 ± 0.20 log CFU/g (means ± SD) for *E. coli* O157:H7 ATCC 43895 and ATCC 35150, respectively. The recovery and reduction in bacterial populations after dipping inoculated strawberry fruit in distilled water alone are presented in Table 4.

**NaOCl.** The widely used sanitizing agent NaOCl was evaluated at two concentrations in aqueous solution: 100 (65 ppm Cl) and 200 ppm (130 ppm Cl). The concentrations of active chlorine are within the range commonly used on produce *(5)*. Treatment of the fruit with NaOCl caused approximately a 1.3-log reduction in *E. coli* O157:H7 levels compared to initial inoculation levels (Table 4). Both concentrations of NaOCl were equally effective on both strains of test bacteria.

Because NaOCl is such a widely used disinfecting agent, subsequent tests were conducted with 10-fold higher concentrations of this reagent—i.e., 1,000 (650 ppm free chlorine) and 2,000 ppm (1,300 ppm free chlorine). However, the reduction of *E. coli* O157:H7 populations observed was only about 1.7 log with the 2,000-ppm treatment (data not shown).

TABLE 4. E. coli *O157:H7 populations after dipping inoculated strawberry fruit in sodium hypochlorite, Tween 80, acetic acid, sodium phosphate, and hydrogen peroxide aqueous solutions*

| Treatment | | Population recovered (log CFU/g)[a] | | Population reduction (log CFU/g) | |
|---|---|---|---|---|---|
| | | ATCC 43895 | ATCC 35150 | ATCC 43895 | ATCC 35150 |
| Water | | 5.73 ± 0.21[b] | 5.59 ± 0.21 | 0.75 ± 0.16 J[c] | 0.86 ± 0.17 IJ |
| Sodium hypochlorite | 100 ppm | 5.28 ± 0.23 | 5.23 ± 0.39 | 1.29 ± 0.19 E–H | 1.30 ± 0.34 D–H |
| Sodium hypochlorite | 200 ppm | 5.23 ± 0.20 | 5.19 ± 0.39 | 1.34 ± 0.17 C–G | 1.34 ± 0.41 C–G |
| Tween 80 | 100 ppm | 5.51 ± 0.16 | 5.28 ± 0.28 | 1.06 ± 0.13 HI | 1.25 ± 0.32 E–H |
| Tween 80 | 200 ppm | 5.40 ± 0.29 | 5.37 ± 0.30 | 1.17 ± 0.23 GH | 1.16 ± 0.29 GH |
| Acetic acid | 2% | 5.10 ± 0.34 | 5.19 ± 0.21 | 1.47 ± 0.30 CDE | 1.34 ± 0.19 C–G |
| Acetic acid | 5% | 5.01 ± 0.24 | 4.96 ± 0.20 | 1.55 ± 0.20 CD | 1.57 ± 0.26 C |
| Sodium phosphate | 2% | 4.99 ± 0.26 | 4.98 ± 0.36 | 1.58 ± 0.22 C | 1.55 ± 0.27 CD |
| Sodium phosphate | 5% | 4.72 ± 0.42 | 4.95 ± 0.28 | 1.85 ± 0.38 B | 1.58 ± 0.24 C |
| Hydrogen peroxide | 1% | 5.27 ± 0.18 | 5.06 ± 0.25 | 1.20 ± 0.13 FGH | 1.42 ± 0.18 C–F |
| Hydrogen peroxide | 3% | 4.29 ± 0.36 | 4.33 ± 0.28 | 2.18 ± 0.28 A | 2.15 ± 0.43 A |

[a] Initial inoculation levels on the fruit were 6.48 ± 0.19 and 6.45 ± 0.20 log CFU/g (mean ± SD) for *E. coli* O157:H7 ATCC 43895 and ATCC 35150, respectively. Variations were from inoculum quantities and sample fruit weight.

[b] Data are means ± standard deviation for three samples per experiment and three experiments.

[c] Data followed by different letters are significantly different by least significant difference at $P < 0.05$.

**Tween 80.** Treatment of the inoculated fruit with Tween 80 solution at concentrations of 100 and 200 ppm resulted in an approximately 1.1- to 1.2-log reduction of *E. coli* O157:H7 populations on strawberries (Table 4). The action of Tween 80 is thought to be due to its surfactant properties. In studies of lettuce, Adams et al. *(3)* found that adding Tween 80 at 100 ppm to chlorine solution increased the effectiveness of the latter in reducing microflora.

**Acetic acid.** Experiments with acetic acid at concentrations of 2 and 5% in aqueous solution showed that *E. coli* O157:H7 populations could be reduced as much as approximately 1.6 log units compared to initial inoculation levels (Table 4). In studies with *E. coli* O157:H7-contaminated apples, Wright et al. *(36)* found that 5% acetic acid caused a 3-log CFU/g reduction in pathogen compared to unrinsed controls and a 2-log CFU/g reduction compared to water-rinsed controls. The greater effectiveness on apples is likely due to the relatively smooth surface of the fruit.

**Sodium phosphate.** Tests with sodium phosphate at 2 and 5% concentration in aqueous solution led to a reduction of pathogen population on the fruit of approximately 1.6 to 1.9 log CFU/g. The results with this alkaline sanitizing agent were similar to those observed with acetic acid. The results with strawberry differed from those obtained by Somers et al. *(31),* who found that *E. coli* O157:H7 at $10^6$ CFU/ml in suspension or $10^5$ CFU/ml as a biofilm was killed by 1% sodium phosphate.

**Hydrogen peroxide.** The results from treating contaminated fruit with 1% hydrogen peroxide solution revealed a 1.2- to 1.4-log reduction in *E. coli* O157:H7 (Table 4). Increasing the concentration of hydrogen peroxide to 3% resulted in an additional reduction in bacterial counts to around 2.2 log CFU/g. This represents a 0.8- to 1-log CFU/g decrease in bacterial population when $H_2O_2$ concentrations were increased from 1 to 3%, which was the only compound that was clearly more effective at the higher concentration tested. Park and Beuchat *(25)* observed that 1% $H_2O_2$ reduced *E. coli* O157:H7 on cantaloupes by 2.3 logs compared to a water rinse of inoculated fruit.

Collectively, the results with the five compounds showed that none of these materials was especially effective in removing *E. coli* O157:H7 from strawberries. The chemical treatments generally yielded similar results with both bacterial strains. Of the materials studied, hydrogen peroxide at 3% concentration reduced the bacterial populations by the greatest amount. None of the compounds at the concentrations tested caused noticeable changes, such as bleaching, in the appearance of the strawberry fruit. Dipping the strawberry fruit in distilled water alone removed about 0.8 log CFU/g of *E. coli* O157:H7. The relative ineffectiveness of the compounds on strawberries is likely due to the rough surface of strawberries and the presence of numerous surface-borne achenes (seeds), which provide sites for the bacteria to attach and become less accessible to sanitizing solutions.

In summary, the results obtained from the present work have shown that *E. coli* O157:H7 strains on the surface and inside strawberry fruit have the capacity to survive at room temperature, two refrigeration temperatures, and frozen storage. Bacteria injected into the fruit survive as well as or better than those placed on the surface. Generally, the bacterial populations decreased the most after freezing the fruit and on the surface after refrigeration. Tests with two strains of *E. coli* O157:H7, ATCC 43895 and ATCC 35150, showed that when differences in recovery occurred, the former strain was recovered in greater quantities than the latter strain. Comparisons made with two strains of *E. coli* demonstrated that these bacteria also had the capacity to survive on the surface and especially within the fruit.

Tests with five chemical agents each tested at two concentrations on strawberries inoculated with two *E. coli*

O157:H7 strains revealed that the pathogen was not readily killed or removed from the fruit. Hydrogen peroxide tested at 3% concentration in aqueous solution reduced the pathogen level approximately 2.2 log CFU/g. Water dipping alone decreased the bacterial population by about 0.8 log. The other agents tested—i.e., NaOCl, Tween 80, acetic acid, and sodium phosphate—reduced *E. coli* O157:H7 populations within the range from approximately 1.1 to 1.9 log units. Thus, these experiments indicate that if strawberry fruit were contaminated with *E. coli* O157:H7, they could present a health hazard due to the survivability of the pathogen, the relatively low effectiveness of common sanitizing agents, and the previously established low cell number required to cause illness.

## ACKNOWLEDGMENTS

We thank Ms. Katherine Akers for her technical assistance, Dr. Joe O'Leary for his critical review, and Pam Wingate for manuscript preparation. This work has been supported by the Graduate Center for Nutritional Sciences at the University of Kentucky.

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