Reduction in Levels of *Escherichia coli* O157:H7 in Apple Cider by Pulsed Electric Fields

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ABSTRACT

Many studies have demonstrated that high voltage pulsed electric field (PEF) treatment has lethal effects on microorganisms including *Escherichia coli* O157:H7; however, the survival of this pathogen through the PEF treatment is not fully understood. Fresh apple cider samples inoculated with *E. coli* O157:H7 strain EC920026 were treated with 10, 20, and 30 instant charge reversal pulses at electric field strengths of 60, 70, and 80 kV/cm, at 20, 30, and 42°C. To accurately evaluate the lethality of apple cider processing steps, counts were determined on tryptic soy agar (TSA) and sorbitol MacConkey agar (SMA) to estimate the number of injured and uninjured *E. coli* O157:H7 cells after PEF treatment. Cell death increased significantly with increased temperatures and electric field strengths. A maximum of 5.35-log10 CFU/ml (*P* < 0.05) reduction in cell population was achieved in samples treated with 30 pulses and 80 kV/cm at 42°C. Cell injury measured by the difference between TSA and SMA counts was found to be insignificant (*P* > 0.05). Under extreme conditions, a 5.91-log10 CFU/ml reduction in cell population was accomplished when treating samples with 10 pulses and 90 kV/cm at 42°C. PEF treatment, when combined with the addition of calcium or nisin, triggered cell death, resulting in a reduction in *E. coli* O157:H7 count of 6 to 8 log10 CFU/ml. Overall, the combination of PEF and heat treatment was demonstrated to be an effective pasteurization technique by sufficiently reducing the number of viable *E. coli* O157:H7 cells in fresh apple cider to meet U.S. Federal Drug Administration recommendations.

*Escherichia coli* O157:H7 was first identified as a human pathogen in 1982 (17). It is now considered to be an important pathogen that can cause serious illness, which may be expressed as hemorrhagic colitis, hemolytic uremic syndrome, or thrombotic thrombocytopenic purpura (8). Outbreaks of *E. coli* O157:H7 have been associated with a variety of foods, including raw milk, raw vegetables, ground beef, pork, lamb, poultry, and contaminated water supplies (3, 6, 8, 16, 17, 36). Highly acidic foods, such as apple cider, mayonnaise, and yogurt, have been major sources of *E. coli* O157:H7 foodborne outbreaks (3, 4, 16). Recent outbreaks of *E. coli* O157:H7 infection involving apple cider and apple juice have highlighted the acid tolerance and probable low infective dose of this pathogen (1, 4).

One currently recommended pasteurization treatment for apple juice is heating to 71.1°C for 6 s (23). Commonly used food additives in cider, such as potassium sorbate and sodium benzoate, have been shown to have a minimal lethal effect on *E. coli* O157:H7. The more potent additive, sodium benzoate (0.1%), has been shown to cause a 5-log10 reduction in *E. coli* O157:H7 numbers during storage for 2 to 10 days at 8°C (39). However, concentrations of benzoate higher than 0.0125% reportedly impart noticeable tastes (32). Another relatively simple way to kill *E. coli* O157:H7 in apple juice is freezing and thawing. Freezing could extend the shelf life of juice and caused a 0.63- to 3.43-log10 CFU/ml reduction in the number of *E. coli* O157:H7 (31). Producers of apple juice are usually reluctant to pasteurize juice using the above methods due to high cost and decrease in flavor (12).

Thermal inactivation studies of acidic foods (pH < 4.5) have revealed that *E. coli* O157:H7 has no unusual heat and pressure resistance (10, 20, 27). Most studies with regard to *E. coli* O157:H7 susceptibility to stress were applied to ground beef due to its high risk for contamination. However, there is limited information on the response of *E. coli* O157:H7 to stress in apple cider, especially when subjected to electrical stress.

In 1998, the U.S. Food and Drug Administration published regulations requiring a warning statement on all unprocessed packaged juices. They suggested that at least a 5-log10 unit reduction in the target microorganism should be achieved for a period of at least as long as the shelf life of the product when stored under normal conditions (35). As a result, the interest in inactivating *E. coli* O157:H7 by pulsed electric field (PEF) treatment of apple juice has increased, because the treatment has little effect on flavor, color, and nutritive value (11, 14). Electrical pulses with high electric field intensity can cause permanent cell membrane breakdown, and this forms the basis for microbial inactivation in nonthermal liquid food pasteurization (15). Juice samples can be treated by PEF with selected parameters, including electric field strength, number of pulses, and treatment temperature (14). Because PEF treatment has...
been demonstrated to be very effective in inactivating E. coli O157:H7 in apple juice samples with a 5-log$_{10}$ unit reduction (12). PEF treatment can be a possible alternative for pasteurization of fresh apple cider.

There are many qualitative methods for detecting E. coli O157:H7 in foods and beverages (13, 22, 25, 28, 29, 36), but relatively few quantitative methods are available. To evaluate the lethality of PEF treatment to E. coli O157: H7 in apple cider, a reliable method for enumeration of this pathogen is required. E. coli O157:H7 cells may be injured but still viable after electrical stress, but it is not known whether cells injured by this type of stress are infective (31). Because it is well reported that injured E. coli O157: H7 cells are unable to form colonies on sorbitol MacConkey agar (SMA) plates, the difference in population deaths determined by TSA and SMA spread platings can be used to estimate the extent of cell injury (9, 31). In fact, there has been no report on the extent of E. coli O157:H7 cell injury in apple cider after PEF treatment. The objective of this study was to determine the ability of E. coli O157: H7 to survive electrical stresses under a variety of conditions.

**MATERIALS AND METHODS**

**Bacterial strains.** Two toxin-negative strains of E. coli O157:H7 (EC920026 and EC920027) were obtained from the Food Microbiology Laboratory, University of Guelph. One of them (EC920026) was chosen for this study after comparing its acid tolerance to that of two toxin-positive strains of E.coli (31). In fact, there has been no report on the extent of E. coli O157:H7 cell injury in apple cider after PEF treatment. The objective of this study was to determine the ability of E. coli O157: H7 to survive electrical stresses under a variety of conditions.

**Preparation of apple cider.** Thirty bottles (1 liter each) of freshly squeezed, unpasteurized, and preservative-free apple cider were purchased from a local juice store in Markham, Ontario. The cider was mixed in five 6-liter volumetric flasks to ensure consistency in the overall composition. An average pH value of 4.22 ± 0.03 was obtained for the five flasks of cider by an electronic pH meter (model AP5; Fisher Scientific, Pittsburgh, Pa.). The cider, after being dispensed into 50-ml disposable screw-cap tubes, was kept in a cold room at 5°C for 3 days. Before transporting the cider to a walk-in freezer (−20°C), pH measurements were performed on five random tubes of cider, and an average value of 4 ± 0.01 was obtained, which again indicated a relatively constant pH among samples. Tubes of apple cider were thawed overnight in the refrigerator at 4°C before use.

To determine the total number of background microorganisms present in the apple cider at the initial and final stages of the study, samples were tested for total microbial count using Petrifilm aerobic count plates (3M; London, Ontario, Canada). To each of three samples, 1 ml of cider was diluted with 0.1% peptone water (Difco Laboratories, Detroit, Mich.) in serial dilutions, and 1 ml of each dilution was pipetted onto a Petrifilm, which was then incubated at 30°C for 3 days.

**Survival studies.** For safety, nonpathogenic strains of E. coli O157:H7 (EC920026 and EC920027) were used in this study. However, for results to be valid, the acid tolerance of the chosen toxin-negative strain of E. coli O157:H7 must be similar to that of toxin-positive strains. Four hundred milliliters of cider was adjusted to pH 3.72 with 1.7 N citric acid (Fisher) or to pH 4.52 with 2.5 N NaOH (Fisher). The cider was filter sterilized using a disposable filter (Nalgene, Rochester, N.Y.), with a pore size of 0.45 μm and a diameter of 90 mm. Each of the four strains of E. coli O157:H7 was used to inoculate the cider, and these were incubated for 0, 2, 4, 18, and 24 h at 37°C. For each sample, 1 ml of inoculum in tryptic soy broth (TSB; Difco) was mixed with 9 ml of apple cider before incubation. After each prescribed time interval, appropriate serial dilutions were made in 0.1% peptone water, followed by spread plating 0.1 ml of selected dilutions in duplicate on TSB using a sterile glass rod. Colonies were counted after incubation at 37°C for 24 h. Cultures for frozen storage at −80°C were prepared by transferring 0.5 ml of a stationary-phase culture in TSB to a cryovial containing 0.5 ml of 99.5% glycerol (Fisher).

In addition to acid tolerance, the temperature tolerance of strain EC920026 at 4, 42, and 43°C for 0, 1, 2, 3, and 4 h was also investigated. This was to ensure that no further growth of microorganisms occurred when the samples were being stored on ice in a styrofoam cooler during travel and to ensure that the temperature used during the PEF treatment did not kill E. coli O157:H7. Two samples of apple cider were employed for each temperature treatment. The samples were stored at 4°C on ice and in two water baths (model ISOTEMP 228; Fisher) at 42 and 43°C, respectively. For each sample, 1 ml of inoculum in TSB was mixed with 9 ml of apple cider before incubation. The temperatures within the styrofoam cooler and the temperature of the inoculated samples were monitored at intervals using thermocouples. Timing started when samples in the test tubes were immersed in the ice or water bath. At each prescribed time interval, a set of duplicate tubes was removed and spread plated on SMA plates immediately.

**Inoculation of apple cider.** To prepare for inoculation of apple cider, a frozen culture was subcultured overnight in 10 ml TSB at 37°C. This overnight culture (0.1 ml) was again subcultured overnight in 10 ml TSB at 37°C, resulting in a stationary-phase culture. Forty milliliters of this culture in TSB was then centrifuged at 10,000 × g for 8 min at 4°C (model J2-MC; Beckman Coulter, Mississauga, Ontario, Canada). After washing the culture twice with 40 ml of 0.1% peptone water and pouring off the supernatant, the cell pellets were resuspended in 200 ml of apple cider, resulting in a bacterial suspension of approximately 7.30 log$_{10}$ CFU/ml.

**PEF treatment.** A low-energy PEF treatment system at the University of Guelph, which consists of a high voltage pulse generator and a circular treatment chamber, was used to treat the inoculated apple cider samples (14). This unit can generate short duration pulses of 2 μs with electric field strengths of up to 100 kV/cm. The circular treatment chamber (25 cm in diameter) has two circular and parallel stainless steel electrodes (16.5 cm) (14). The distance between the electrodes was adjusted to 0.3 cm, thereby allowing a process volume of approximately 50 ml. For each treatment, approximately 200 ml of apple cider was required to fill and wash the chamber. Before and after each treatment, the chamber was cleaned with 50% bleach and rinsed with sterile water at appropriate temperatures to ensure sterility.

Before PEF treatment, the initial number of microorganisms was obtained by surface inoculation on SMA plates in duplicate. Two cider samples were also passed through the treatment chamber, with no voltage applied, and were spread plated on SMA plates to obtain two control values for comparison. In this study,
several parameters were assessed, including electrical field strengths at 0 (control), 60, 70, and 80 kV/cm with 10, 20, and 30 pulses at 20, 30, and 42°C. Each treatment combination involved three trials. The desired temperatures of the cider were achieved by heating in a microwave oven (model NN-S759BC; Panasonic, Mississauga, Ontario, Canada), and the desired temperatures of the chamber were achieved by passing hot water through the chamber. Cider samples were heated to determine the amount of time needed to reach the desired temperatures. It usually took 25, 45, and 60 s to heat the apple cider from 4°C to 20, 30, and 42°C, respectively, while it usually took 3, 6, and 12 liters of 80°C hot water to heat the chamber to 20, 30, and 42°C, respectively. Initial pH values of all samples were measured and found to be relatively constant at 4 ± 0.05. The temperatures of the cider entering and leaving the chamber were recorded. Treated samples were collected in sterile containers and were stored on ice until plated on tryptic soy agar (TSA) and SMA plates.

Because the optimum treatment combination was found to be 10 pulses, 80 kV/cm, and 42°C, further tests were done using different conditions to evaluate the survival of E. coli O157:H7. These included increasing the electric field strength to 90 kV/cm. Second, 2% (wt/vol) cinnamon powder was added to the cider before PEF treatment. Third, nisin (2.5% nisin, 97.5% NaCl, and lactose; Canadian Innovatech, Abbotsford, B.C., Canada) was also added to a final concentration of 2% wt/vol of the cider. Appropriate serial dilutions were made in 0.1% peptone water, followed by spread plating of selected dilutions in duplicate on TSA and SMA plates. Colonies were counted after incubation at 37°C for 24 h.

To study the effect of lethality on other microorganisms present in the apple cider, one sample (200 ml) was treated with 10 pulses and 80 kV/cm at 42°C and was also analyzed for yeasts and molds, E. coli, coliforms, and lactic acid bacteria, as described below. Finally, to determine the effect of PEF treatment with 10 pulses and 80 kV/cm at 42°C on E. coli O157:H7 alone, autoclaved apple cider was used to eliminate any background microorganisms. The dilution, spread plating, and incubation procedures were the same as for the TSA and SMA spread platings.

Enumeration of surviving E. coli O157:H7 cells. Colonies on TSA and SMA plates were counted, and log10 CFU/ml values were calculated. These values were subtracted from the control inoculum (log10 CFU/ml) to determine the extent of cell death and injury in the culture.

To determine the amount of each microorganism present in apple cider before and after the PEF treatment, potato dextrose agar (PDA; Difco), E. coli Petrifilm (3M), and deMan Rogosa Sharpe agar (Difco) were used to detect yeasts and molds, E. coli and coliforms, and lactic acid bacteria, respectively. The dilution and incubation procedures for E. coli Petrifilm were similar to those used for Petrifilm aerobic count plates, whereas the procedures for the inoculation of PDA and de Mann Rogosa Sharpe agar were similar to those used for SMA, except that PDA plates required 5 d of incubation at 37°C.

Statistical analysis. Means and variances were calculated for each of the PEF treatment combinations. Since the deviations between all three sets of replicates were very small, mean values were used for calculation of population death in log10 CFU/ml. Analysis of variance was used to test for significant differences (α = 0.05) between any pair of mean population death in log10 CFU/ml of E. coli O157:H7 cells recovered on TSA and SMA plates derived after each sampling occasion. The statistical package used was the Statistical Analysis System, version 6.12 (SAS Institute, Cary, N.C.).

RESULTS AND DISCUSSION

Survival studies. E. coli O157:H7 has a high acid tolerance (2, 33, 34), and growth at pH as low as 4.0 to 4.5 has been recorded in broth studies (5, 7, 10). The survival of E. coli O157:H7 in fresh apple cider has been shown to well exceed the typical 1- to 2-week refrigerated shelf life (24, 30, 39). Other acid-tolerance studies revealed that E. coli O157:H7 could survive for up to 31 days at 8°C in apple cider of pH 3.7 (39). The result of a Student’s t test showed that the acid tolerance of strain EC920026 was similar (P < 0.05) to that of strains C7927 and EC92005 at pH 3.72 and 4.52 (data not shown). However, the acid tolerance of strain EC920027 was significantly different (P > 0.05) under similar conditions. Therefore, strain EC920026 was chosen for this study to represent infective toxin-positive E. coli O157:H7 strains that can survive in fresh cider.

Because E. coli O157:H7 is likely to be sensitive to heat above 42°C (9, 12, 20), the treatment temperature should be kept at or below 42°C to screen out the extraneous effect of temperature and thus provide information about the inactivation of the culture by the electric fields. For confirmation, the temperature tolerance of E. coli O157:H7 strain EC920026 at 42 and 43°C was investigated. Throughout 4 h of incubation, cultures in apple cider at 42°C maintained population levels at about 7 log10 CFU/ml, whereas cultures in apple cider at 43°C showed a decrease in population to less than 5 log10 CFU/ml. The results agree with published literature stating that E. coli O157:H7 does not survive well above 42°C (9, 12, 27).

Uninoculated apple cider. There is no legal or product definition of apple cider. For purposes of this study, apple cider is defined as the liquid resulting from freshly squeezed apples with no further clarification (19). Some common microorganisms that naturally adhere to apples, which can contaminate apple cider, include yeast and molds, E. coli, coliforms, and lactic acid bacteria (27). Apple cider can become contaminated with E. coli O157:H7 through contact with bacteria in fecal material in the soil, improper handling, or contamination in the manufacturing process (19). Fresh apple cider (unpasteurized) was used for this study to reflect the ability of PEF treatment to inactivate E. coli O157:H7 as well as other microorganisms that may be present. Aerobic plate count results indicated a 4.23 ± 0.15 log10 CFU/ml, whereas cultures in apple cider at 43°C showed a decrease in population to less than 5 log10 CFU/ml. The results agree with published literature stating that E. coli O157:H7 does not survive well above 42°C (9, 12, 27).

Inoculated apple cider (control). Before PEF treatment, the initial number of microorganisms in inoculated apple cider at 20, 30, and 42°C was obtained using SMA plates, and counts of approximately 8 log10 CFU/ml were obtained. As a control, cider samples were also passed through the treatment chamber with no voltage applied. There was no difference in counts between the initial sample and the cider that had been passed through the PEF
equipment (data not shown). This reveals that the treatment chamber and treatment procedures would not affect the E. coli O157:H7 counts.

Effects of PEF treatment. It is important to ensure that the cleaning process of the treatment chamber between trials does not influence PEF results. A study on the effects of water and cleaning reagent on inactivation of microorganisms was performed. It was confirmed that cleaning the chamber with 50% bleach followed by rinsing with water was sufficient to sterilize the system.

Electroporation is a widely accepted concept to describe the phenomenon of cell membrane discharge and cell membrane breakdown under the application of short electric field pulses (15). Many researchers have performed high voltage electric pulse treatment on various microorganisms in a variety of liquid and semisolids media, and moderate (2 log_{10} cycles) to significant (9 log_{10} cycles) microbial inactivation has been achieved (15). The microbial reduction rate seems to be a function of numerous process parameters, conditions, and procedures used (12, 21, 26, 37, 38). However, the results obtained are hard to standardize because of the different process conditions and the wide variety of designs of high voltage pulsers and treatment chambers used (14).

As expected, the greatest recovery of E. coli O157:H7 was obtained on TSA plates. Table 1 summarizes the results obtained from PEF treatments. As the treatment temperature increased from 20 to 42°C, cell death increased significantly by greater than 5 log_{10} CFU/ml (P < 0.05). As the electric field strength increased from 60 to 80 kV/cm, cell death also increased significantly by 0.1 to 1 log_{10} CFU/ml (P < 0.05). However, the number of pulses did not show a significant effect (P > 0.05) in reducing cell population at each of the electric field strengths tested. Therefore, the combination of an elevated temperature, i.e., 42°C, and high electric field strength, i.e., 80 kV/cm, with any number of pulses ranging from 10 to 30 should induce significant cell death of E. coli O157:H7. Significantly greater cell death of 5.91 log_{10} CFU/ml (P < 0.05) was achieved when the electric field strength was adjusted to 90 kV/cm at 42°C (Table 1).

Since the amount of microorganisms initially present in the apple cider was found to be about 4 log_{10} CFU/ml, the growth of these microorganisms on TSA and SMA plates during incubation at 37°C was considered negligible when compared to that of the inoculated E. coli O157:H7, which had an initial concentration of about 7.30 log_{10} CFU/ml. Also, the morphology of the resulting colonies formed on TSA and SMA plates after incubation at 37°C resembled that of E. coli O157:H7. Therefore, the effect of these microorganisms on E. coli O157:H7 population count was considered insignificant.

The difference in surviving population count determined on TSA and SMA can be used to estimate the extent of cell injury that occurred during PEF treatments. Cell injury was measured by TSA count minus SMA count. However, there was no significant difference between TSA and SMA counts under any combination of treatment conditions (P > 0.05). Thus, the extent of E. coli O157:H7 cell injury in apple cider after PEF treatment appeared to be minimal.

To determine the effect of PEF treatment with 10 pulses and 80 kV/cm at 42°C on E. coli O157:H7 alone, autoclaved apple cider was used to eliminate any background microorganisms. In autoclaved products, E. coli O157:H7 underwent a 5.17-log_{10} CFU/ml decrease in cell population. Since the data using unpasteurized apple cider under similar conditions also reveal a 5.20-log_{10} CFU/ml decrease in cell population, this indicates that all the inoculated E. coli O157:H7 bacteria were destroyed. Perhaps the difference between the two values of cell death may represent a reduction in count of other background microorganisms present in the unpasteurized apple cider.

In addition to E. coli O157:H7, the survival of the other microorganisms that were initially present in apple cider was determined (Table 2). PEF treatment produced a significantly greater effect on E. coli, coliforms, and lactic acid bacteria than on yeasts and molds (P < 0.05). This could

### Table 1. Survival of E. coli O157:H7 EC920026 in unpasteurized apple cider after PEF treatment

<table>
<thead>
<tr>
<th>Electric field strength (kV/cm)</th>
<th>No. of pulses</th>
<th>Mean surviving E. coli O157:H7 population (log_{10} CFU/ml)^a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>20°C</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8.25 ± 0.07 x1</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>8.22 ± 0.07 x1</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>8.13 ± 0.02 x2</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>8.10 ± 0.02 x3</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>8.07 ± 0.04 x3</td>
</tr>
</tbody>
</table>

^a Mean of three experiments. ND, not determined. Means followed by the same number within a column are not statistically significant (P > 0.05), and means followed by the same letter within a row are not significantly different (P > 0.05).
be because yeasts and molds survive better in an acidic environment and often require a higher temperature to be destroyed (27). Another possibility is that gram-negative bacteria, such as E. coli and coliforms, are more susceptible to the PEF process than gram-positive species and yeasts and molds (16, 27).

The treatment combination of 10 pulses and 80 kV/cm at 42°C resulted in a 3.58 ± 0.17 log_{10} CFU/ml surviving population. This provides a reduction in count of greater than 5 log_{10} CFU/ml, in which the value includes the death of all bacteria. The remaining population might be yeasts and molds because they both showed about a 3-log_{10} CFU/ml surviving population under the same set of treatment conditions. Once again, this has further supported the assumption that all the E. coli O157:H7 would have been killed under the same set of PEF treatment conditions.

**Effect of cinnamon and nisin on PEF treatment.**

Cinnamon is a well-known flavoring agent that is added to impart a taste or aroma in food. Since microorganisms are often sensitive to food additives, cinnamon may play a role in reducing the cell population of E. coli O157:H7 in apple cider (5, 18). Similarly, nisin is an antimicrobial agent that is used to preserve food by acting on the cell membrane of microorganisms, thereby preventing growth of microorganisms and subsequent spoilage (18).

The effect of cinnamon alone in inactivating cells is not great (Table 3). The addition of cinnamon to apple cider in combination with a PEF treatment of 10 pulses and 80 kV/cm at 42°C induced further cell death by only 1 log_{10} CFU/ml. However, the addition of nisin together with a PEF treatment caused a significantly greater reduction in E. coli O157:H7 cells of greater than 3 log_{10} CFU/ml (P < 0.05). These results suggest that nisin acts synergistically with PEF treatments to reduce E. coli O157:H7 load in apple cider. Although nisin is known to be ineffective against gram-negative bacteria (18, 27), like E. coli O157: H7, it may enhance the effectiveness of PEF treatments by permeabilizing the cell membrane and thus making cells more susceptible to electroporation (15). Furthermore, the combination of PEF treatment and cinnamon or nisin was found to induce cell death of about 6 to 8 log_{10} CFU/ml when compared to the control conditions. This indicates that PEF treatment, when combined with either cinnamon or nisin, can be a very effective pasteurization technique in inactivating E. coli O157:H7 cells in apple cider.

**CONCLUSIONS**

This study reveals that PEF treatment is very effective in inactivating E. coli O157:H7 cells in apple cider. A greater than 5-log_{10} CFU/ml reduction in count was observed as the treatment temperature increased from 20 to 42°C with 10 electrical pulses at 80 kV/cm. PEF treatment, when combined with either cinnamon or nisin, could induce cell inactivation by 6 to 8 log_{10} CFU/ml. The combination of PEF and heat treatment is an effective pasteurization technique, as it reduces the number of viable E. coli O157:H7 cells in fresh apple cider by at least 5 log_{10} cycles, the degree of inactivation recommended by the U.S. Food and Drug Administration. This study also contributes to the understanding of the electrical stress response of E. coli O157:

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**TABLE 2. Cell deaths of the different microorganisms present in apple cider after PEF treatment**

<table>
<thead>
<tr>
<th>Count</th>
<th>Medium</th>
<th>No treatment at 42°C</th>
<th>80 kV/cm, 10 pulses, 42°C</th>
<th>Mean (n = 3) decrease in count (log CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeasts</td>
<td>PDA</td>
<td>3.89 ± 0.36</td>
<td>3.20 ± 0.19</td>
<td>0.696 a</td>
</tr>
<tr>
<td>Molds</td>
<td>PDA</td>
<td>3.89 ± 0.31</td>
<td>3.16 ± 0.14</td>
<td>0.733 a</td>
</tr>
<tr>
<td>E. coli</td>
<td>E. coli Petrifilm</td>
<td>2.27 ± 0.05</td>
<td>&lt;1</td>
<td>1.271 b</td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>MRS agar</td>
<td>2.89 ± 0.47</td>
<td>&lt;1</td>
<td>1.894 b</td>
</tr>
<tr>
<td>Coliforms</td>
<td>E. coli Petrifilm</td>
<td>2.18 ± 0.00</td>
<td>&lt;1</td>
<td>1.176 b</td>
</tr>
</tbody>
</table>

* Means followed by the same letter within a column are not significantly different (P > 0.05).

**TABLE 3. Effects of cinnamon (2% wt/vol) and nisin (2% wt/vol) on PEF treatment in inactivation of E. coli O157:H7 EC920026**

<table>
<thead>
<tr>
<th>Electric field strength (kV/cm)</th>
<th>No. of pulses</th>
<th>Temperature (°C)/ special conditions</th>
<th>Mean (n = 3) surviving population on TSA plates (log_{10} CFU/ml)</th>
<th>mean (n = 3) surviving population on SMA plates (log_{10} CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>42</td>
<td>8.78 ± 0.78 x</td>
<td>7.64 ± 0.20 x</td>
</tr>
<tr>
<td>80</td>
<td>10</td>
<td>42</td>
<td>3.58 ± 0.17 y</td>
<td>3.47 ± 0.31 y</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>42/cinnamon</td>
<td>6.78 ± 0.24 x</td>
<td>6.51 ± 0.24 x</td>
</tr>
<tr>
<td>80</td>
<td>10</td>
<td>42/cinnamon</td>
<td>2.55 ± 0.27 y</td>
<td>2.43 ± 0.12 y</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>42/nisin</td>
<td>4.15 ± 0.11 z</td>
<td>3.97 ± 0.09 z</td>
</tr>
<tr>
<td>80</td>
<td>10</td>
<td>42/nisin</td>
<td>&lt;1 wv</td>
<td>&lt;1 wv</td>
</tr>
</tbody>
</table>

* Means followed by the same letter within a column are not significantly different (P > 0.05).

b Less than 1, no detectable survivors in undiluted sample by a plating procedure.
H7, thereby providing strategies for the control of *E. coli* O157:H7 contamination in food and beverages.

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**REFERENCES**


