A combined treatment of UV-light and radio frequency electric field for the inactivation of Escherichia coli K-12 in apple juice

Dike O. Ukuku *, David J. Geveke

Food Safety Intervention Technologies Research Unit, Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture 1,
600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

A R T I C L E   I N F O

Article history:
Received 23 July 2009
Received in revised form 18 December 2009
Accepted 6 January 2010

Keywords:
UV-light
RFEF
Injury
Inactivation
UV-absorbing substances
Escherichia coli
Apple juice

A B S T R A C T

Radio frequency electric fields (RFEF) and UV-light treatments have been reported to inactivate bacteria in liquid foods. However, information on the efficacy of bacterial inactivation by combined treatments of RFEF and UV-light technologies is limited. In this study, we investigated the relationship between cell injury and inactivation of Escherichia coli K-12 in apple juice treated with a combination of RFEF and UV-light. Apple juice purchased from a wholesale distributor was inoculated with E. coli K-12 at 7.8 log CFU/ml, processed with a laboratory scale RFEF unit at 20 kHz, 15 kV/cm for 170 μs at a flow rate of 540 ml/min followed by UV-light treatment (254 nm) for 12 s at 25, 30 and 40 °C. Treated samples were analyzed for leakage of UV-substances as a function of membrane damage and were plated (0.1 ml) on Sorbitol MacConkey Agar (SMAC) and Trypticase Soy Agar (TSA) plates to determine the viability loss and percent injury. At 40 °C, UV-light treatment alone caused 5.8 log reduction of E. coli in apple juice while RFEF caused only 2.8 log reduction. A combination of the two processing treatments did not increase cell injury or leakage of intracellular bacterial UV-substances more than that from the UV-light treatment. Similarly, the viability loss determined was not significantly (P>0.05) different than UV-light treatment alone. However, the UV-substances determined in apple juice treated with RFEF was significantly (P<0.05) different than UV-light treated samples. The results of this study suggest that RFEF treatment causes more injury to the bacterial cells leading to more leakage of intracellular UV-substances than cells treated with UV-light alone. Also, the effect of the two processing treatment combination on bacterial inactivation was not additive.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Contamination of juices with pathogenic microorganisms and the pathogens ability to survive in low acid fruit juices (Besser et al., 1993; CDC, 1996a,b, 1999) have caused few cases of foodborne illnesses and some fatalities (Besser et al., 1993; Doyle, 1991; Madden, 1992; Mason, 1994; Padhye and Doyle, 1992). From 1923 to 2000, consumption of contaminated fruit juices has been implicated in at least 28 foodborne illness outbreaks (Cody et al., 1999). Eleven out of 28 (almost 40%) outbreaks were associated with Salmonella spp. Eight out of 28 (close to 30%) outbreaks were caused by Escherichia coli especially E. coli O157:H7. All these outbreaks involving E. coli O157: H7 and Salmonella have raised concerns about the safety of consuming unpasteurized fruit juices. Thermal processing is used by the juice industry to inactivate foodborne pathogens in foods however; it impairs the characteristic flavor of juices (Mazzotta, 2001). People are becoming more health conscious and tend to opt for less heat processed foods. Therefore there is a need for alternative processing treatments that can achieve a 5 log reduction of these pathogens (Mazzotta, 2001; Sizer and Balasubramaniam, 1999; Anonymous, 1999) without causing adverse effect on the flavor of the juice.

Several nonthermal technologies for food processing have been commercialized, including ultraviolet processing of apple cider (FDA, 2002). Several UV apparatus for inactivation of bacteria has been tested (Basaran et al., 2004; Duffy et al., 2000; Hanes et al., 2002; Koutchma et al., 2004; Koutchma and Parisi, 2004; Quintero-Ramos et al., 2004; Shama, 1999; Tandon et al., 2003; Wright et al., 2000). However, the juice processing industries have not fully explored these innovative technologies due to limited information on the mechanism of inactivation of bacteria by these technologies. A simple UV apparatus was designed and assembled at the Eastern Regional Research Center (ERRC), Agricultural Research Services (ARS) of United States Department of Agriculture (USDA). The UV lamp was surrounded by a coil of tubing. Inactivation of bacteria by this apparatus has been reported (Geveke, 2008, 2005). Similarly, a pasteurization process that uses radio frequency electric fields (RFEF) for inactivation of bacteria in foods was developed (Geveke et al., 2002; Geveke and Brunkhorst, 2003, 2004a,b). In these studies, a

* Corresponding author. Tel.: +1 215 233 6427; fax: +1 215 233 6406.
E-mail address: dike.ukuku@ars.usda.gov (D.O. Ukuku).

1 Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.
set of RFEF operating parameters that achieved 99.999% (5 logs) reduction of *E. coli* in apple cider was determined, and the kinetics of bacterial inactivation established. The effect of RFEF treatment on bacterial membrane was investigated and the presence of injured *E. coli* cells in apple juice was determined (Ukuku et al., 2008). In this study, we reported 2 to 4 log reductions of bacteria in apple juice and the population of injured *E. coli* cells determined among the surviving bacterial cells ranged from 70 to 80%. In our current study, we proposed that combining the RFEF treatment of apple juice contaminated with *E. coli* with UV-light treatment may lead to higher bacterial inactivation and less population of damaged cells in the juice. The combined non-thermal treatment may cause the membrane structure of the damaged bacteria to be beyond self-repair, leading to complete inactivation than when either treatment is used alone. Therefore, the objective of this study was to combine RFEF and UV light treatment for inactivation of *E. coli* cells inoculated in apple juice to produce a non-thermal microbial safe apple juice. Similarly, the behavior of injured cells per treatment was monitored during storage at 5 and 23 °C. Also leakage of biological intracellular materials from damaged/injured *E. coli* cells was measured per individual treatments and then the results were compared against combined treatments. Results presented in this study are intended to provide a scientific basis for an effective comparison of each treatment or its combination on bacterial inactivation in apple juice.

2. Materials and methods

2.1. Test strains and preparation of inocula

*E. coli* K-12 (ATCC 23716) from the USDA, ARS, ERRC culture collection was used in this study. Cell culture was maintained on tryptic soy agar (TSA; Remel, Inc., Lenexa, KS) at 4 °C. Prior to use the cells were inoculated by loop in tryptic soy broth (TSB: Remel, Inc., Lenexa, KS) with incubation at 37 °C for 16–18 h with shaking. A 0.1 ml cell aliquot was transferred to 100 ml of TSB and incubated at 37 °C for 24 h. The overnight cell suspension was centrifuged at 3000 g for 10 min at 5 °C. The cell pellets were washed with equal volume (100 ml) of sterile phosphate-buffered saline (PBS, pH 7.2) solution. Finally, the washed cells were resuspended in 100 ml PBS (10^9 CFU/ml) and used as the inoculum.

2.2. Sample preparation

Pasteurized apple juice concentrate (1.30 L) purchased from a local store was mixed with deionized sterile water (8.30 L) at room temperature (∼23 °C). *Escherichia coli* K-12 prepared as stated above (10^9 CFU/ml) was added to the apple juice, mixed and a serial dilution was prepared. A 0.1 ml of these diluted samples was plated in duplicate on TSA and Sorbitol MacConkey Agar (SMAC; BBL/Difco, Sparks, MD) plates with incubation at 35 °C for 24 h to determine the initial bacterial count.

2.3. RFEF treatment and processing variables

Inoculated apple juice was allowed to stand at room temperature for up to 2 h to allow the *E. coli* to adjust to the acidic environment before being pumped through the radio frequency electric field (RFEF) chamber (0.1 cm diameter, 0.2 cm gap, Geveke and Brunckhorst, 2004a) at a rate of 540 ml/min. The residence times of inoculated apple juice in the treatment chamber and the holding tube after the treatment chamber were 170 µs and 1.6 µs, respectively. The radio frequency used throughout the entire test was set at 20 kHz with a supplied peak to peak voltage of 6 kV. The resultant electric field strength was 15 kV/cm. The inlet temperatures to the treatment chamber were adjusted to achieve outlet temperatures of 25, 30 and 40 °C.

2.4. UV-light treatment and processing variables

A UV system with a higher flow rate closer to industrial scale was assembled in our lab and used for this study. The description of this equipment and the procedure used for this process have been reported (Geveke, 2008). The experimental system included a feed tank, pump, heat exchanger, tubular UV unit, and a cooling coil. A progressing cavity pump (model ADBP 8.3, Allweiler, Radolfzell, Germany) pumped the apple juice through a plate heat exchanger (model FT74-30-MKIII-33-34, Armfield, Jackson, NJ, USA) where the temperature was adjusted to 25, 30 and 40 °C and the feed rate was 540 ml/min. The apple juice flowed through the UV transparent Chemfluor tubing wrapped around a 30 W UV bulb. The UV bulb generated 90% energy at a wave length of 254 nm. The Chemfluor tubing has an internal diameter of 3.3 mm and a thickness of 1.6 cm. The tubing has a UV transmission of 89% at 254 nm and the length of the tubing in contact with the bulb was 14 m. The tubing was secured to the bulb with a self fusing silicone rubber tape (McMaster-Carl, Robbinsville, NY, USA). The rubber tape wrapped around the Chemfluor tubing and the UV bulb also ensured that no UV-light escaped. The UV treatment time was 12 s. Passage of apple juice through the UV reactor resulted in a slight temperature increase from the initial temperature. At inlet temperatures of 25, 30 and 40 °C, the temperatures of the apple juice increased by approximately 0.4, 0.5 and 0.1 °C, respectively. The temperatures of the apple juice entering and exiting the tubular UV bulb unit were measured with 3.2 mm diameter chrome-constantan thermocouples (Omega Engineering, Inc., Stamford, CT, USA). After UV treatment, the apple juice was cooled to <25 °C in approximately 2 s using a stainless-steel cooling coil submerged in a water bath. Controls were performed to determine the effect of temperature alone. The UV bulb was turned off, and the apple juice was heated to the specific temperature tested using the heat exchanger and then cooled using the cooling coil. Processed samples were collected in polypropylene tubes and placed on ice in a dark location to prevent photoreactivation (Clarke and Berman, 1983). Each experiment was performed in duplicate. Appropriate dilutions of the samples were plated on TSA and SMAC plates to determine colony forming units (CFU).

2.5. Microbial injury and viability loss

Aliquot (1 ml) of apple juice treated above was plated on TSA (non selective), and SMAC (selective) plates and incubated at 36 °C for 48 h. When necessary, depending on the treatment, samples were diluted in 0.1% peptone water (PW) before plating onto the agar plates. The difference in populations of *E. coli* enumerated on selective versus non-selective media was considered as injured cells, and the percent injury was calculated using this formula:

\[
\text{[1-colonies on selective agar/counts on nonselective]} \times 100
\]

The number of colony forming unit (CFU/ml) determined on non-selective and selective agar media was used to calculate the viability loss which is defined as the differences in log CFU/ml of bacteria between control and treated samples (Linton et al., 1999). Untreated inoculated apple juice was used as controls for each experiment.

2.6. Leakage of bacteria intracellular Ultraviolet (UV)-materials

To quantify the intracellular UV materials released from *E. coli* K-12, treated and untreated apple juice containing the UV materials were measured at 260 and 280 nm with a Spectrophotometer (DUR 530, Beckman Coulter, Fullerton, CA) (Burrin, 1986; Virto et al., 2005; Woo et al., 2000).
2.7. Bioluminescence ATP assay

To determine any increase in extracellular (somatic) ATP of apple juice after RFEF treatment, portions (0.1 ml) of sample prepared for microbiological analysis were mixed with 0.1 ml luciferin–luciferase (Sigma) reagent, using an ATP bioluminescent assay kit (Turner Design, Sunnyvale, CA). Bioluminescence ATP assays were performed using a TD-20/20 (DL Ready) Luminometer (Turner Design). The generated light signal was measured after a 3 s delay time and a 14 s integration time. Assays of standard amounts of purified ATP (Turner Design) were used to calculate ATP levels, and ATP concentrations in samples were expressed as log₁₀ femtogram (fg)/ml. Controls, for background luminescence, consisting of 50 μl apple juice, were run, and the readings were subtracted from readings for ATP determination. Possible inhibition of the luciferase reaction by residues from the apple juice and/or RFEF treatment was corrected by addition of known amounts of ATP standard to the reaction vial followed by addition of the luciferase enzyme (Ukuku et al., 2008).

2.8. Data analysis

All experiments were done in triplicate with duplicate samples being analyzed at each sampling time. Data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System Program (SAS Institute, Cary, NC, USA). Significant differences (P<0.05) between mean cell survival, viability loss, and leakage of UV-absorbing substances and ATP concentrations were determined by the Bonferroni least significant difference (LSD) method (Miller et al., 1981).

3. Results and discussion

There was no background microflora recovered in the uninoculated diluted apple juice concentrate plated on TSA and SMAC. The population of E. coli cells inoculated in apple juice determined before RFEF and UV-light treatment is shown in Table 1. The average population of E. coli cells determined after inoculation averaged 7.8 log₁₀ CFU/ml and 7.7 log₁₀ CFU/ml on TSA and SMAC plates, respectively. Thermal treatment at 25, 30 and 40 °C did not cause significant (P>0.05) reduction in the initial E. coli population determined however, the percent injury determined were significantly (P<0.05) different. Percent injury as a result of the thermal treatment alone at 25 °C, 30 °C and 40 °C was 8%, 69% and 80%, respectively.

3.1. Survival and injury of E. coli cells after UV-light treatment

The initial populations of E. coli cells determined before UV-light and RFEF treatment on TSA plates averaged 7.8 log. The UV treatment at 25 °C, 30 °C and 40 °C reduced the surviving population of E. coli cells to 3.8 log, 3.0 log and 2.0 log, respectively (Fig. 1). Among the surviving populations of E. coli cells in apple juice treated with UV-light at 25 °C and 30 °C reported above, 99% and 90% of the populations were injured, respectively. Treatment at 40 °C leads to higher inactivation and the population of injured E. coli cells determined was below 5%. The viability loss of E. coli cells determined in apple juice treated with UV light alone at 25 °C, 30 °C and 40 °C was 4.0 log, 4.8 log and 5.8 log. The results of this data suggest that UV-light treatment at 30 °C or below resulted in higher injury to the surviving E. coli cells in apple juice than treatment at 40 °C. Also, treatment at 40 °C was found to be appropriate for inactivating E. coli and reducing the population of injured E. coli cells in the juice.

3.2. Survival and injury of E. coli cells after RFEF treatment

The RFEF treatment at 25 °C, 30 °C and 40 °C alone decreased the surviving E. coli population to 7.6 log, 7.2 log and 5 log, respectively (Fig. 2). The surviving E. coli population determined at 25 °C was not significantly (P>0.05) different than the numbers determined in juice treated at 30 °C. Percent injury as a result of differences in plating media used and any other physiological response of E. coli cells to the environment in control samples averaged <5%. The percent RFEF injured populations of E. coli cells determined at 25 °C, 30 °C and 40 °C were 55%, 76% and 84%, respectively. With this treatment, three major observations were noted; a combination of RFEF and thermal treatment at 25 °C and 30 °C increased the population of injured E. coli cells.

Table 1

<table>
<thead>
<tr>
<th>Waterbath (°C)</th>
<th>TSA</th>
<th>SMAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.81±0.32</td>
<td>7.78±0.22</td>
</tr>
<tr>
<td>25</td>
<td>7.81±0.31</td>
<td>7.77±0.22</td>
</tr>
<tr>
<td>30</td>
<td>7.72±0.22</td>
<td>7.21±0.12</td>
</tr>
<tr>
<td>40</td>
<td>7.61±0.22</td>
<td>6.91±0.14</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation of three experiments with duplicate determinations. TSA = Tryptic Soy Agar. SMAC = Sorbitol MacConkey Agar.
a higher microbial inactivation and low number of viable and cultural able population of *E. coli* cells was determined at 40 °C suggesting that increasing the RFEF treatment temperature would enhance microbial inactivation. Another observation noted in this study was that a higher percentage of injured bacteria were determined within the viable and cultural able population of *E. coli* cells.

3.3. Effect of treatment combination on cell survival and injury

The results on the combined treatment of RFEF followed by UV-light for inactivation of *E. coli* cells and percent population of injured cells is shown in Fig. 3. Treatment combination (UV-light + RFEF) at 25 °C, 30 °C and 40 °C, caused a decline in the surviving populations of *E. coli* cells determined in the juice to 4.0 log, 3.3 and 1.5 log, respectively (Fig. 3). All treatment temperatures tested caused a significant (*P* < 0.05) decline in the surviving population of *E. coli* cells.

The results of UV treatment alone (Fig. 1), RFEF treatment alone (Fig. 2) and a combination of UV and RFEF treatment (Fig. 3) showed differences on where significant log reduction of *E. coli* cells occurred at a particular treatment temperature. For example, the figure on UV treatment alone by interpolation, suggest that treatment temperature at 20 °C could result in a viability loss of 2.8 log and approximate 70% injury to the *E. coli* cells, and that increasing the treatment temperature to about 37 °C would increase cell viability loss and at the same time decreased the percentage of injured population (Fig. 1). With RFEF treatment, the figure suggests treatment temperature above 30 °C to be enough to cause significant viability loss of *E. coli* with a high population of injured cells in the population (Fig. 2). When both treatments (UV and RFEF) were combined, the treatment temperature that led to significant cell viability loss was determined to be at 25 °C (Fig. 3), and was about 5 °C higher than when UV light was used alone. A higher bacterial inactivation was expected when the two treatments were combined; however, the number determined was only approximately 0.6 log higher than UV-light treatment alone. The data also suggest that combining the two treatments would reduce the treatment temperature required for significant inactivation of bacteria in apple juice. The UV-light treatments alone caused a significant (*P* < 0.05) inactivation of *E. coli* populations in the apple juice in contrast to RFEF treatment. However, results on viability loss of *E. coli* populations in apple juice treated with UV-light and RFEF treatments combination was not significantly (*P* > 0.05) different than when UV-light was used alone. At 40 °C UV-light treatment alone, the injured population determined was below 4% while the population determined in apple juice treated with RFEF was at 84%. The injured *E. coli* population determined in apple juice treated with RFEF at 25 °C was 55% and at 40 °C treatment, the injured populations increased to 84%.

3.4. Effect of storage on injured *E. coli* population

The effect of storage temperatures at 5 and 23 °C for 24 h on the surviving populations of RFEF, UV-light and a combined RFEF and UV treatment on injured *E. coli* populations in apple juice is shown in Table 2. The population of *E. coli* cells determined in apple juice immediately after treatments averaged 4.9 log with RFEF, 2 logs with UV-light and 1.4 log with RFEF+UV-light combine treatment. These populations decreased to <1 log CFU/ml in RFEF treated juice during storage at 5 °C for 24 h. The population determined in samples stored at room temperature (23 °C) for 24 h averaged <1 log. In UV-light and RFEF+UV-light combination, the population of surviving *E. coli* cells in the juices could not be determined. The best results were achieved with UV-light treatment alone and in combination with RFEF treatment.

3.5. Leakage of cellular materials of injured *E. coli*

The linear relationship between treatments and leakage of UV-materials from injured *E. coli* cells is shown in Fig. 4A and B. The average UV-absorbing materials determined at Ab260nm were 0.11. This value increased in all treatments and the correlation coefficient determined in juices treated with individual technology separately was *R*² = 0.985 for RFEF, 0.968 for UV-light and 0.930 for the combined UV-light and RFEF treatment (Fig. 4A). When the UV-materials were measured at Ab280nm the averaged value determined in untreated apple juice was 0.04. Again, this value increased in all treatments and the correlation coefficient determined in juices treated with individual technology separately was *R*² = 0.957 for RFEF, 0.993 for UV-light and 0.994 for the combined UV-light and RFEF treatment (Fig. 4B). The data suggest better correlation of UV-materials associated with protein (Ab280nm) than nucleic acid materials determined at 260 nm, with all treatments tested. Leakage of intracellular ATP of damaged *E. coli* cells and its accumulation in the media suggest membrane damage by the treatment (Fig. 5). Differences of ATP values determined between UV-light and RFEF treatment suggest that specificity of inactivation of bacteria by UV-light and RFEF treatment are not the same. The RFEF targets surface structures while UV-light targets membrane materials and the slight increase in the ATP determined at 40 °C could be attributed to temperature effect on the membrane. Injured bacterial cells are also known to release intracellular nucleic acid and proteins into a suspension (Ukuku and Shelef, 1996; Ukuku et al., 2008). Leakage of nucleic acid and its related compounds, such as pyrimidines and purines, absorb UV light at a wavelength of 260 nm (Virto et al., 2005; Woo et al., 2000). The level of protein related material determined at 280 nm was dependent on the type of treatment used. All treatment at 30 °C led to significant (*P* < 0.05) membrane damage and leakage of UV-materials however, higher values of nucleic acid and protein related materials were determined when treatment was performed at 40 °C.

Table 2

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>RFEF</th>
<th>UV-light</th>
<th>RFEF + UV-light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediately after treatment</td>
<td>4.9 ± 0.15</td>
<td>2.0 ± 0.04</td>
<td>1.4</td>
</tr>
<tr>
<td>5 °C storage</td>
<td>&lt;10 CFU/ml</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>23 °C storage</td>
<td>&lt;1 log</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = No CFU was determined.

Determination were made after storage for 24 h at designated temperature. Values are means ± standard deviation of three experiments with duplicate determinations.

---

**Fig. 3.** Relationship between survivals of *Eschericha coli* cells and percent injured populations in apple juice caused by a combined treatment of UV-light and Radio frequency electric field (RFEF) at 25, 30 and 40 °C. Values are means ± standard deviation of duplicate determination. Determinations on TSA and SMAC were performed immediately after treatment.
In our previous study, we reported low levels of nucleic acid related material determined in apple juice treated with RFEF at 45 °C and above (Ukuku et al., 2008). In that study we attributed the rapid decline of these substances at higher temperature to possible interaction of electrical charge produced by the RFEF as they leak out of the bacterial membrane. In our current study, we did not want to encounter this problem; therefore the maximum treatment temperature tested was at 40 °C. Also we wanted to make sure that any bacterial inactivation by these technologies can only be attributed to non-thermal effects. This is the first study to document the efficacy of using a combined UV-light and RFEF technology for inactivation of bacteria. Similarly, we showed differences on the effect of these treatments on injury and leakage of UV-materials and the fate of injured E. coli cells in treated apple juice during storage. Earlier studies have used model system of lipid vesicle consisting of phosphatidyl choline for determination of cellular leakages and for effective comparison. In that study, the authors observed an increased leakage of phosphatidyl choline throughout the course of the study (Ponne et al., 1996).

The inactivation of E. coli population in apple juice by the UV-light exposure alone was significantly \( P<0.05 \) different than treatments using RFEF alone. When the treatment temperature was increased to 40 °C, bacterial inactivation determined was significantly \( P<0.05 \) different than treatments using RFEF at 40 °C. Other researchers have reported inactivation of approximately 5 log or greater when annular-type UV apparatuses were used (Basaran et al., 2004; Wright et al., 2000) and their results were also similar to those determined with California Day-Fresh Foods tubular UV apparatus (Morris, 2000). In our study, we achieved a viability loss of 5.8 log using the UV treatment alone and 6.4 log when the treatment was combined, suggesting an increase in viability loss of 0.6 log. In our previous study, we reported that RFEF treatment at 27 °C did not cause significant \( P>0.05 \) change in the population of E. coli cells inoculated in apple juice (Ukuku et al., 2008). The results of our current study clearly showed the differences in bacterial inactivation by UV light treatment alone at 25 and 30 °C. In conclusion, the RFEF treatment at 25 and 30 °C did not cause significant reduction of cell populations; however, treatment at 40 °C did cause significant reduction. The combined UV-light and RFEF treatment resulted to 0.6 log higher microbial inactivation in contrast to UV-light treatment alone at 40 °C. The percent population of injured E. coli cells and leakage of intracellular UV-materials determined when treatment was combined was significantly \( P<0.05 \) different than UV-light treatment alone. Cold storage at 5 °C for 24 h inactivated the injured cells. Further studies are needed to establish the optimum processing variables that could increase bacterial inactivation, and at the same time reducing the populations of injured cells in treated samples when stored at an abusive temperature. With only 0.6 log inactivation higher than UV-light treatment alone, the results of this study seem to suggest that bacterial inactivation by combining these two non-thermal technologies at the parameters tested was not additive.

Acknowledgments

The authors thank Timothy Schurmann and Donyel Jones for their technical assistance and Dr. John G. Phillips for the assistance in statistical analysis of the data.

References
