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Effect of Thermal and Radio Frequency Electric Fields Treatments on Escherichia coli bacteria in Apple Juice

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Abstract

The need for a nonthermal intervention technology that can achieve microbial safety without altering nutritional quality of liquid foods led to the development of the radio frequency electric fields (RFEF) process. However, insight into the mechanism of bacterial inactivation by this technology is limited. In this study, we investigated membrane damage of *Escherichia coli* bacterial (7.8 log CFU/ml) and leakage of intracellular membrane materials in RFEF treated apple juice at 25 kV/cm and operated at 25°C, 55°C and 75°C for 3.4 milliseconds at a flow rate of 540 ml/min. Damage to cell membrane was detected with Transmission Electron Microscopy (TEM) and leakage of cellular materials was determined with ATP luminometer (20 D) and electrostatic and hydrophobic interaction chromatography used to characterize changes in bacterial cell surfaces. RFEF treatment caused a significant decrease in bacterial cell surface hydrophobicity and loss of relative negative ions compared to heat treatment alone at 55°C and 75°C. Leakage of cellular materials into the media indicated cell damage and TEM observation showed altered intracellular membrane structure in RFEF treated *E. coli* cells. The results of this study suggest that the mechanism of inactivation of RFEF is by disruption of the bacterial cell surface hydrophobicity and loss of relative negative ions which led to injury and leakage of cellular materials and death.

Keywords: Injury; Heat; *Escherichia coli*; Surface charge; Hydrophobicity; Apple juice; Radio Frequency Electric Field (RFEF)

Introduction

Physical and chemical treatments have been used in food processing to eliminate or reduce the presence of pathogenic and spoilage microorganisms in foods [1-5]. Thermal processing is used by the juice industry to inactivate food borne pathogens; however; it impairs the characteristic flavor of juices [6-8]. Therefore there is a need for alternative processing treatments that can achieve a 5-log reduction of these pathogens [9,10], without causing adverse effect on the flavor of the juice. Several nonthermal technologies have been commercialized, including ultraviolet processing of apple cider [11,12]. A pasteurization process using radio frequency electric fields (RFEF) has recently been developed [11,13-15]. In these studies, a set of RFEF-operating parameters that achieved 5-logs reduction of *E. coli* in apple cider was determined, and the kinetics of bacterial inactivation established.

Enterohemorrhagic *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* are recognized foodborne pathogens [3,6,10,16,17], and are capable of surviving in low acid foods like fruit juices [6,18-20]. Outbreaks involving *E. coli* O157:H7 in apple cider [2,21], and *Salmonella* in orange juices [20,22], have raised concerns about the safety of consuming unpasteurized fruit juices. The effect of RFEF on sublethal injury of *E. coli* and leakage of intracellular adenosine triphosphate (ATP) and Ultraviolet (UV)-absorbing materials have been reported [12,23]. The authors, using scanning electron microscopy showed differences in *E. coli* cell surface damage. Bacterial cell surfaces have a net negative charge due to the presence of ionized phosphoryl and carboxylate moieties on the outer envelope exposed to the extracellular environment [24], used hydrophobic interaction chromatography (HIC) and electrostatic interaction chromatography (ESIC) to estimate changes on net negative charge and hydrophobicity of *E. coli* cells treated with pulsed electric fields. In that study, the authors reported that pulsed electric fields, a similar non-thermal processing treatment to RFEF led to changes in *E. coli* cell surface charge. Data on the exact mechanism of bacterial inactivation using RFEF are limited. We hypothesized that RFEF inactivation occurs through irreversible alterations in the organization of the outer and inner cell membranes with subsequent internal cytoplasmic reorganization as a consequence of the high voltage electric field exerted on the bacterial cells.

In this study, we used hydrophobic interaction chromatography (HIC) and electrostatic interaction chromatography (ESIC) to estimate changes on the net negative charge and surface hydrophobicity of *E. coli* cells treated with RFEF with or without heat. The effect of RFEF and thermal processing on intracellular membrane organelles of *E. coli* was investigated using transmission electron microscopy (TEM). Results presented in this study are intended to serve as a guide in understanding the mechanism of inactivation of bacteria by RFEF and will provide a scientific basis for an effective comparison of this technology against conventional thermal processing technology. Similarly, the results may help juice processors, research institutions and the regulatory agency to understand the mechanism of bacteria inactivation by RFEF processing.

Materials and Methods

Test strains and preparation of inocula

E. coli K-12 (ATCC 23716) from the U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research

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Center culture collection were used in this study. Individual cell cultures were maintained on Tryptic Soy Agar (TSA) at 4°C. Prior to use the cells were inoculated by loop in Tryptic Soy Broth (TSB: Remel, Inc., Lenexa, KS) with incubation at 36°C for 16-18 h with shaking. A 0.1-ml cell aliquot was transferred to 100 ml of TSB and incubated at 36°C for 24 h. The overnight cell suspensions were centrifuged at 3,000 *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 resuspended in 100 ml PBS was plated (0.1 ml) on TSA to determined the number of Colony Forming Unit (CFU) after serial dilution in 0.1% Peptone water. After 48 h incubation of TSA plates at 36°C, the average bacterial count was approximately 10^9 CFU/ml and this was used as the inoculum.

Sample preparation

A 1.30 L volume of apple juice concentrate purchased from a local store was mixed with 8.30 L of deionized sterile water at room temperature (~23°C). *Escherichia coli* K-12 prepared as above (10^9 CFU/ml) was added to the apple juice, mixed and a serial dilution was prepared. A 0.1 ml aliquot of these diluted samples was plated in duplicate on TSA with incubation at 36°C for 24 h to determine the initial bacterial colony-forming units (CFU).

RFEF treatment and processing variables

Inoculated apple juice was allowed to stand at room temperature for up to 2 h before being pumped through the radio frequency electric field (RFEF) chamber (0.1-cm diameter, 0.2-cm gap) [15], at a rate of 540 ml/min. The residence time of inoculated treated and untreated apple juice in the holding tube after the treatment chamber was 1.6 s. The radio frequency used throughout the entire test was set at 20 kHz with a supplied peak to peak voltage of 6 kV. The inlet temperatures to the treatment chamber were adjusted to achieve outlet temperatures of 25°C, 55°C and 75°C. In another study designed to investigate the influence of temperature alone, the 0.2-cm gap treatment chamber was replaced with a 20-cm gap treatment chamber to reduce the electric field by a factor of 100 [14]. The water bath temperature was adjusted to give a similar outlet temperatures stated above up to 75°C. Samples (~ 20- ml) at each outlet temperatures were individually collected and analyzed for survivors, viability loss, injury and changes in cell surface hydrophobicity.

Chromatography

Hydrophobic interaction chromatography (HIC) and electrostatic interaction chromatography (ESIC) columns were prepared according to the procedure described by [25,26], with slight modification [24]. For the HIC, Pasteur capillary pipettes (14.59 cm long, Macalaster Bicknell Co., Millville, NJ) were plugged with glass wool, and washed sequentially with 5-ml of 75 % ethanol and 10-ml of 0.02 M sodium phosphate ($NaPO_4$), pH 6.8 buffer. Columns for HIC were packed with 8-ml of Octyl-Sepharose CL-4B gel (Sigma, St. Louis, MO) equilibrated overnight at 4°C in 12-ml of 1 M ammonium sulfate (NH_4SO_4), pH 6.8 buffer. Approximately 4 ml of the equilibrated gel was added to the column to obtain a 0.7-ml bed volume. The gel bed was washed with 12 bed volumes of 1 M (NH_4SO_4), pH 6.8 buffers to remove traces of ethanol added to the gel as preservatives. The ESIC columns were packed with 2 -ml distilled water (1:2 wt/vol) suspension of the ion-exchange resin (Dowex chloride form, Sigma) for the anionic resin and Dowex hydrogen form (capacity, 1.7 meq/ml, 50 by 8) for the cation resin (Bio-Rad Laboratories, Richmond, CA). The mesh size was 100 to 200 μm for both resins. Chromatography was done according to [27].

Surface hydrophobicity and the bacterial surface charge

For bacterial surface hydrophobicity, a sample (0.1-ml) of washed bacterial cell suspension for each strain stated above was loaded onto the surface of the column followed by elution with 10-ml of 1 M NH_4SO_4 buffer at a flow rate of 2.6 ml/min. The eluted fractions were collected at 2-ml interval and the bacterial populations in each ml fraction were determined by plating on TSA. From this experiment, it was established that approximately 5-ml of the buffer was needed to desorb most of the bacteria from the gel. For the rest of the study, 0.1 ml of the RFEF treated cells was placed on the gel, 5-ml of 2 M NH_4SO_4 buffered with sodium phosphate ($NaPO_4$, pH 6.8) was passed continuously through the gel and the eluted fraction was collected and named eluate (e). Similarly, the gel also was collected and named g. Finally, 0.1 ml of washed or RFEF treated cells was mixed with 4-ml of 2 M NH_4SO_4 buffer solutions and were individually named X [28]. The same procedure was used for the ESIC study and the relative ion values for RFEF treated and untreated *E. coli* cells were determined and expressed as r/e. "r" represent the number of bacteria retained by the gel in the columns while "e" is the numbers eluted from the gel. The bacterial cell populations in all the suspensions including each eluted sample and the populations remaining in the gel were determined using TSA (BBL/Difco). The relative hydrophobicity of RFEF treated and untreated *E. coli* cells were expressed as the g/e ratio. According to [22], a log g/e ratio of < 0 is considered to indicate hydrophilicity.

Microbial injury and viability loss

An aliquot (1 ml) of apple juice treated as described above was plated on TSA and Violet Red Bile Agar, with 5 ml overlay of the same agar containing 4-methylunbelliferyl-beta-D-glucuronide ((BBL/Difco) and Sorbitol McConkey Agar (SMAC, (BBL/Difco) incubated at 36°C for 48 h to determine the number of CFU/ml. When necessary, depending on the RFEF 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:

[1- colonies on selective agar/ colonies on nonselective agar] × 100----------(1)

The number of colony forming unit (CFU/ml) on nonselective 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, thermal and RFEF- thermal treatment combination [8]. Untreated RFEF inoculated apple juice was used as controls for each experiment.

Transmission electron microscopy (TEM)

Ten microliter (10 uL) aliquots of 25% glutaraldehyde were added to 0.99 mL volumes of treated and untreated apple juice. The mixture was rapidly vortexed and centrifuged in Eppendorf tubes to sediment the cells into a pellet and stored after sealing the tubes. Subsequently the supernatant fluid was decanted and pellets washed with 0.1 M imidazole HCl buffer at pH 7.2, then post-fixed with 2% osmium tetroxide solution- imidazole buffer for 2 hours, dehydrated in 50%, 80% and absolute ethanol for an hour, infiltrated with epoxy resin overnight and cured for two days at 60°C. Thin sections of the pellets were cut with diamond knives and stained with 2% uranyl acetate and lead citrate solutions before finally observing cell profiles in the thin sections with a transmission electron microscope (model CM12, FEI Corp., Hillsboro, OR) operated at 80 kV in the bright field mode. Photographic images were collected at an instrumental magnification of 22,000X and digitized with a film scanner.

Leakage of bacteria intracellular Ultraviolet (UV)-absorbing materials

To quantify the intracellular UV materials released from *E. coli* K-12, the extracts from treated and untreated cells containing the UV materials were measured at 260 and 280 $_{nm}$ with a Spectrophotometer (DUR 530, Beckman Coulter, Fullerton CA) according to published reports [29,30].

Bioluminescence ATP assay

Extracellular (somatic) ATP content of treated and untreated apple juice before and after inoculation with *E. coli* were determined. Portions (0.1 ml) of apple juice samples were mixed with 0.1 ml luciferin-luciferase (Sigma, St Louis, MO.) and the somatic ATP content of the samples were measured as relative light unit (RLU) using an ATP bioluminescent assay kit (Turner Design, Sunnyvale, CA). The generated light signal (RLU) was measured using a TD-20/20 (DL Ready) Luminometer (Turner Design) after a 3 s delay time and a 14 s integration time. Assays of standard amounts of purified ATP were used to calculate ATP levels, and ATP concentrations in samples were expressed as $\log_{10}$ femtogram (fg)/ml. Controls, for background luminescence, consisting of 0.1 ml of the apple juice, were run, and the readings were subtracted from readings for ATP determination. Possible inhibition of the luciferase reaction by any residues from the apple juice was corrected by addition of known amounts of ATP standard to the reaction vial followed by addition of the luciferase enzyme [31].

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). The SAS program was used to determine significant differences in survival, viability loss, injury and changes in bacterial cell surface charge and hydrophobicity. Significant differences ($p < 0.05$) between mean values of number of surviving cells and viability loss were determined by the Bonferroni least significant difference (LSD) method [32].

Results and Discussion

Effect of RFEF and heat treatment on E. coli populations

There was no background microbiota recovered in the uninoculated apple juice plated on TSA and VRBA. To investigate the effect of heat treatment alone, the RFEF treatment chamber was changed from 0.2 cm gap (25 kV/cm) to 20 cm gap (0.25 kV/cm) and the chamber temperature set at 25°C, 55°C and 75°C. It is widely understood that application of heat pasteurization would inactivate most bacteria in liquid food. In this study, we investigated the effect of minimal heat treatment alone by allowing inoculated apple juice samples to pass through the 20 cm RFEF chamber. The population of *E. coli* cell recovered in apple juice after inoculation and before heat and RFEF treatment averaged 7.8 $\log_{10}$ CFU/ml on agar plates. This population remained the same in apple juice treated at 25°C without RFEF being on. The heat treatment at 55°C and 75°C for 1.6 s alone reduced the surviving populations of *E. coli* to approximately 7.3 $\log_{10}$ CFU/ml and 6.6 log CFU/ml, respectively. RFEF treatment at 25°C caused approximately 0.8 log viability loss in *E. coli* cell populations in treated apple juice. Surviving populations of *E. coli* cells in apple juice treated at 55°C and 75°C were significantly ($p < 0.05$) reduced. At 75°C treatment, the viability loss for *E. coli* in heat and RFEF combination averaged approximately 7 log CFU/ml and, the healthy population of *E. coli* cells recovered in VRBA plates averaged < 15 CFU/ml. The viability loss and percent injury for *E. coli* decreased significantly when the RFEF was turned on and operated at the set temperatures.

Injured populations of E. coli populations

The background population of injured *E. coli* cells of the total population before treatments averaged approximately 4%. Heat treatment alone at 25°C did not cause any injury to the *E. coli* populations however, treatment at 55°C and 75°C led to increased populations of injured *E. coli* cells to approximately 38 % and 70%, respectively (Figure 1). When the RFEF treatment was operated at 25°C and 55°C, the injured populations in samples increased to approximately 50% and 60%, respectively. Leakage of intracellular membrane materials from injured bacteria is shown in Figure 2. Heat treatments alone resulted to a slight increase in ATP leakage in samples treated at 55°C and 75°C. Intracellular membrane materials determined at Ab_{260} and Ab_{280} were not significantly ($p > 0.05$) different. However, treatment at 55°C in combination with RFEF showed a significant ($p < 0.05$) leakage of ATP and intracellular membrane materials at Ab_{260}. Also, leakage of intracellular membrane materials in 55°C+RFEF samples determined at Ab_{280} was slightly higher than heat treatments at 55°C and 75°C.

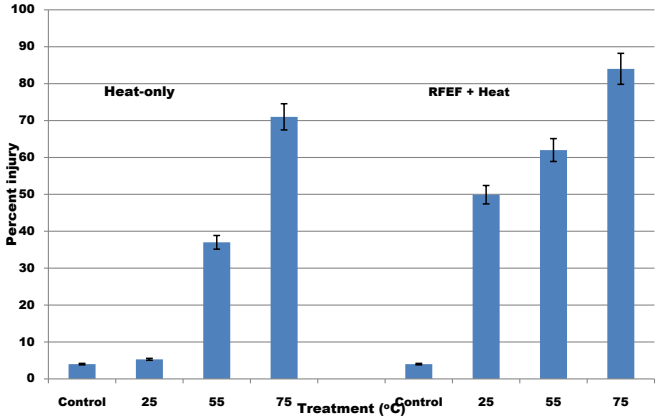

Figure 1: Percent injury of *Escherichia coli* K-12 (ATCC 23716) in apple juice treated with RFEF (25 kV/cm) at 25 °C, 55°C and heat at 75°C. Values are means of three determinations ± standard deviation.

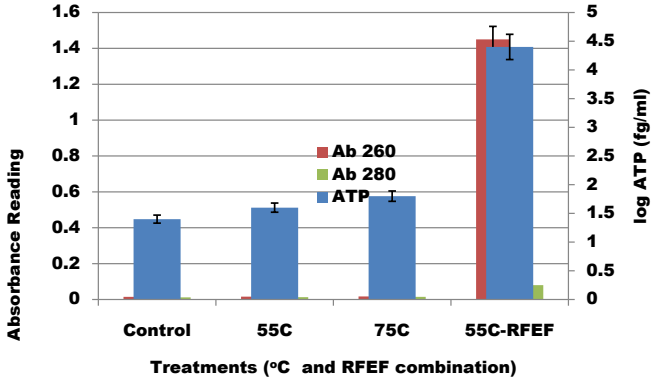

Figure 2: Leakage of intracellular membrane materials of *Escherichia coli* K-12 (ATCC 23716) in apple juice treated with heat alone at 55°C and 75°C and RFEF (25 kV/cm) +55°C combination. Values are means of three determinations ± standard deviation.

TEM observation of intracellular structure of E. coli cells

Previously, we used SEM to visualize the outer membrane structures of RFEF treated *E. coli* bacteria and determined formations of blebs on the cell surfaces [12]. The effect of heat and RFEF treatments on the cytoplasmic structure of the *E. coli* K-12 cells suspended in apple juice visualized by TEM is shown in Figure 3. At room temperature, the outer membranes of the regular, rod-shaped control *E. coli* bacteria were smooth and the internal organelles densely dispersed (Figure 3A). Heat treatment alone at 75°C resulted in condensed and aggregated cytoplasm (Figure 3B), but the outer membranes lacked the smooth regular, rod shaped membrane that were typical of the control *E. coli* cells (Figure 3A). The effect of treatment temperature at 75°C alone caused changes in the internal alignment of the cellular organs and these changes were significantly ($p < 0.05$) different than *E. coli* cells in control samples. TEM images of RFEF-treated samples (Figure 3C) revealed an altered outer membrane structure; although distinctly trilaminar, the electron-density of the cytoplasm was markedly condensed and aggregated as compared to controls. Cytoplasmic membrane materials of *E. coli* cells treated with heat and RFEF combination (Figure 3C) appeared more separated and disrupted than *E. coli* cells treated with heat only (Figure 3B). It is possible that the condensed and aggregated electron-density of the cytoplasm by the RFEF treatment led to the separation and disruption of cytoplasmic membranes and the formation of surface blebs or vice versa as reported earlier [12]. Since surface blebs occurred on the cell membrane, we investigated the effect of RFEF treatment on *E. coli* cell surface charge and hydrophobicity.

Another experiment designed to investigate the effect of heat and RFEF treatments on the *E. coli* cell surface charge and hydrophobicity is shown in Table 1. The relative surface charge and hydrophobicity of *E. coli* cells determined immediately before treatment averaged 33.3 and 0.240, respectively. These values did not change in *E. coli* cells treated at 25°C. These results are consistent with our earlier studies on relationship between bacterial cell surface charge and attachment to cantaloupe surfaces [33]. In our current study, when the RFEF treatment was set at 25°C the *E. coli* cell surface hydrophobicity increased slightly while the relative surface charge declined. Similarly, heat treatment alone at 55°C and 75°C increased the surface hydrophobicity to 0.268 and 0.348, respectively. Both heat alone and in combination with RFEF treatments increased changes in surface hydrophobicity of *E. coli* bacteria in apple juice (Figure 4). Increase in surface hydrophobicity of *E. coli* bacteria in apple juice was moderate when heat treatment alone was used compared to when used in combination with RFEF. There was an inverse relationship between negative ion loss and percent injury in *E. coli* bacteria treated with heat alone and in combination with RFEF (Figure 5). Heat alone, also decreased the relative negative ion charge on the surface of *E. coli* bacteria; however, the relative loss of these negative ions on the surface of *E. coli* cells treated with heat and RFEF combination was significantly ($p < 0.05$) different than heat treatment alone.

The most widely used techniques to study bacterial cell surface charge are HIC and ESIC because bacterial cell surface properties can only be measured indirectly, through phenomena that reflect more or less the nature of molecular interactions with surfaces [28,34,35]. The results of our HIC and ESIC studies were similar to those of Dickson and Koohmaraie [27], who used phenyl-Sepharose CL-4B gel (less hydrophobic than Octyl-Sepharose CL-4B used in this study) for HIC and Dowex resins (chloride and hydrogen form) for ESIC. Their study reported g/e values of 0.203 for *E. coli* O157:H7 (no strain designation given). The relationship between bacterial cell surface charge and injury as a function of treatment was investigated and is shown in Figure 4. With heat treatment alone, we can extrapolate that injury and changes in surface hydrophobicity of *E. coli* cells occurred at approximately 50°C while these changes in RFEF+ heat treatment combination occurred at approximately 12°C. We further estimated the relationship between injury and loss of negative ions on *E. coli* cell surface (Figure 5). The result of these treatments suggests that surface hydrophobicity of *E. coli* cells are affected first before the negative ion charges. This is consistent with our earlier report where changes in bacterial cell surface structure and shape where reported. In that study, most cells had "pimpled" surfaces with a few to many circular and irregularly shaped spots (20-

		Surface charge (r/e)	
Treatment (°C)	Hydrophobicity (g/e)	ESIC (-)	ESIC (+)
Control at 23°C	0.240 + 0.022^{E}	33.30 ± 0.14^{A}	0.09 ± 0.02^{A}
25°C only	0.245 + 0.023^{E}	33.27 ± 0.14^{A}	0.09 ± 0.02^{A}
RFEF + 25°C	0.274 + 0.018^{C}	28.26 ± 0.12^{B}	0.00 ± 0.01^{A}
55°C only	0.268+ 0.022^{D}	22.41 ± 0.16^{C}	ND
RFEF + 55°C	0.524 +0.032^{A}	4.82 ± 0.12^{F}	ND
Heat at 75°C	0.348 +0.020^{B}	16.12 ± 0.14^{D}	ND

[a] Values are means ± standard deviation of three separate experiments with duplicate determinations. Mean in each column not followed by the same letter are significantly ($p<0.05$) different

ND = None determined

No positive (+) ion value was determined when 2 M $(NH_4)_2SO_4$ buffer was used to desorb the *E. coli* cells from the hydrogen ion gel

Table 1: Effect of Thermal, RFEF and Thermal-RFEF combination treatments on bacterial cell surface charge and hydrophobicity of Escherichia coli in apple juice.

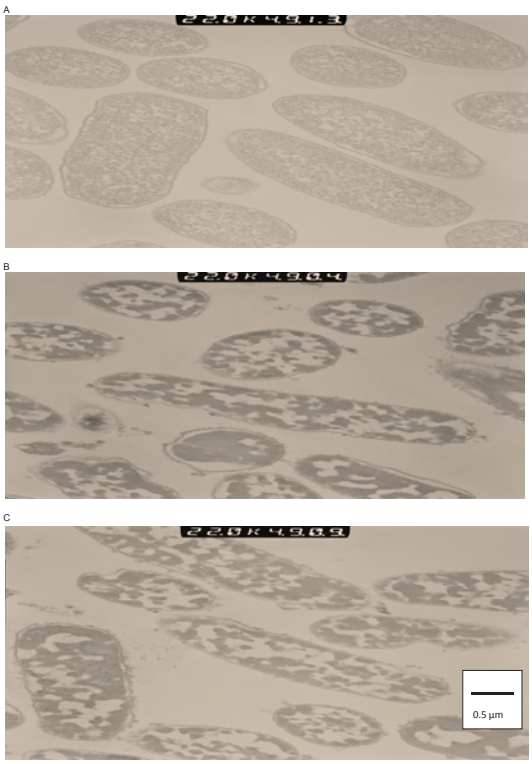

Figure 3: TEM observation of changes in RFEF (25 kV/cm) at 55°C and heat (75°C) treated *Escherichia coli* K-12 (ATCC 23716) in apple juice (A = control; B = 75°C and C = 55°C + RFEF, Bar = 0.5 µm). Values are means of three determinations ± standard deviation.

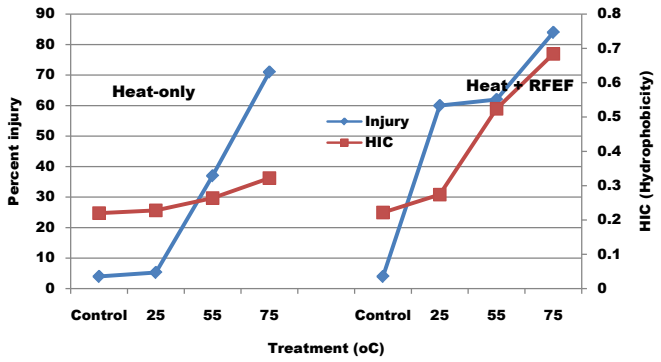

Figure 4: Effect of RFEF (25 kV/cm) treatment at 25°C, 55°C and heat (75°C) on the relationship between injury and bacterial cells surface hydrophobicity *Escherichia coli* K-12 (ATCC 23716). Values are means of three determinations ± standard deviation.

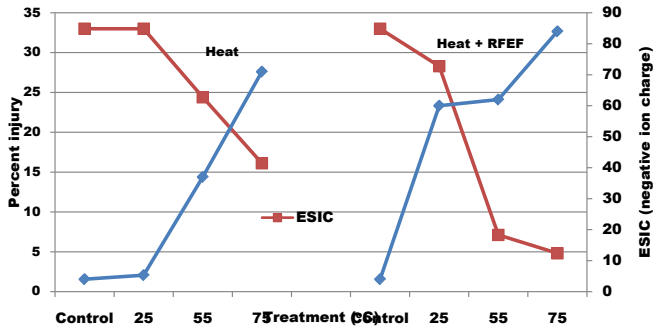

Figure 5: Effect of RFEF (25 kV/cm) treatment at 25°C, 55°C and heat (75°C) on the relationship between injury and relative negative ion loss of *Escherichia coli* K-12 (ATCC 23716). Values are means of three determinations ± standard deviation.

30 nm) in diameter after RFEF treatment at 40°C [12]. In that study, the authors also reported leakage of intracellular ATP of *E. coli* bacteria exposed to RFEF treatment at 27°C.

The electric field strength of the RFEF at all temperature tested deformed the bacterial surface structure leading to the observed changes on both external cell surfaces and intracellular membranes of *E. coli* cells. Gross effects of electrical interaction with biological cells are well known and a macroscopic intravascular electrode maintained at a constant current intensity of 1 mA was found to induce thrombosis and injury on the vascular wall, ranging from minimal lesion of endothelium to almost total necrosis of the vascular wall [36,37]. *Escherichia coli* K-12 used in this study is a gram-negative bacteria which has lipopolysaccharide (LPS) and protein units on its outer layer. The (LPS) and protein forms a highly charged surface that is stabilized by cation binding [38]. It is possible that the disruption of the surface structure of *E. coli* in this study was due to charge-charge interactions between the bacterial negative charge and the energy charge produced by the RFEF. For example, when the RFEF treatment chamber was changed from 0.2 cm gap (25 kV/cm) to 20 cm gap (0.2 kV/cm) and the chamber temperature set at 25°C, the population reduction determined with selective vs. non selective agar plates were not significantly ($p > 0.05$) different than when the chamber was 0.2 cm. Similarly, when the temperature inside the RFEF treatment chamber was reduced, viability loss and percent injury for *E. coli* decreased significantly and at 25°C the population of bacteria recovered and the percent injury were not significantly ($p > 0.05$) different than the control. Changes on the surfaces of *E. coli* cells treated at <50°C were significantly ($p < 0.05$) different than bacterial cells treated with RFEF at similar temperatures, and this differences can only be attributed to the electrical effect of the RFEF. The electric energy for the RFEF process used was calculated at 300 J/ml which was enough to cause changes on bacterial cell surface charge and hydrophobicity as seen in this study. When the intensity of the electric field was decreased to <1 kV/cm and the outlet temperature maintained at 25°C, the viability loss for *E. coli* averaged 0.3 $\log_{10}$ CFU/ml. Above this temperature, thermal effects contributed to minimal inactivation and less sub-lethal injury. A similar finding on inactivation of yeast in water and *E. coli* in apple juice at near ambient temperatures by RFEF has been reported [13-15].

The RFEF technique utilized in this study is non-thermal due to the fact that bacterial inactivation does not rely on heat application alone. However, an application of moderate heat to the RFEF treatment as seen in this study provides a much greater effect to the inactivation of bacteria than when used alone. The results of this study suggest that inactivation of bacteria by RFEF treatment was in part, as a result of induced changes on bacterial cell surface hydrophobicity and loss of negative ions which led to changes in structural surfaces, injury and leakage of intracellular membrane materials of treated *E. coli* cells. The results of this study will contribute to the understanding of the mechanism of inactivation of bacteria by RFEF and will aid both the regulatory agency and industry in their decision making towards approval and recommendation of this process.

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References

1. Benkerroum N, Sandine WE (1988) Inhibitory action of nisin against Listeria monocytogenes. J Dairy Sci 71: 3237-3245.
2. Hite B N, Giddings N J Weakly CE (1914) The effect of certain microorganisms encountered in the preservation of fruits and vegetables. Bull. West Virginia Agr. Exp. Sta., 146:3-67.
3. Padhye NV, Doyle MP (1992) Escherichia coli O157:H7: Epidemiology, pathogenesis and methods for detection in food. J Food Prot 55: 555-565.
4. Sizer CE, Balasubramaniam VM (1999) New intervention processes for minimally processed juices. Food Technol. 53: 64-67.
5. Ukuku DO, Pilizota V, Sapers GM (2004) Effect of hot water and hydrogen peroxide treatments on survival of Salmonella and microbial quality of whole and fresh-cut cantaloupes. J Food Prot 67: 432-437.
6. Besser RE, Lett SM, Weber JT, Doyle MP, Barrett TJ, et al. (1993) An outbreak of diarrhea and hemolytic uremic syndrome from Escherichia coli O157:H7 in fresh-pressed apple cider. JAMA 269: 2217-2220.
7. FDA. (2002) Thermally-processed low-acid foods packaged in hermetically sealed containers. Federal Register, 21CFR113. US Food and Drug Administration, Rockville, MD.
8. Linton M, McClements JM, Patterson MF (1999) Inactivation of Escherichia coli O157:H7 in orange juice using a combination of high pressure and mild heat. J Food Prot 62: 277-279.
9. Mazzotta AS (2001) Thermal inactivation of stationary-phase and acid-adapted Escherichia coli O157:H7, Salmonella, and Listeria monocytogenes in fruit juices. J Food Prot 64: 315-320.
10. Mason J (1994) Salmonella Enteritidis control programs in the United States. Int J Food Microbiol 21: 155-169.
11. Geveke DJ, Kozempel M, Scullen OJ, Brunkhorst C (2002) Radio frequency energy effects on microorganisms in food. Innov Food Sci Emer Technol 3: 133-138.

12. Ukuku DO, Geveke DJ, Cooke P, Zhang HQ (2008) Membrane damage and viability loss of E. coli K-12 in apple juice treated with radio frequency electric field. J Food Prot 71: 684-690.

13. Geveke DJ, Brunkhorst C (2003) Inactivation of Saccharomyces cerevisiae with radio frequency electric fields. J Food Prot 66: 1712-1715.

14. Geveke DJ, Brunkhorst C (2004) RFEF pilot plant for inactivation of Escherichia coli in apple juice. Fruit Processing 14: 167-170.

15. Geveke DJ, Brunkhorst C (2004) Inactivation of Escherichia coli in apple juice by radio frequency electric fields. J Food Sci 69: 134-138.

16. Doyle MP (1991) Escherichia coli O157:H7 and its significance in foods. Int J Food Microbiol 12: 289-301.

17. Madden JM (1992) Microbial pathogens in fresh produce-the regulatory perspective. J Food Prot 55: 821-823.

18. CDC (1996) Outbreaks of Escherichia coli O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider-Connecticut and New York. MMWR 46: 4-8.

19. CDC (1996) Outbreak of Escherichia coli O157:H7 infections associated with drinking unpasteurized commercial apple juice-British Columbia, California, Colorado, and Washington. MMWR 45: 975.

20. CDC (1999) Outbreak of Salmonella serotype Muenchen infections associated with unpasteurized orange juice-United States and Canada. MMWR 48: 582-585.

21. Cody SH, Glynn MK, Farrar JA, Cairns KL, Griffin PM, et al. (1999) An outbreak of Escherichia coli O157:H7 infection from unpasteurized commercial apple juice. Ann Intern Med 130: 202-209.

22. Dahlback B, Hermansson S, Kjelleberg S, Norkrans B (1981) The hydrophobicity of bacteria- an important factor in their initial adhesion at the air-water interface. Arch Microbiol 128: 267-270.

23. Ukuku DO, Geveke DJ (2010) A combined treatment of UV-light and radio frequency electric field for the inactivation of Escherichia coli K-12 in apple juice. Int J Food Microbiol 138: 50-55.

24. Ukuku DO, Yuk HG, Zhang H (2011) Hydrophobic and electrostatic interaction chromatography for estimating changes in cell surface charge of Escherichia coli cells treated with pulsed electric fields. Foodborne Pathog Dis 8: 1103-1109.

25. Mafu AA, Roy D, Savoie L, Goulet J (1991) Bioluminescence Assay for estimating the hydrophobic properties of bacteria as revealed by hydrophobic interaction chromatography. Appl Environ Microbiol 57: 1640-1643.

26. Pedersen K (1981) Electrostatic interaction chromatography, a method for assaying the relative surface charge of bacteria. FEMS Microbiol Lett 12: 365-367.

27. Dickson JS, Koohmaraie M (1989) Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. Appl Environ Microbiol 55: 832-836.

28. Mozes N, Rouxhet PG (1987) Methods for measuring hydrophobicity on microorganisms. J Microbiol Methods 6: 99-112.

29. Virto R, Manas P, Alvarez I, Condon S, Raso J (2005) Membrane damage and microbial inactivation by chlorine in the absence and presence of a chlorine-demanding substrate. Appl Environ Microbiol 71: 5022-5028.

30. Woo IS, Rhee IK, Park HD (2000) Differential damage in bacterial cells by microwave radiation on the basis of cell wall structure. Appl Environ Microbiol 66: 2243-2247.

31. Ukuku DO, Shelef LA (1996) Bioluminescence measurements of the antilisterial activity of nisin: comparision with ampicillin and streptomycin. J Biolumin Chemilumin 11: 169-173.

32. Miller RG Jr (1981) Simultaneous Statistical Inference, 2nd Edition. Springer-Verlag New York pp: 67-70.

33. Ukuku DO, Fett WF (2002) Relationships of cell surface charge and hydrophobicity with strength of attachment of bacteria to cantaloupe rind. J Food Prot 65: 1093-1099.

34. Bayer ME, Sloyer JL Jr (1990) The electrophoretic mobility of gram-negative and gram-positive bacteria: an electrokinetic analysis. J Gen Microbiol 136: 867-874.

35. Noda Y, Kanemasa Y (1986) Determination of hydrophobicity on bacterial surfaces by nonionic surfactants. J Bacteriol 167: 1016-1019.

36. Weidenbach H, Sedlarik K, Reimann G, Wilde J (1978) Light and electron microscopy studies on experimental arterial thrombosis in dwarf swine. Z Exp Chir 11: 230-237.

37. Ponne CT, Balk M, Hancioglu O, Gorris LGM (1996) Effect of radio frequency energy on biological membrane and microorganisms. LWT 29: 41-48.

38. Peterson AA, Hancock RE, McGroarty EJ (1985) Binding of polycationic antibiotics and polyamines to lipopolysaccharides of Pseudomonas aeruginosa. J Bacteriol 164: 1256-1261.