Radio frequency electric fields inactivation of *Escherichia coli* in apple cider

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Received 23 January 2007; received in revised form 26 June 2007; accepted 28 June 2007
Available online 2 August 2007

Abstract

A nonthermal process using radio frequency electric fields (RFEF) was developed to pasteurize apple cider. An 80 kW RFEF pilot plant system was used to process cider at flow rates of 1.5 and 1.9 l/min. *Escherichia coli* K12 in apple cider was exposed to electric field strengths of 20–30 kV/cm at frequencies of 21, 30, and 41 kHz. Treatment times varied from 140 to 420 s. Electrical energy costs were calculated using the measured voltages and currents. Energy balances were performed using the inlet and outlet temperatures. RFEF processing at an outlet temperature of 60 °C reduced the population of *E. coli* by 4.8 log, whereas thermal processing at the same conditions had no effect. Varying the frequency between 21 and 41 kHz had no effect on the level of microbial inactivation; however, increasing the treatment time, field strength and outlet temperature enhanced inactivation. The inactivation data at 20 kV/cm and 60 °C follow first order kinetics with a calculated D values of 74 μs. The inactivation data are represented well by the electric field strength model; the calculated critical electric field strength, \( E_c \), for 60 °C was 4.0 kV/cm. The electrical energy for RFEF pasteurization was 260 J/ml. The electrical cost was $0.0050/l of apple cider. Processing temperature had the greatest influence on energy efficiency. A RFEF nonthermal process has been developed to pasteurize apple cider. The effect of varying processing conditions on energy efficiency was investigated and at the optimum condition, the electrical cost appears to be minor. In addition, the RFEF process can be correlated using first order kinetic models.

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Keywords: Radio frequency electric fields; Apple cider; Nonthermal pasteurization; Electrical costs; Kinetics

1. Introduction

Recently, the New York State Department of Health and the New York State Department of Agriculture and Markets alerted residents and health care providers statewide of a possible link between unpasteurized cider and 41 cases of illness among consumers (New York State Department of Health, 2004). At least five of these cases required hospital treatment. Laboratory tests confirmed the presence of Shiga toxin-producing bacteria in specimens obtained from four patients. After the outbreak,

New York State considered passing a law that would outlaw the sale of unpasteurized cider (Goodman, 2006).

The radio frequency electric field (RFEF) nonthermal process has been developed for inactivating bacteria in apple juice (Geveke & Brunkhorst, 2004a, 2004b). In an electric field, a voltage is formed across the cell membrane (Zimmermann, 1986). The opposite charges on either side of the membrane are attracted to each other and the membrane becomes thinner. At sufficiently high field strengths, pores are formed in the membrane and the cell ruptures. Hulsheger, Potel, and Niemann (1981) proposed a model that related microbial inactivation to the electric field strength above a critical value, \( E_c \). The electric field strength model is

\[
\ln(\text{inactivation}) = a(E - E_c)
\]
where $a$ is a constant, $E$ is the electric field strength, and $E_c$ is the critical electric field strength.

Treatment times are less than 1 ms in RFEF processing. The electric field raises the temperature of the liquid food by ohmic heating due to electrical resistance. The final temperature is less than 70°C and the juice is cooled within several seconds using a heat exchanger. The RFEF process is similar to the pulsed electric field (PEF) process, except that in RFEF processing, the field is applied continuously using an AC generator, whereas in PEF processing, it is applied in pulses using a pulse generator.

RFEF processing at 30 kV/cm reduced the population of *Saccharomyces cerevisiae* in water by 3.8 log at 35°C (Geveke & Brunkhorst, 2003). RFEF processing at 21 kV/cm and 55°C inactivated *Escherichia coli* K12 in apple juice by 1.9 log relative to the control (Geveke & Brunkhorst, 2004a). Raising the temperature increased inactivation. The flow rate was limited to 0.55 l/min by the RF power supply. The RFEF process was successfully scaled up from 0.55 l/min to 1.4 l/min using an innovative pilot plant consisting of an 80 kW power supply and novel matching network (Geveke & Brunkhorst, 2004b). RFEF processing reduced *E. coli* in apple juice by 2.7 log at 60°C, whereas conventional heating at the same conditions had no effect. Nonthermal inactivation of *E. coli* K12 was dependent upon the electric field strength, frequency, treatment time and temperature.

The electric field strength within the RFEF treatment chamber was modeled using finite element analysis software (Geveke & Brunkhorst, 2004a). The results indicated that the field is nearly uniform which promotes evenly processed foods.

Apple cider generally contains more particulates than apple juice and it is more challenging to process with RFEF. Apple cider has been successfully processed with PEF (Evrendilek et al., 2000; Iu, Mittal, & Griffiths, 2001). The objective of this work was to extend the RFEF process to apple cider. Additional objectives were to develop microbial inactivation kinetics and to evaluate the processing energy.

### 2. Materials and methods

*E. coli* K12 (ATCC 23716) was maintained on Tryptic Soy Agar (Remel, Lenexa, KS) at 4°C. Prior to inoculation of product, the organism was cultured in Tryptic Soy Broth (Remel) with shaking at 37°C for 16-18 h. Pasteurized apple cider containing no preservatives was obtained from a local producer, Zeigler Beverage (Lansdale, PA). The cider was checked for background flora and the levels were consistently below 2 log. The cider was inoculated from the stationary phase culture to give an approximately 6–7 log cfu/ml population. Processing began after a 2 h waiting period at 10°C to allow the *E. coli* to become adapted to the acidic environment. The pH and conductivity of the cider was 3.8 and 2.16 mS/cm, respectively.

Geveke and Brunkhorst (2004b) have previously described the radio frequency electric fields (RFEF) power supply system used in this investigation. It consisted of an 80 kW RF power supply (Ameritherm, Scottsville, NY, model L-80) and a custom designed matching network (Ameritherm) that enabled the RF energy to be applied to a resistive load over a frequency range of 21.3-40.6 kHz. The RFEF power supply was connected to one or more treatment chambers.

The chambers were made of Rexolite, a transparent cross-linked polystyrene copolymer (C-Lec Plastics, Philadelphia, PA). The treatment chambers were designed to converge the flowing apple cider into a narrow area in order to reduce the power requirement (Geveke & Brunkhorst, 2004a; Matsumoto, Satake, Shioji, & Sakuma, 1991; Sensoy, Zhang, & Sastry, 1997). Cider entered and exited each Rexolite chamber through the annuli of cylindrical stainless steel electrodes (Swagelok, Solon, OH, part no. SS-400-1-OR) as shown in Fig. 1. Between the electrodes in the treatment chamber, there was a thin partition with a channel of circular cross section through the center. The diameter and length of the channel were 0.14 cm and 0.23 cm, respectively. The chamber design included a 0.20 cm space between the end of each of the electrodes and the central channel to reduce the potential for arcing. The volume formed by the space is 0.19 cm³.

The output of the RFEF power supply was connected to the electrodes such that the electric flux lines were approximately perpendicular to the direction of the liquid flow. One electrode on each of the treatment chambers was grounded. The remaining electrodes on the treatment chambers were connected to the RFEF power supply in parallel. Upon exiting the treatment chamber, the cider flowed through a 1.8 m section of plastic tubing having an internal diameter of 3.2 mm. The purpose of this plastic tubing was to electrically isolate the treatment chamber from the surrounding equipment and ensure that the maximum field is achieved within the chamber.

The supplied voltage and current to the RFEF treatment chambers were measured using an oscilloscope (Tektronix, Beaverton, OR; model TDS224), current probe (Pearson Electronics, Palo Alto, CA, model 411), and a voltage divider (Ross Engineering, Campbell, CA; model

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**Fig. 1.** Cross-section of a RFEF converged treatment chamber including Rexolite insulation and stainless steel (SS) electrodes. The diameter and length of the cylindrical channel through the Rexolite, the high electric field zone, were 0.14 cm and 0.23 cm, respectively.
The maximum voltage applied was limited to 6.9 kV\textsubscript{peak} in order to control the temperature rise of the apple cider. Therefore, the nominal maximum electric field strength used in the study was 30 kV/cm obtained by dividing the peak voltage, 6.9 kV, by the length of the central gap, 0.23 cm. The minimum voltage applied was 4.6 kV\textsubscript{peak} and was limited by the RFEF power supply and matching network operating requirements. Hence, the minimum electric field strength used in the study was 20 kV/cm.

The experimental system included a stainless steel feed tank and a progressing cavity pump (Moyno, Springfield, OH; model 2FG3) that supplied apple cider to the RFEF system through stainless steel tubing as shown in Fig. 2. The inlet temperature to the first RFEF treatment chamber was controlled using a 0.24 m\textsuperscript{2} stainless-steel heat exchanger (Madden Manufacturing, Elkhart, IN; model SC0004) with a residence time of 8.5 s (at a flow rate of 1.9 l/min) and a temperature controller (Cole-Parmer, model CALL 9400). Depending on the number of treatments desired, the system contained one RFEF chamber alone or two or three chambers in series with intercooling. The minimum flow rate was 1.5 l/min in order to limit the temperature rise of the apple cider. The Reynolds number ($Re$) within the treatment chamber was calculated based on a flow rate of 1.5 l/min, a density of 1 g/ml, and a viscosity of 0.01 g/(cm s). The $Re$ was 22,000 which is in the turbulent flow region; this improved the processing uniformity. The maximum flow rate was restricted to 1.9 l/min because of the system’s pressure drop and pump limitations with three treatment chambers in series. The cider was exposed to RFEF in the chambers for 140 s at 1.5 l/min and 110 s at 1.9 l/min. A back pressure of 1 atmosphere gauge minimized arcing.

The temperature of the apple cider rises during RFEF processing due to ohmic (resistance) heating. The temperatures of the cider entering and exiting the RFEF treatment chambers were measured with 3.2 mm diameter chromo-constantan thermocouples (Omega Engineering, Inc., Stamford, CT). The temperatures were continuously logged to a data acquisition system (Dasylab USA, Amherst, NH, Dasylab version 7). The RFEF treatment chamber outlet temperatures ranged from 50 to 60 °C. In the cases where successive treatments were desired, the cider exiting the chamber was cooled in a heat exchanger (Madden Manufacturing, model SC0004), equipped with a temperature controller (Cole-Parmer, model CALL 9400), before entering the next chamber. The inlet and outlet temperatures of all of the RFEF treatment chambers were identical.

The apple cider was quickly cooled after exiting the final treatment chamber to less than 25 °C using a stainless-steel heat exchanger sample cooler (Madden Manufacturing, model SC0004). The lengths of (holding) time for the cider to travel from each of the treatment chambers through tubing to the heat exchangers ranged from 1.4 to 1.8 s, depending on the flow rate.

Controls were performed to determine the bactericidal effect of temperature alone. In order to ensure that the control cider received the same time and temperature history as the treated cider, the high electric field treatment chambers were replaced with low electric field ohmic heating chambers. The chambers consisted of two stainless steel electrodes (Swagelok, Solon, OH, part no. SS-400-1-OR) inserted into a 10.2 cm length of 0.64 cm ID plastic tubing. The ohmic chamber quickly heated the cider to the desired temperature. The control cider was held for the same time as the RFEF treated cider, 1.4–1.8 s, before cooling to less than 25 °C.

The processing energies for each of the RFEF operating conditions were calculated by two means. The first was based on the applied voltage and current as measured by an oscilloscope. The second method was based on the energy calculated to raise the temperature of the apple cider from the treatment chamber inlet temperature to the outlet temperature. Energy densities were calculated by dividing the energy by the flow rate. Normalized energy densities were calculated by dividing the energy densities by the log reduction in the population of $E.\ coli$.

Appropriate dilutions of the product samples were made in Butterfield’s Phosphate Buffer (Hardy Diagnostics, Santa Maria, CA) with a minimum 1 mL transfer. Duplicate samples were then pour plated with Tryptic Soy Agar (Remel, Lenexa, KS) and the plates incubated at 37 °C for 24 h. Plates with 30–300 colonies were enumerated using a manual colony counter (Bantex, Burlingame, CA, model Colony Counter 920).

Each RFEF experiment was performed in duplicate. Results were expressed as the means of these values ± the standard deviations calculated using Microsoft Excel statistical analysis algorithms. Bon multiple comparisons were conducted using SAS software (SAS Institute, Cary, NC, version 9) to differentiate the means at a significant level of 0.05.

3. Results

RFEF processing successfully inactivated $E.\ coli$ K12 in apple cider at nonthermal conditions. The extent of
microbial inactivation is dependent on the temperature, treatment time, as well as electric field strength and is independent of frequency. The electrical energy to process apple cider by RFEF was calculated.

3.1. Frequency

The effect of frequency on microbial inactivation was determined. The RFEF system is capable of operating at 21.3, 30.0, and 40.6 kHz and all three frequencies were investigated. The inactivations at all of the frequencies were not significantly different \((P > 0.05)\) at RFEF conditions of 60°C treatment chamber outlet temperature, 140 µs treatment time, and both 25 and 30 kV/cm peak electric fields (data not shown). A frequency of 21.3 kHz was chosen to use for all of the remaining experiments as the effect of frequency was insignificant and because nearly all of the previous studies on RFEF have used a frequency close to 20 kHz (Geveke & Brunkhorst, 2003; Geveke & Brunkhorst, 2004a; Geveke & Brunkhorst, 2004b; Uemura & Isobe, 2002; Uemura & Isobe, 2003).

3.2. Temperature

A series of experiments were performed at 21.3 kHz to determine the effect of temperature on inactivation. The population of E. coli in apple cider was reduced by 1.3 ± 0.2 log after being RFEF processed at a peak electric field of 20 kV/cm, treatment time of 420 µs, treatment chamber outlet temperature of 50°C, and hold time of 5.4 s (Fig. 3). When the cider was ohmically heated (<1 kV/cm) to 50°C and held for 5.4 s, the population of E. coli was unaffected. RFEF inactivation improved as the temperature increased from 50 to 60°C. The RFEF inactivation at 55°C was 2.4 ± 0.7 log, while the 55°C control again determined that there was no thermal inactivation. The inactivation at 60°C was 5.0 ± 0.1 log. Although at 60°C there was some thermal inactivation, 0.2 ± 0.1 log, the vast majority of the RFEF inactivation was due to nonthermal effects.

3.3. Treatment time

A series of experiments were performed at a field of 20 kV/cm to determine the effect of treatment time on inactivation. Using either one, two, or three treatment chambers in series, the total treatment times investigated were 140, 280, and 420 µs. The effect of treatment time on inactivation was studied at two outlet temperatures, 55 and 60°C (Fig. 4). The data follow first order kinetics with a multiple regression correlation coefficient \((r^2)\) of 0.972 and 0.986 for 55 and 60°C, respectively. The calculated \(D\) values for 55 and 60°C are 194 and 74 µs, respectively. When the cider was ohmically heated (<1 kV/cm) to 55°C for the identical hold times, the E. coli was unaffected. Likewise, the E. coli was unaffected when the cider was ohmically heated to 60°C, except for at the longest hold time, 5.4 s, which resulted in a thermal inactivation of 0.2 ± 0.1 log.

3.4. Electric field strength

The effect of electric field strength on microbial inactivation was determined. The field strengths investigated were 20, 25, and 30 kV/cm. The effect of field strength on inactivation was studied at two outlet temperatures, 55 and 60°C (Fig. 5). In order to limit the outlet temperature while operating at a 30 kV/cm field, the apple cider flow rate was increased from 1.5 l/min to 1.9 l/min. The population of E. coli in apple cider was reduced by 4.8 ± 0.1 log after being RFEF processed at a peak electric field of 30 kV/cm, treatment time of 220 µs, treatment chamber outlet temperature of 60°C, and hold time of 2.8 s. The population of E. coli was unaffected when the cider was ohmically heated (<1 kV/cm) to 60°C and held for 2.8 s as
a control. The data were described well using the electric field strength model (Hulsheger et al., 1981). The calculated critical electric field strengths, $E_c$, for 55 and 60 °C are 13.5 and 4.0 kV/cm, respectively. The correlation coefficients ($r^2$) are 0.900 and 0.958 for 55 and 60 °C, respectively.

### 3.5. Energy density

The RFEF processing energies were calculated for each of the conditions presented in Figs. 3–5 (Table 1). The energy was calculated based on the applied voltage and current. For instance, for the operating conditions of 60 °C outlet temperature (25 °C inlet temperature), 30 kV/cm electric field strength, and 220 µs treatment time, the voltage and current measured by an oscilloscope were 13.8 kV peak–peak and 5.8 A peak–peak, respectively. The calculated energy for this particular condition was 9.9 kW. In continuous processes, a common method of comparing efficiencies is to calculate the energy per flow rate, or energy density. In this case, the energy density was 310 J/ml which was obtained by dividing the energy by the flow rate, 1.9 l/min. A second method for calculating energy is based on the temperature rise of the apple cider from the inlet to the outlet of the treatment chamber. Using this method, the energy density was 300 J/ml. This is in good agreement with the energy calculated using the voltage and current. A similar analysis was performed using additional data and the electrical method yielded consistently higher energy densities than the temperature method did. On average, the energy densities calculated by the electrical method were 11% higher than those calculated by the temperature method. In order to conservatively evaluate the energy density of the RFEF process, the electrical method was used.

Each of the operating conditions in (Table 1) resulted in a different microbial inactivation. Thus, the energy densities were normalized on a per log basis to facilitate a fair comparison. For instance, for the example previously discussed, the energy density was 310 J/ml and the microbial inactivation was 4.8 log, so the normalized energy density was 65 J/(ml log).

### 4. Discussion

The effect of frequency on microbial inactivation was not significant ($P > 0.05$). For a 140 µs residence time within the treatment chamber, the number (and duration) of half-cycles at frequencies of 21.3, 30.0, and 40.6 kHz was 6.0 (23 µs), 8.5 (16 µs), and 11.5 (12 µs), respectively. The half-cycles were calculated by taking the reciprocal of the frequencies and dividing by two. Each half-cycle contained a peak field strength of at least 25 kV/cm. In this investigation, the microbial inactivation was the same applying 6.0 half-cycles of 23 µs or 11.5 half-cycles of 12 µs. In designing RFEF systems, it is necessary to have a minimum of 1 half-cycle in each chamber. It is safer to design for a minimum of 2 half-cycles to insure against

![Fig. 5. Effect of electric field strength on the inactivation of E. coli at 220 µs RFEF treatment time and 2.8 s hold time. Means of two replicate experiments. Error bars indicate standard deviations.](image)

### Table 1: Energy density of RFEF processing at the operating conditions used in the present study

<table>
<thead>
<tr>
<th>Outlet temperature °C</th>
<th>Field kV/cm</th>
<th>Treatment time µs</th>
<th>Current A peak–peak</th>
<th>Voltage kV peak–peak</th>
<th>Energy density a J/ml</th>
<th>Inactivation log cfu/ml</th>
<th>Energy density per log J/(ml log)</th>
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<tr>
<td>60</td>
<td>20</td>
<td>420</td>
<td>5.7</td>
<td>9.2</td>
<td>260</td>
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<td>60</td>
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<td>9.4</td>
<td>160</td>
<td>3.0</td>
<td>52</td>
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<tr>
<td>60</td>
<td>20</td>
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<td>9.2</td>
<td>240</td>
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<td>190</td>
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Ranked in descending order of energy efficiency.

*a* Energy delivered to the product. The energy measured at the power supply was approximately 20% greater.
non-uniform particle velocities. Therefore, the minimum frequency that could be used with the present treatment chambers is 7.1 kHz. The corresponding half-cycle duration would be 69 µs. The RFEF equipment used in this study was only capable of operating between approximately 20 and 40 kHz. It is probable that nonthermal microbial inactivation is feasible at frequencies well above 40 kHz; however, there may be an upper limit due to the capacitance of the bacterial cell membrane. A capacitor consists of two conductors (such as the cytoplasm and the medium) insulated from each other by a dielectric (such as the membrane) which stores electrical energy, blocks the flow of direct current, and permits the flow of alternating current to a degree dependant on the capacitor’s capacitance and the current frequency. There is a lag between the applied voltage and the induced voltage across the membrane. Although it is very difficult to predict the peak value of the induced transmembrane voltage, it is clear that the voltage is significantly reduced as the frequency is increased from 100 kHz to 1 MHz (Kotnik, Miklavcic, & Slivnik, 1998). In theory, if the frequency is raised much above 40 kHz, the induced transmembrane voltage will be reduced to a point where pores in the membrane are no longer formed and the cell does not rupture. At a frequency of 250 kHz, the half-cycle is 2 µs. Interestingly, in PEF processing at electric field strengths in the range of 15-35 kV/cm, pulse widths of 2 µs are typically used and pulse widths of less than 1 µs are rarely used. It is probable that membrane capacitance plays a role in limiting the minimum pulse width in PEF processing.

As with nearly all nonthermal processes, microbial inactivation increased with increasing temperature (Fig. 3). At an outlet temperature of 50 °C, all of the inactivation, 1.3 ± 0.2 log, was attributed to nonthermal effects. At 60 °C, the thermal component inactivation was 0.2 ± 0.1 log and the nonthermal component inactivation was 4.8 ± 0.2 log. This synergism of temperature and RFEF was previously observed during RFEF processing of apple juice (Geveke & Brunkhorst, 2004b). The same phenomenon has been reported in PEF processing (Bazhal, Ngadi, Raghavan, & Smith, 2006; Wouters, Dutreux, Smelt, & Lelieveld, 1999). Using synergistic effects of elevated treatment temperature, 65 °C, and PEF, the processing energy was reduced by more than 60% for a microbial inactivation of 6 log (Heinz, Toepfl, & Knorr, 2003). The nonthermal inactivation is believed to be due to electroporation of the cells as a result of high electric fields (Zimmermann, Pilwat, & Riemann, 1974).

The effect of treatment time on microbial inactivation was successfully modeled using first order kinetics (Fig. 4). Similar results have been obtained with PEF and thermal treatments (Espachs-Barroso, Barbosa-Canovas, & Martin-Belloso, 2003). The effect of electric field strength on microbial inactivation also followed first order kinetics (Fig. 5). The critical electric field strength, \( E_c \), is the minimum field required to irreversibly rupture the cell membrane according to the electric field strength model (Hulsheger et al., 1981). The calculated \( E_c \) substantially decreased as the temperature increased. The \( E_c \) depends upon the compressibility and the permittivity of the membrane (Heinz et al., 2003). It seems logical that the temperature would affect the compressibility and the permittivity of the membrane, and therefore the \( E_c \).

The microbiological inactivation results obtained in this study using cider can be compared to those obtained employing nearly similar RFEF equipment to process apple juice (Geveke & Brunkhorst, 2004b). The population of \( E. coli \) in apple juice was reduced by 2.7 log after being exposed to a 20 kV/cm peak electric field for 270 µs with an outlet temperature of 60 °C. In the present study, the population of \( E. coli \) in apple juice was reduced by 3.6 log after being exposed to a 20 kV/cm peak electric field for 280 µs with an outlet temperature of 60 °C. The variation in results may be due to the use of different methods to connect the RFEF power supply to the treatment chambers (Geveke, Brunkhorst, Cooke, & Fan, 2006). In the experiments with apple juice, two chambers were joined by stainless steel tubing. The inner electrodes between the chambers were connected to the RFEF power supply. The outer electrodes were grounded. The advantage of this setup is that there is no concern about isolating the chambers from the surroundings. The disadvantage is that there is no intercooling between the treatment chambers. In the present study with apple cider, there was intercooling between the chambers. This was possible because the first electrode on each of the treatment chambers was grounded and the remaining electrode on each of the treatment chambers was connected to the RFEF power supply in parallel. Upon exiting each treatment chamber the cider flowed through a section of plastic tubing that electrically isolated the treatment chamber from the surrounding equipment.

As is typical with processing, various combinations of operating conditions may yield the same results. The applied energies, for the different conditions, may not be the same. The RFEF processing energies were calculated for each of the conditions presented in Figs. 3–5 (Table 1). As is readily apparent from Table 1, processing temperature has the greatest influence on energy efficiency. All of the cases in which the temperature was 60 °C were more efficient than those in which the temperature was 55 °C. Likewise, 55 °C was more efficient than 50 °C. In fact, the average normalized energy density for the 60 °C conditions was less than one-third that for the 50 °C condition. This same effect has been observed in PEF processing of apple juice to obtain a 6 log inactivation of \( E. coli \) (Heinz et al., 2003). Raising the treatment temperature from 35 to 65 °C reduced the energy from 100 to 40 J/g.

The energy required for a 5 log reduction using RFEF at a processing temperature of 60 °C was approximately 300 J/ml. For PEF processing, the estimated energy ranges from 100 to 400 J/ml (Barsotti & Cheftel, 1999; Schoenbach, Katsuki, Stark, Buescher, & Beebe, 2002). The
The estimated energy cost for RFEF pasteurization is $0.0050/l of apple cider employing the US Department of Energy’s data for the average industrial electric price for the first six months of 2006 of $0.0586/kWh. For comparison, the energy costs for conventional thermal pasteurization with heat regeneration is approximately $0.00057/l (Kozempel, McAloon, & Yee, 1998).

In the future, as the RFEF process is scaled up, the dimensions of the treatment chambers will be increased. Preliminary designs indicate that the present 80 kW RFEF system can supply power to four chambers, each having a 3 cm internal diameter and 3 cm gap. The larger dimensions will enable the processing of larger particulates and greater flow rates. A complete economic analysis including equipment, maintenance, labor, and utilities costs is needed. In addition, the quality of fresh apple cider, thermally pasteurized cider, and RFEF pasteurized cider should be compared. Various methods to quantify differences could include color analysis, vitamin analysis, aroma compound analysis, and panel sensory tests. Finally, shelf life studies should be performed to determine if RFEF processing can extend the shelf life of apple cider, particularly organic products that do not contain preservatives.

In summary, the RFEF process was capable of inactivating *E. coli* K12 in apple cider at nonthermal conditions. The level of inactivation was dependent on the electric field, treatment time and outlet processing temperature and was independent of the frequency. The effects of electric field strength and treatment time on microbial inactivation followed first order kinetics. The electrical energy density for the RFEF process, that reduced the population of *E. coli* by 5.0 ± 0.1 log, was 260 J/ml of cider. The results of the present study successfully extend the application of nonthermal RFEF processing to apple cider.

**Acknowledgements**

The authors thank O.J. Scullen, G. Boyd, and K.Y. Snipes for microbiological support, and R.E. Radewonuk for engineering support, all of the US Department of Agriculture, Wyndmoor, PA. Princeton Plasma Physics Laboratory is funded by the US Department of Energy and managed by Princeton University.

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