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Ultraviolet and Pulsed Electric Field Treatments Have Additive Effect on Inactivation of *E. coli* in Apple Juice

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Abstract

Apple juice inoculated with *Escherichia coli* ATCC 23472 was processed continuously using either ultraviolet (UV), high-voltage pulsed electric field (PEF), or a combination of the PEF and UV treatment systems. Apple juice was pumped through either of the systems at 3 flow rates (8, 14, and 20 mL/min). *E. coli* was reduced by 3.46 log CFU/mL when exposed in a 50 cm length of UV treatment chamber at 8 mL/min (2.94 s treatment time with a product temperature increase of 13 °C). *E. coli* inactivation of 4.87 log CFU/mL was achieved with a peak electric field strength of 60 kV/cm and 11.3 pulses (average pulse width of 3.5 μs, product temperature increased to 52 °C). *E. coli* reductions resulting from a combination treatment of UV and PEF applied sequentially were evaluated. A maximum *E. coli* reduction of 5.35 log CFU/mL was achieved using PEF (electrical field strength of 60 kV/cm, specific energy of 162 J/mL, and 11.3 pulses) and UV treatments (length of 50 cm, treatment time of 2.94 s, and flow rate of 8 mL/min). An additive effect was observed for the combination treatments (PEF and UV), regardless of the order of treatment ($P > 0.05$). *E. coli* reductions of 5.35 and 5.30 log CFU/mL with PEF treatment (electrical field strength of 60 kV/cm, specific energy of 162 J/mL, and 11.3 pulses) followed by UV (length of 30 cm, treatment time of 1.8 s, and flow rate of 8 mL/min) and UV treatment followed by PEF (same treatment conditions), respectively. No synergistic effect was observed.

Keywords: electrical field strength, nonthermal, pulse number, pulsed electric field, specific energy, treatment length, treatment time, ultraviolet

Introduction

Apple juice is consumed by people of all ages for its sensory and nutritional qualities. The United States produced more than 115,759 metric tons of apple juice in 2004 and 2005 (U.S. Apple Assn. 2006). The apple juice contains a wide array of phytonutrients, is a rich source of antioxidants has good antioxidant properties and is devoid of sodium, cholesterol, and fat (Lee *et al.* 2003; Leontowicz *et al.* 2003). Apple juice has been implicated in several foodborne illness outbreaks (Goverd *et al.* 1979; Steele *et al.* 1982; Besser *et al.* 1993; CDC 1996, 1997). The U.S. Food and Drug Administration requires apple juice processors to implement effective food safety programs such as Hazard Analysis and Critical Control Point program, sanitation and Good Manufacturing Practices during production of juice and juice products and requires that the processes meet a performance standard of 5 log reduction of the most resistant pathogen (FDA 2000). Thermal treatment has been the predominant technology for assuring food safety and improving the shelf life of fruit juices. However, the thermal processing potentially results in loss of sensory attributes like flavor and taste as well as a reduction in its nutritional quality.

Consumer demand for natural, minimally processed foods is increasing and consumers are willing to pay a premium for minimally processed food that retains original, fresh, nutritional, and sensory qualities. This has led to the development of alternate nonthermal technologies such as high hydrostatic pressure (Wood *et al.* 2001); cold plasma (Laroussi *et al.* 2006); dense phase CO_2 (Erkmen 2001); ultraviolet (UV) light (Guerrero-Beltran and Barbosa-Cánovas 2004); and pulsed electric field (PEF) (Ho and Mittal 2000; Fernandes-Molina *et al.* 2006).

UV treatment has been successfully used for disinfection of water and air due to greater transparency of the medium. While UV light has been used in food processing (Koutchma *et al.* 2004), its efficacy is reduced due to the lower medium transparency. To improve treatment uniformity and the resulting consistency in microbial destruction, the treatment chamber must be designed to present a thin film of juice or the medium to the UV light. The mechanism of destruction by UV involves disruption of the microbial DNA, preventing replication of microorganisms (Guerrero-Beltran and Barbosa-Cánovas 2004). The degree of microbial inactivation depends on the UV dosage (intensity times the exposure time) applied to the product although minimal increase in product temperature can occur (Bintsis *et al.* 2000).

UV dosage in excess of 6500 $\mu J/cm^2$ is required to achieve a 5 log reduction in *Escherichia coli* in apple cider (Quintero-Ramos *et al.* 2004). Geveke (2005) reported a 3.4 log reduction in *E. coli* K-12 and 2.5 log reduction in *Listeria innocua* with an exposure time of 19 and 58 s, respectively. The energy used in the treatment was comparable to thermal processing (Geveke 2005). Bacterial strain and cultivar (apple cider) differences were observed for UV inactivation of *E. coli* O157:H7 (Basaran *et al.* 2004).

PEF treatment of foods results in minimal changes in sensory and nutritional quality of foods (Ho and Mittal 2000). Application of PEF involves exposure of food products to high

electric field (>30 kV/cm) at relatively low temperatures for very short time, typically in the order of microseconds. While the applied electrical energy is partially converted to thermal energy, the increase in product temperature alone is not sufficient to cause microbial destruction. The primary mechanism of lethality from PEF treatments is cell membrane compression and pore formation resulting in increased membrane permeability, leakage of cytoplasmic contents, and lysis (Aronsson and Ronner 2001). The degree of inactivation by PEF is related to electric field intensity, pulse number, and pulse duration (Barbosa-Cánovas *et al.* 1998). Iu *et al.* (2001) reported a 5.35 log reduction for *E. coli* O157:H7 in apple juice using 30 pulses at 80 kV/cm and the temperature of the liquid was 42 °C. Evrendilek *et al.* (1999) reported a 5 log reduction in *E. coli* O157:H7 and *E. coli* 8739 in apple juice using bipolar pulses with an electric field of 30 kV/cm treatment time of 172 μs. In a subsequent report, the authors (Evrendilek *et al.* 2000) reported *E. coli* O157:H7 reductions of 4.5 logs at 35 kV/cm electric field strength and 94-μs treatment time with improvement in microbial shelf life and retention of apple juice color and vitamin C content.

Factors that influence effectiveness of PEF or UV light include pH, temperature, and pressure (Ross *et al.* 2003). Some of these factors were shown to provide synergistic effect on microbial destruction. Synergism refers to the phenomenon in which 2 or more distinct influences acting together achieve an effect greater than the result of their individual effects on the process. Ngadi *et al.* (2004) evaluated the combined effect of PEF and UV treatment and reported *E. coli* O157:H7 reductions of >6 log in poultry chiller water using a combination of PEF and UV treatments. The authors reported synergistic antimicrobial effect between PEF and UV treatments, with a pulse number <50 for the PEF treatment, while larger pulse numbers resulting in an additive effect. Ngadi *et al.* (2004) attributed this synergism to complete inactivation of the injured cells resulting from the UV treatment within the 1st 50 pulses.

The objective of this study is to investigate the destruction of *E. coli* ATCC 23472 in apple juice by PEF and UV treatments individually and in combination.

Materials and Methods

Pasteurized apple juice was obtained from a local grocery store and stored under refrigeration until use (5 °C, 1 wk). The sugar content was found to be 11.7 °Brix using digital handheld "Pocket" refractometer (PAL-α, ATAGO, Carnation, Wash., U.S.A.). The pH of the apple juice was 3.8, measured using Accumet Basic (AB 15, Fisher Scientific, Pittsburgh, Pa., U.S.A.). The electrical conductivity of apple juice was 2.36 mS/cm measured using CON 6/TDS 6 (OAKTON Instruments, Vernon Hills, Ill., U.S.A.), respectively.

Microorganism and growth condition

E. coli ATCC 23472 was maintained on tryptic soy agar (Bacto Tryptic Soy Agar, Beckton Dickinson and Co., Sparks, Md., U.S.A.) under refrigeration with monthly transfers. Fresh culture was prepared by inoculating 10-mL sterile tryptic soy broth (Beckton Dickinson and Co.) and incubating at 35 °C for 18 h. An aliquot of the culture was added to the apple juice and mixed to obtain *E. coli* population of approximately 6 log CFU/mL in the apple juice.

UV treatment

A continuous flow UV treatment chamber was constructed by placing a germicidal UV lamp (GML 440; American Ultraviolet, Inc., Lebanon, Ind., U.S.A) inside a PVC pipe (12 cm dia) fitted with a fan to provide air circulation (Figure 1). The inner surface of the pipe was covered with aluminum foil to serve as a reflector. The lamp specifications are: 25 UV W, 86 cm long, 2.5 cm dia, and primary light emission at a wavelength of 253.7 nm. Quartz tubes (1.00 × 3.00 mm ID × OD, GE 214 fused quartz, Technical Glass Products Inc., Painesville, Ohio, U.S.A.) of 10, 20, 30, 40, and 50 cm length were placed around the lamp at equal distance from the lamp. The apple juice was pumped continuously using a peristaltic pump (EW-07523-60, Barnant Co., Barrington, Ill., U.S.A.) through the treatment chamber at flow rates of 8, 14, or 20 mL/min through one of the quartz tubes of 30, 40, or 50 cm length, depending on the treatment. Treatment time was adjusted by varying the flow rate and the length of the tubing. The temperature of the juice before and after treatment was measured using J-type thermocouples (Barnant Co., Barrington) and temperature reader (HH23, Omega Engineering, Conn. U.S.A.).

Pulsed electric field system

The PEF system was designed using an exponential decay pulse generator, cofield continuous treatment chamber, and measurement system. Figure 2 shows the diagram of the pulse applied to the treatment chamber. A DC power supply (CF60/25-12C, Hipotronics, Inc., Brewster, N.Y., U.S.A.) 60 kV and 100 mA was used to charge a high-voltage capacitor of 20 nF (General Atomics Electronic Systems, San Diego, Calif., U.S.A.) and a spark gap was used as a switch. The electrical fields of 40, 50, and 60 kV/cm were used. PEF treatment of apple juice was preformed in a cofield continuous treatment chamber (Figure 3) constructed of stainless steel cylindrical electrodes separated by an insulator (1 mm thick; Polythermide, Ultem, McMaster-Carr, Chicago, Ill., U.S.A.). The treatment chamber has 2 treatment regions each with a volume of 0.0126 mL. The apple juice was pumped through the treatment chamber using the peristaltic pump described previously. The power supply charged the capacitor. The energy

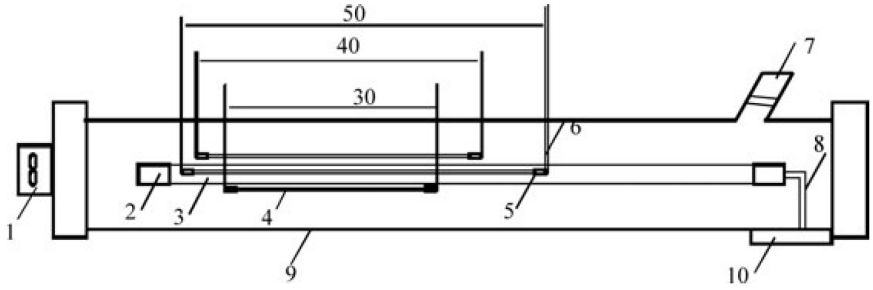

Figure 1. UV treatment chamber: 1) fun; 2) lamp holders; 3) UV lamp; 4) quartz tube; 5) switch lock connector; 6) plastic tube; 7) output for cooling; 8) wires; 9) plastic corpus of the treatment chamber; and 10) power supply for the UV lamp.

stored in the capacitor is given by $CV^2/2$, where C is the capacitance of the capacitor and V is the voltage supplied by the power supply. When the voltage on the capacitor exceeds the dielectric breakdown voltage between the spark gap, a spark connected the treatment chamber to the capacitor, and the capacitor discharged its energy as an exponential decay pulse into liquid in the 2 treatment regions. Care was taken to adjust the flow rate such that the fastest flowing particle in the treatment region receives at least 1 pulse so that each and every particle is treated. The time constant or pulse width of the exponential decay pulse is calculated by RC, where R is the resistance of the treatment chamber. The pulse width was 3.5 μs. The number of pulses received by the liquid in each treatment region is given by f*v/Q, where f is the pulse frequency (60 Hz), v is the volume of the treatment region (0.0126 mL), and Q is the flow rate (8, 14, and 20 mL/min). The total energy received by the liquid is given by the product of energy per pulse and number of pulses. Specific energy (SE) is calculated by the total electrical energy applied per unit volume of the treated liquid and is given by:

$$SE = CV^2f/2Q \quad (1)$$

The treatment voltage was measured using a high-voltage probe (P6015 A, Tektronix, Inc., Beaverton, Oreg., U.S.A.) and oscilloscope (TDS 2024, Tektronix, Inc.). A data acquisition system (Model: U3; LabJack Corp., Lakewood, Colo., U.S.A.) was connected to another high-voltage probe PR-28A (BK Precision, Yorba Linda, Calif., U.S.A.) to count the number of pulses. A simple user-graphical interface was developed in the LabView software to count the number of pulses for 10 s and display the average frequency real-time.

Processing juice through the UV and PEF treatment chambers

The system was flushed with hot, distilled water (75 °C) for 5 min to remove food residues, and subsequently with 10% (v/v) low foaming chlorinated alkaline cleaner/sanitizer (LFC; Spartan Chemical Co., Inc. Maumee, Ohio, U.S.A.) followed by 70% ethyl alcohol (Fisher Scientific) for 1 min. Finally, the system was flushed with sterile distilled water for 5 min and the water sample was collected and plated to evaluate the efficacy of sanitation. All the cleaning and sanitizing operations were done at a flow rate of 60 mL/min. The system consisted of two 50-mL burettes; one was filled with inoculated juice *et al.* filled with noninoculated liquid. The noninoculated apple juice was pumped through the system to fill the system and to remove entrapped air before switching the pulse generator. Once the PEF parameters were fixed, the inoculated apple juice was pumped through the system. A heat exchanger (cold water maintained at 1 °C) was connected between the 2 treatment systems to minimize temperature variations between the combination treatments.

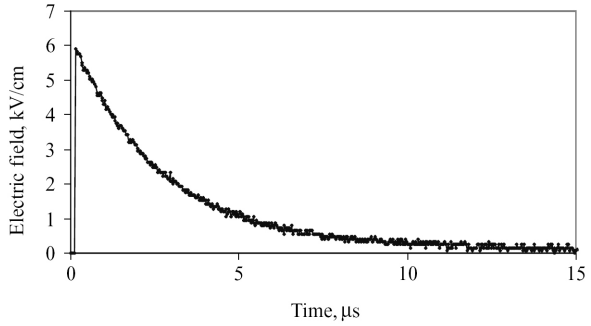

Figure 2. Shape of the pulse applied to the treatment chamber.

Microbial enumeration

The samples were collected aseptically and chilled immediately by placing them in an ice water bath and transferred to a refrigerator. Serial dilutions were prepared using sterile 0.1% peptone water (PW, Bacto Peptone, Beckton Dickinson). Appropriate dilutions were plated on *E. coli* Petrifilm™ (3M, St. Paul, Minn., U.S.A.) in duplicate, incubated, and colonies were counted following the manufacturer's instructions.

Statistical analysis

Three independent trials were performed for all treatments. The $\log_{10}$ transformed microbial counts were analyzed by analysis of variance using general linear model procedure with Statistical Analysis System (Release 9.1, SAS Inst. Inc., Cary, N.C., U.S.A.). The experimental design is a split plot design with the main factor (treatment) in a randomized complete block with 3 replications (blocks). The subplot factor is flow rate.

Results and Discussion

UV treatment

The inoculated juice (4 °C) was passed through the UV treatment chamber for the different treatment lengths of 30, 40, or 50 cm, with different treatment times by varying the flow rates. Microbial inactivation as a function of length of the UV treatment chamber at different flow rates is presented in Figure 4. Flow profile was laminar in quartz tubes. The residence time for the different flow rates of 8, 14, and 20 mL/min corresponds to 1.8, 1.0, and 0.7 s for 30-cm treatment length; 2.4, 1.3, and 0.9 s for 40-cm treatment length, and 2.9, 1.7, and 1.2 s for 50-cm treatment length, respectively. A maximum temperature increase of 13 °C was observed with the longest treatment length of 50 cm and the slowest flow rate of 8 mL/min, with the longest residence time of 2.3 s. Therefore, thermal effect on microbial inactivation was negligible. A maximum reduction of 3.46 log CFU/mL in *E. coli* population was achieved under the same conditions having a treatment time of 2.3 s.

E. coli destruction was dependent on the UV dosage, which in turn was affected by the flow rate and treatment time (Figure 4). Geveke (2005) reported 3.4 log reduction in *E. coli* population in apple cider with 19-s exposure time with a product temperature of 25 °C using flow rates varying from 27 to 83 mL/min, whereas similar reductions were achieved with an exposure time of 3 s in the current study. This may be attributed to higher intensity (25 W) of the UV lamp used in the current study compared to that (15 W) used by Geveke (2005). The quartz tubing used in this study had greater transmission of UV light compared to the chemflour tubing used by Geveke

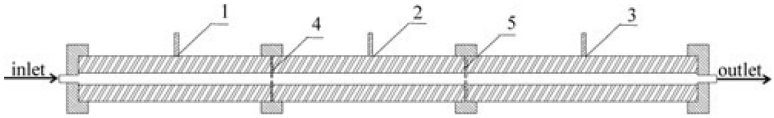

Figure 3. PEF cofield treatment chamber: 1) ground electrode, 10 cm length; 2) high-voltage electrode, 10 cm length; 3) ground electrode, 12 cm length; 4) and 5) insulator/spacer, 1 mm length.

(2005). In addition, the internal diameter of the quartz tubing (1 mm) used in this study was smaller than the chemflour tubing (1.6 mm) used by Geveke (2005), thereby resulting in lower film thickness and providing more uniform exposure of the liquid to the UV for better penetration.

With the same treatment time, an increase in length of the treatment chamber resulted in an increase in *E. coli* reductions. For example, the reductions of *E. coli* for the 3 treatment lengths 30, 40, and 50 cm and same treatment time of 1.5 s were 0.35, 0.80, and 2.75 log CFU/mL, respectively. This could be attributed to the better mixing of the juice, which resulted in more uniform treatment and exposure of each part of the liquid to the UV for inactivation. The current food additive regulation for UV light treatment of fresh juices requires the use of turbulent flow. Koutchma *et al.* (2004) attributed flow rate and mixing of the liquid in the treatment chamber as critical factors affecting the microbial inactivation. Therefore, it is better to have a greater velocity and longer treatment length for the same desired treatment time to achieve greater turbulence and inactivation.

PEF treatment

The inoculated apple juice was passed through a cofield PEF treatment chamber with electrical field strengths of 40, 50, and 60 kV/cm and 3 levels of flow rate (8, 14, and 20 mL/min). For a continuous flow treatment chamber, the number of the applied pulses is a function of the treatment chamber volume v, (mL), pulse frequency f, (Hz) and the flow rate Q (mL/s) of the liquid food passing through the chamber. The number can be calculated (Equation 2) as:

$$n = vf \div Q \tag{2}$$

The flow rates correspond to pulse numbers of 11.3, 6.5, and 4.5, respectively, at 60-Hz frequency. The effect of electrical field intensity and pulse numbers on the microbial inactivation is presented in Figure 5. A maximum temperature increase to 52 °C (from an initial temperature of 5 °C) was observed during the most severe treatment having electrical field intensity of 60 kV/cm and slowest flow rate of 8 mL/min (approximately 11.3 pulses). Splittstoesser *et al.* (1995) reported thermal D-values for *E. coli* O157:H7 in apple juice as 12, 5, and 1 min at 52, 55, and 58 °C, respectively. The apple juice was cooled to 4 °C within 2 min after the PEF treatment, minimizing the thermal effect on microbial reduction.

Figure 5 shows that the inactivation of *E. coli* is a function of the pulse number (treatment time) and electrical field applied to the product. A maximal *E. coli* reduction of 4.87 log CFU/mL was observed with 60 kV/cm electrical field at 8 mL/min flow rate having 11.3 pulses (treatment time of 40 μs). Similarly, Iu *et al.* (2001) reported a 4.73 log reduction in *E. coli* O157:H7 using 60 kV/cm electric field and 10 pulses in apple cider using a static PEF treatment chamber. Evrendilek *et al.* (1999) reported approximately 5.0 log reduction in *E. coli* O157:H7 in apple cider using 30 kV/cm electric field strength and 172-μs treatment time. Increase in the number of pulses

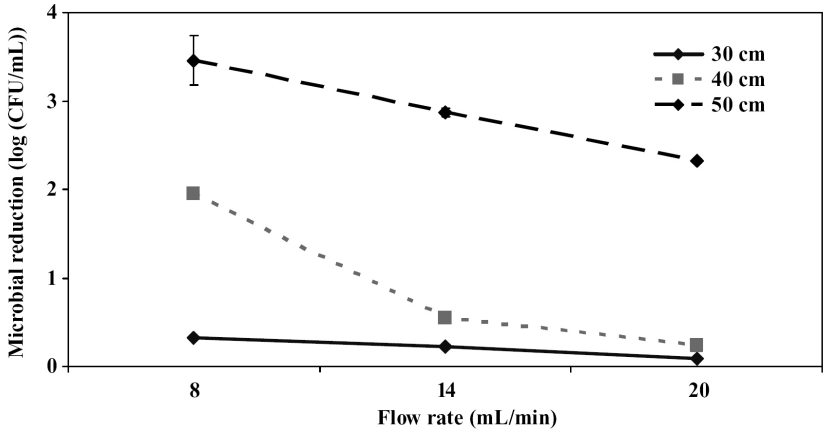

Figure 4. Inactivation of *E. coli* using UV for 3 treatment lengths.

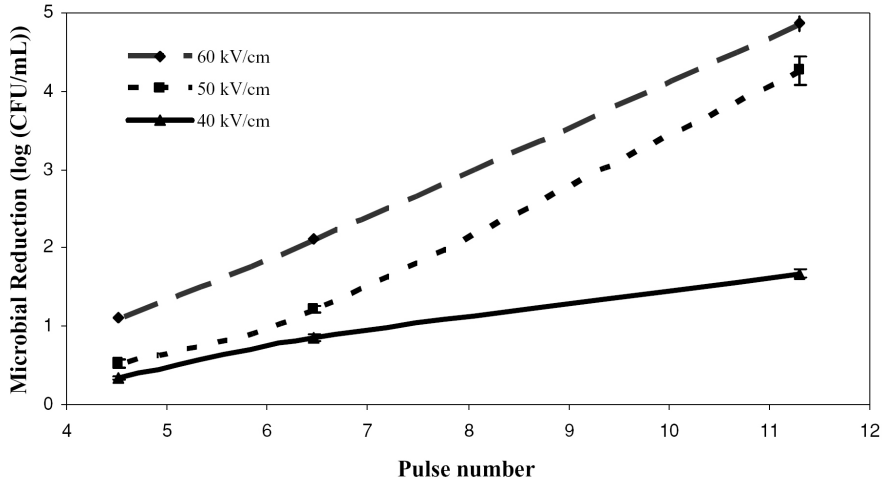

Figure 5. Inactivation of *E. coli* using exponential decay pulses with 3.5-μs pulse width.

(treatment time) resulted in an increase in the microbial inactivation. Similar results were reported by others in different food products (Martin *et al.* 1997; Garcia *et al.* 2003; Amiali *et al.* 2006) using PEF treatment.

E. coli inactivation in apple juice with PEF treatment was proportional to the specific energy applied, with greater inactivation observed at higher electric field strengths, with the same number of pulses (Figure 6). For example, the log reduction of *E. coli* with the 3 electric fields 40, 50, and 60 kV/cm and the same number of pulses (10) were 1.5, 3.6, and 4.2 log CFU/mL, respectively. Iu *et al.* (2001) reported similar reductions in *E. coli* O157:H7 in apple cider, with higher lethality of the PEF process with increasing electric field strength.

Combined PEF and UV treatment

Application of higher electric field intensities to achieve higher microbial inactivation of microorganisms may also cause adverse effects in sensory or functional properties of the food (Wood and Bruhn 2000). Increasing electric field strength to more than 60 kV/cm with frequency 60 Hz and flow rate 8 mL/min resulted in an increase in the juice temperature to >60 °C, which is not desirable. During the PEF treatment, the applied electrical energy is converted to thermal energy. The resulting increase in temperature will decrease the solubility of the gas leading to the formation of bubbles (Zhang *et al.* 1995). Presence of bubbles in the liquid during the PEF treatment leads to dielectric breakdown resulting in sparking in the treatment region. During sparking, all electrical energy passes through the spark and therefore treatment is not uniform. In addition, liquid around the spark can reach a very high temperature, which results in loss of sensory properties. Electric field strengths greater than 60 kV/cm led to dielectric breakdown in apple juice for the treatment chamber used in this study.

UV is an economical method to pasteurize apple juice, but may result in a large population of injured cells (Sharma 1999; Lado and Yousuf 2002). As the mode of microbial inactivation for UV differs from that of PEF, using PEF in combination with UV treatment may result in synergistic activity and result in greater microbial inactivation. PEF and UV treatments were sequentially applied to evaluate synergistic effects, thus reducing the severity of the individual treatments to achieve 5 log reduction of *E. coli*. Several studies showing synergism and/or additive effects have been reported with PEF treatment in combination with low pH, antimicrobial agents, ultrasonication, high hydrostatic pressure, and temperature (Crawford *et al.* 1996; Jin *et al.* 1998; Pagan *et al.* 1998). Liu *et al.* (1997) reported synergistic effect between PEF and benzoic or sorbic acid on the inactivation of *E. coli* O157:H7. Fernandez-Molina *et al.* (2006) showed the synergistic effect of temperature and PEF on the microbial inactivation. The combination of PEF, mild heat, and antimicrobials exhibited synergism compared to the individual effects on the inactivation of microorganisms in raw milk (Smith *et al.* 2002). Calderon-Miranda *et al.* (1999) showed that PEF treatment of liquid whole egg followed by exposure to nisin exhibited an additive effect in the overall inactivation of *L. innocua*. Thus, use of combination nonthermal treatments may achieve the microbial destruction necessary to assure food safety while maintaining product quality and sensory properties.

E. coli inactivation in apple juice with combination treatments (PEF followed by UV and vice versa), as well as additive results for their individual treatment inactivation are presented in Figure 7. Differences between either of the combination treatments and the additive treatments were not observed ($P > 0.05$), indicating an additive effect of PEF and UV treatments. Huang *et al.* (2006) evaluated the combined efficacy of PEF, high pressure, and ultrasound to inactivate *Salmonella* Enteritidis in liquid whole egg and reported only additive effects. Similarly, Ngadi *et al.* (2004) evaluated combinations of UV, PEF, and ozone treatments for inactivation of *E. coli* O157:H7 in poultry chiller water. The authors reported synergistic effect for the combined treatments until 50 pulses and additive effects beyond 50 pulses. Reductions in *E. coli* of 5.33 log CFU/mL were achieved using PEF treatment (electrical field strength of 60 kV/cm; 11.3 pulses) followed by UV treatment (30 cm length; 1.8-s treatment time; flow rate of 8 mL/min). The effects of combination treatments on the sensory quality and shelf life of the treated product will be evaluated in the future. Also, the effect of further parameters of PEF (electrical field and time constant) and UV (exposure time and diameter of quartz tube) will be investigated.

Conclusions

The *E. coli* inactivation in apple juice increased with an increase in the UV radiation dose which is a function of treatment time. Increasing the PEF treatment energy (a function of electrical field strength and pulse number) resulted in an increase in the *E. coli* inactivation. *E. coli* population reductions of up to 4.87 log was observed in PEF treatment using 60 kV/cm and flow rate 8 mL/min (11.3 pulses). A maximum log reduction of 3.46 was observed for UV treatment of apple juice using 50-cm treatment length and flow rate 8 mL/min (2.94 s treatment time). Differences in microbial inactivation between the order of combined treatments of PEF and UV were not ob-

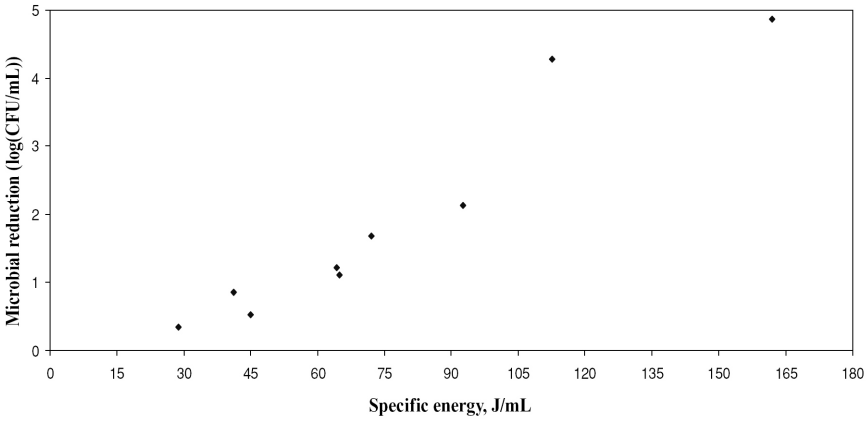

Figure 6. Inactivation of *E. coli* as function of specific energy for different PEF treatments.

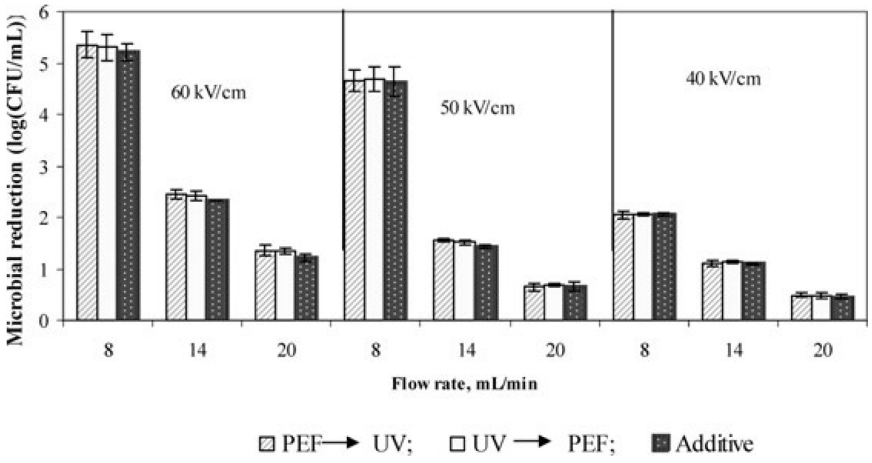

Figure 7. Inactivation of *E. coli* in apple juice using PEF and UV combination treatments. (For PEF treatment, the pulse numbers were approximately 11.3, 6.5, and 4.5 for 8, 14, and 20 mL/min flow rates, and the length of the quartz tube for UV treatment was 30 cm).

served. There was approximately the same log reduction of 5.2 log CFU/mL with the PEF condition of 60 kV/cm (11.3 pulses) and UV treatment length of 30 cm with flow rate of 8 mL/min irrespective of the order of treatment. An additive but not synergistic effect was observed for PEF and UV treatments for inactivating *E. coli* in apple juice.

References

Amiali M, Ngadi MO, Smith JP, Raghavan GSV. 2006. Synergistic effect of temperature and pulsed electric field on inactivation of *Escherichia coli* O157:H7 and *Salmonella* Enteritidis in liquid egg yolk. J Food Eng 79(2):689–94.

Aronsson K, Ronner U. 2001. Influence of pH, water activity and temperature on the inactivation of *Escherichia coli* and *Saccharomyces cerevisae* by pulsed electric fields. Inn Food Sci Emerg Technol 2(2):105–12.

Barbosa-Cánovas GV, Gòngora-Nieto MM, Swanson BG. 1998. Nonthermal electrical methods in food preservation. Food Sci Technol Int 4(5):363–70.

Basaran N, Quintero-Ramas A, Moake JJ, Worobo RW. 2004. Influence of apple cultivars on inactivation of different strains of *Escherichia coli* O157:H7 in apple cider by UV irradiation. Appl Environ Microbiol 70(10):6061–5.

Besser RE, Lett SM, Weber JT, Doyle MP, Barret TJ, Wells JG, Griffin PM. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh pressed apple cider. JAMA 269:2217–20.

Bintsis T, Litopoulou-Tzanetaki E, Robinson RK. 2000. Existing and potential applications of ultraviolet light in the food industry—a critical review. J Sci Food Agric 80(6):637–45.

Calderon-Miranda ML, Barbosa-Cánovas GV, Swanson BG. 1999. Inactivation of *Listeria innocua* in liquid whole egg by pulsed electric fields and nisin. Int J Food Microbiol 51(1):7–17.

CDC. 1996. Outbreak of *Escherichia coli* O157:H7 infections associated with drinking unpasteurized commercial apple juice—British Columbia, California, Colorado, and Washington, October 1996. MMWR 45(44):975.

CDC. 1997. Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider—Connecticut and New York, October 1996. MMWR 46(44):4–8.

Crawford YJ, Murano EA, Olson DG, Shenoy K. 1996. Use of high hydrostatic pressure and irradiation to eliminate Clostridium sporogenes spores in chicken breast. J Food Prot 59(7):711–5.

FDA. 2000. 21 CFR Par 179: irradiation in the production, processing and handling of food. Federal Register 65:71056–8.

Erkmen O. 2001. Effects of high-pressure carbon dioxide on *Escherichia coli* in nutrient broth and milk. Int J Food Microbiol 65(1–2):131–5.

Evrendilek GA, Zhang QH, Richter ER. 1999. Inactivation of *Escherichia coli* O157:H7 and *Escherichia coli* O157:H7 in apple juice by pulsed electric fields. J Food Prot 62(7):793–6.

Evrendilek GA, Jin ZT, Ruhlman KT, Qiu X, Zhang QH, Richter ER. 2000. Microbial safety and shelf-life of apple juice and cider processed by bench and pilot scale PEF systems. Inn Food Sci Emerg Technol 1(1):77–86.

Fernandez-Molina J, Bermudez-Aguirre D, Altunakar B, Swanson B, Barbosa-Cánovas GV. 2006. Inactivation of *Listeria innocua* and *Pseudomonas fluorescens* by pulsed electric field in skim milk: energy requirements. J Food Process Eng 29(6):561–73.

Garcia D, Gomez N, Raso CJ, Pagan R. 2003. Pulsed electric field cause sublethal injury in *Escherichia coli*. Lett Appl Microb 36(3):140–4.

Geveke DJ. 2005. UV Inactivation of bacteria in apple cider. J Food Prot 68(8):1739–42.

Goverd KA, Beech FW, Hobbs RP, Shannon R. 1979. The occurrence and survival of coliforms and salmonellas in apple juice and cider. J Appl Bacteriol 46(3):521–30.

Guerrero-Beltran JA, Barbosa-Cánovas GV. 2004. Advantages and limitations on processing foods by UV light. Food Sci Technol Int 10:137–47.

Huang E, Mittal GS, Griffiths MW. 2006. Inactivation of *Salmonella* Enteritidis in liquid whole egg using combination treatments of pulsed electric field, high pressure and ultrasound. Biosyst Eng 94(3):403–13.

Ho S, Mittal GS. 2000. High voltage pulsed electrical field for liquid food pasteurization. Food Reviews Intl 16(4):395–434.

Iu J, Mittal GS, Griffiths MW. 2001. Reduction in levels of *Escherichia coli* O157:H7 in apple cider by pulsed electric fields. J Food Prot 64(7):964–9.

Jin ZT, Su Y, Tuhela L, Singh B, Zhang QH. 1998. Inactivation of *Bacillus subtilis* using high voltage pulsed electric fields and ultrasonication. IFT Annual Meeting Book of Abstracts, Session 59C-15.

Koutchma T, Keller S, Stuart C, Parisi B. 2004. Ultraviolet disinfection of juice products in laminar and turbulent flow reactors. Inn Food Sci Emerg Technol 5(2):179–89.

Lado B, Yousuf A. 2002. Alternative food-preservation technologies: efficacy and mechanisms. Microbes Infect 4(4):433–40.

Laroussi M, Tendero C, Lu X, Alla S, Hynes W. 2006. Inactivation of bacteria by the plasma pencil. Plasma Proc Polym 3(6–7):470–3.

Lee KW, Kim YJ, Kim DO, Lee HJ, Lee CY. 2003. Major phenolics in apple and their contribution to the total antioxidant capacity. J Agri Food Chem 51(22):6516–20.

Leontowicz M, Gorinstein S, Leontowicz H, Krzeminski R, Lojek A, Katrich E, Cizm M, Martin-Bellooso O, Soliva-Fortuny R, Haruenkit R, Trakhtenberg S. 2003. Apple and pear peel and pulp and their influence on plasma lipids and antioxidant potentials in rats fed cholesterol-containing diets. J Agri Food Chem 51(19):5780–5.

Liu X, Yousef AE, Chism GW. 1997. Inactivation of *Escherichia coli* O157:H7 by the combination of organic acids and pulsed electric field. J Food Safety 16(4):287–99.

Martin O, Qin BL, Chang FJ, Barbosa-Cánovas GV, Swanson BG. 1997. Inactivation of *Escherichia coli* in skim milk by high intensity pulsed electric fields. J Food Proc Eng 20(4):317–36.

Ngadi M, Jun X, Smith J, Raghavan GSV. 2004. Inactivation of *Escherichia coli* O157:H7 in poultry chiller water using combined ultraviolet light, pulsed electric field and ozone treatments. Int J Poult Sci 3(11):733–7.

Pagan R, Esplugas S, Gongora-Nieto MM, Barbosa-Cánovas GV, Swanson BG. 1998. Inactivation of *Bacillus subtilis* spores using high intensity pulsed electric fields in combination with other food conservation technologies. Food Sci Technol Int 4(1):3–44.

Quintero-Ramos A, Churey JJ, Hartman P, Barnard J, Worobo RW. 2004. Modeling of *Escherichia coli* inactivation by UV irradiation at different pH values in apple cider. J Food Prot 67(6):1153–6.

Ross AIV, Griffiths MW, Mittal GS, Deeth CS. 2003. Combining nonthermal technologies to control foodborne microorganisms. Int J Food Microbiol 89(2–3):125–38.

Sharma G. 1999. Ultraviolet light. In: Robinson RK, Batt C, Patel P, editors. Encyclopaedia of food microbiology 3. London : Academic Press. p 2208–14.

Smith K, Mittal GS, Griffiths MW. 2002. Pasteurization of milk using pulsed electric field and antimicrobials. J Food Sci 67(6):2304–8.

Splittstoesser DF, McLellan MR, Churey JJ. 1995. Heat resistance of *Escherichia coli* O157:H7 in apple juice. J Food Prot 59(3):226–9.

Steele BT, Murphy N, Arbus GS, Rance CP. 1982. An outbreak of hemolytic uremic syndrome associated with the ingestion of fresh apple juice. J Pediatr 101(6):963–5.

US Apple Assn. 2006. Production and utilization analysis. Available at: http://www.yvgsa.com/yvgsa/pdf/facts/USApple2006ProductionAnalysis.pdf Accessed August 9, 2007.

Wood OB, Bruhn CM. 2000. Position of the American dietetic association: food irradiation. J Am Diet Assoc 100(2):246–53.

Wood J, Capellas M, Pla R, Fung DYC, Mor-Mur M. 2001. High pressure processing for food safety and preservation: a review. J Rapid Meth Autom Microbiol 9(1):1–10.

Zhang Q, Barbosa-Cánovas GV, Swanson BG. 1995. Engineering aspects of pulsed electric field pasteurization. J Food Eng 25(2):261–81.