



Upscaling from benchtop processing to industrial scale production: More factors to be considered for pulsed electric field food processing [☆]



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ABSTRACT

Pulsed electric field (PEF) processing of juice has been intensively studied with benchtop scale experiments. However, there is still limited information regarding critical factors to be considered for PEF efficacy in microbial reduction with PEF processing during pilot or commercial scale production of juice. In the present study, continuous benchtop (3.6–7.2 L/h) PEF processing systems with co-field treatment chambers and bipolar square waveform pulses were used and simulated production conditions were tested for pomegranate juice. Microbial reductions of *Escherichia coli*, as affected by PEF process conditions (field strength, pulse width, pulse frequency, total treatment time, input energy), production conditions (flow rate, juice holding time and temperature), and juice properties (pH, conductivity, particulate), were investigated. Flow rate, PEF process parameters, production conditions, type of target microorganism, and properties of juice significantly affected microbial reductions by PEF treatments. *E. coli* ATCC 35218, a non pathogenic surrogate bacterium, exhibited higher resistance to PEF treatments than *E. coli* O157:H7 and *E. coli* K12 in pomegranate juice. Increase of a single PEF parameter (field strength, pulse width, pulse frequency, total treatment time, or energy input) is insufficient to achieve maximum microbial reduction. Optimal PEF treatment conditions for maximum microbial reduction depend on multiple factors including PEF processing parameters, production conditions and product properties. This study demonstrates that scale-up and validation studies in a specific PEF system for specific products are very important and necessary before successful commercial application of this novel technology is possible.

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1. Introduction

Pulsed electric fields (PEF), one of the novel non-thermal processing technologies, has been studied intensively worldwide for the past two decades, resulting in over thousands of articles published from 1993 to 2013 regarding PEF treatment of foods (Sampedro et al., 2014). PEF treatments not only inhibit pathogenic and spoilage microorganisms, but also result in the retention of flavor, aroma, nutrients, and color of foods when compared to thermal processing (Charles-Rodriguez et al., 2007; Cserhalmi et al., 2006; Elez-Martinez et al., 2006a; Hodgins et al., 2002; Jin and Zhang, 1999; Min et al., 2007; Yeom et al., 2000). This technology shows a promising application for enhancing food safety, improving food

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quality and extending food shelflife. Although most of those studies involved lab scale PEF systems, pilot and commercial scale PEF processing systems are available and have been evaluated for orange juice, tomato juice, and applesauce (Jin et al., 2009; Min et al., 2003a,b). Cost analyses of a commercial scale PEF system using orange juice as a model have been reported by Jin and Zhang (2002) and Sampedro et al. (2013). However, in spite of such achievements, currently there is no commercial food production line using PEF technology. There may be multiple reasons that prohibit the commercialization of PEF technology. Except the initial capital investment, other major reasons may be the complexity of PEF processing technology and lack of consistent data in microbial reduction, as required by FDA for a 5 log reduction of a target pathogen. The microbial inactivation by PEF depends on the various relationships of different treatment parameters, including PEF treatment parameters (electric field strength, treatment time, pulse frequency, pulse width, and treatment temperature); PEF treatment system (batch/static or continuous chamber; coaxial or co-field; square wave, exponential decay, or oscillatory pulses); product

parameters (electric conductivity, density, viscosity, pH, temperature); and microbial characteristics (bacteria or mold/yeast, Gram-positive or negative, vegetable cell or spores), as summarized by Min et al. (2007). Due to so many factors being involved, parameters for microbial reduction in many published papers are not comparable, and some of them are even controversial, because different PEF systems were used for different microorganisms in different liquid media or foods. For instance, some studies reported that most Gram-negative bacterial cells showed a greater PEF resistance at acidic pH than at neutral pH (Alvarez et al., 2000; García et al., 2003; García et al., 2007; Somolinos et al., 2010), while others found that *Escherichia coli* cells were more sensitive to PEF treatments at lower pH (Aronsson and Ronner, 2001; Raso et al., 1998; Vega-Mercado et al., 1996). In addition, other factors may also be involved and must be considered in microbial reduction when PEF is scaled up to commercial production, such as holding time and holding temperature of juices before PEF processing or the efficacy of the PEF system at a higher flow rate. From an industry point of view, PEF as a non-thermal pasteurization technology requires accurately defined treatment conditions to achieve 5 logs or more reduction of pathogens of concern when the system runs at an industrial/commercial scale. The juice industry really wants to know the details as much as possible before they invest in this technology and set up production lines. Therefore, it is necessary to understand and know the situation and information need for scale up and production.

The objective of this study was to investigate the various interdependencies of different treatment parameters that need to be considered in juice production. Pomegranate juice was used as a model food, and a benchtop PEF system with continuous co-field flow tubular PEF treatment chambers was used, so that the data from this study is comparable. In addition, the same designed commercial scale PEF system and PEF treatment chambers were used to treat salted peptone water at two pH levels to evaluate PEF microbial reduction efficacy at a large scale (100 L/h). The information from this study could be useful to overcome some obstacles that block this technology moving from lab scale to full industrial production and promote the commercialization of this technology.

2. Materials and methods

2.1. Juice

Frozen bulk packaged and untreated pomegranate juice was provided by the AMC Group, Spain. The frozen juice was shipped under refrigerated temperature and received within 2 days, then stored at -20°C . The untreated juice was thawed at 4°C for 3 days prior to PEF processing. The pH and total soluble solids content of the juice were measured using a pH meter (TS625, Thermo Electron Corp., Beverly, MA, USA) and a digital refractometer (Reichert, Inc., Depew, New York, USA). The electrical conductivity of pomegranate juice was measured with an Oakton Instruments ECTestr11 conductivity meter (Vernon Hills, IL, USA).

2.2. Preparation of bacterial cells and inoculation of pomegranate juice

Pulsed electric field treatments were carried out on pomegranate juice inoculated with acid-resistant enterohemorrhagic *E. coli* O157:H7 (ATCC 43895), non-pathogenic *E. coli* (ATCC 35218) and *E. coli* K12 (ATCC 23716) similar to the study by Gurtler et al. (2011) on strawberry juice. These cultures were stored on Tryptic Soy Agar (TSA, Difco, Becton Dickinson, Sparks, MD) slants in borosilicate screw-cap test tubes at 4°C . Cultures were grown in Tryptic Soy Broth (TSB, EMD, Merck KGaA, Darmstadt, Germany) (10 mL) at 37°C for 24 h, with a 1 mL transfer at 24 h, into 100 mL of

TSB. This 1 mL suspension was incubated for another 24 h at 37°C to populations of ca. $9\log$ CFU/mL prior to inoculation into pomegranate juice.

Bacterial cultures were inoculated into juice samples to obtain populations of ca. $7\log$ CFU/mL and treated by PEF within 30 min, unless otherwise specified.

2.3. Pulsed electric field processing system and treatment conditions

A benchtop PEF continuous processing system (OSU-4H Model) and a commercial PEF continuous processing system (OSU-6 Model) located at the Eastern Regional Research Center, USDA (Wyndmoor, PA, USA) were used for this study. Both systems provide bipolar square waveform pulses with a maximum peak voltage of $\pm 11\text{ kV}$ and 60 kV , respectively. The high voltage pulse generator operated at a repetition rate of 2000 pulses per second (pps) and a pulse width of $1\ \mu\text{s}$. Pulses were monitored with a high voltage probe (VD-60; Northstar, Albuquerque, NM, USA), current monitors (Model 110; Pearson, Palo Alto, CA, USA) and oscilloscopes (TDS-210; Tektronix, Beaverton, OR, USA).

2.4. PEF treatment chambers and cooling systems

The benchtop system was composed of three pairs of treatment chambers, each containing two stainless electrodes with a diameter of 0.23 cm and a gap distance of 0.29 cm, which were connected in series (electrically in parallel). The treated sample was cooled by passing through a cooling coil submerged in a water bath (Multitemp Water Bath III, Pharmacia Biotech, AB, Uppsala, Sweden) after passing through each pair of treatment chambers in order to control the final outlet temperature. The water bath temperatures ($4\text{--}30^{\circ}\text{C}$) were adjusted based on different treatments in order to control the final outlet temperature at $53\text{--}55^{\circ}\text{C}$ for all treatments in this study. The inlet and outlet temperatures were monitored by type K thermocouples attached to a dual input digital thermometer (Omega HH509, Omega Engineering Inc., Stamford, CT).

For the commercial scale system, each PEF treatment chamber consisted of two boron carbide electrodes and a ceramic insulator. The inner diameter of the chambers was 0.807 cm, and the gap distance between the electrodes was 1.27 cm. Six chambers were connected in series (electrically in parallel), thus enabling the products to flow sequentially through all six treatment zones. Counter flow heat exchangers, controlled by independent PID controllers, maintained the outlet temperature of each chamber at 55°C , which were monitored and recorded by a USDA developed data acquisition system using National Instruments LabVIEW (Austin, TX) software. All temperature probes were calibrated prior to the start of the experiments.

All the PEF treated juice samples were immediately placed in ice boxes and subjected to microbiological analysis on the same day. Fig. 1 shows an overview of continuous co-field PEF treatment chambers for benchtop and commercial systems, and Fig. 2 shows flow charts of each system.

2.5. Enumeration of surviving cells

All samples were serially diluted with sterile 0.1% peptone water (BBL/Difco Laboratories, Sparks, MD, USA) and enumerated by surface-plating ($100\ \mu\text{l}$) onto TSA plates. All plates were incubated at 35°C for 24 h.

2.6. Statistical analysis

Three replicate trials were conducted, duplicate samples from each trial were averaged, and means were transformed to \log_{10}

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