Combination of pulsed electric field processing and antimicrobial bottle for extending microbiological shelf-life of pomegranate juice

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Pomegranate juice was processed using bench top (7.2 L/h flow rate, 35 kV/cm field strength, 72 μs total treatment time) and pilot scale (100 L/h flow rate, 35 kV/cm field strength, 281 μs total treatment time) continuous pulsed electric field (PEF) processing systems. The treated juice was packaged in PET bottles or PET bottles coated with potassium sorbate and sodium benzoate, and stored at 4 °C for 84 days. Samples were assessed every 7 days for total aerobic bacteria and yeast and mold. Untreated juice had less than one week of shelf-life, while untreated juices in antimicrobial bottles had 56 days. Juices treated with PEF alone had a shelf-life of 21 days (bench scale) and over 84 days (pilot scale). Juices treated with PEF and stored in antimicrobial bottles had a shelf-life over 84 days for both scale tests, which significantly extended the microbiological shelf-life of pomegranate juice.

Industrial relevance: Pulsed electric field (PEF), one of novel non-thermal processing technologies, has been studied intensively worldwide for the last decades. However, most of them were done at laboratory scale and few were at pilot or commercial scale. In addition, PEF processing alone may not provide enough shelf-life of juice as juice industry expects. The work in this paper shows the side-by-side comparison of PEF processing at lab and pilot scales and demonstrates that the combination of PEF with antimicrobial battle packaging significantly extended the shelf-life of juice. The use of a large scale PEF processing system and the combination of antimicrobial packaging provide juice manufacturers an innovate approach for enhancing the safety and extending the shelf-life of juice products.

1. Introduction

Pulsed electric field (PEF) processing has been of growing interest owing to its potential to provide consumers with microbiologically-safe and fresh-like quality foods. Inactivation by PEF is dependent on multiple factors relating to the process conditions, medium, and microbial species (Aronsson, Lindgren, Johansson, & Ronner, 2001; Wouters & Smelt, 1997). These factors may limit the application of PEF as a sole preservation method against pathogenic bacteria under acid conditions, and especially against Escherichia coli O157:H7 (Ait-Ouazzou et al., 2012; Garcia, Gomez, Raso, & Pagan, 2005; Garcia, Hassani, Manas, Condon, & Pagan, 2005; Lu, Mittal, & Griffiths, 2001). PEF may therefore be optimally used in combination with other antimicrobial interventions.

The hurdle approach, as described by Leistner (1992), is used to produce minimally processed food by applying several sub-lethal treatments to achieve microbial stability, rather than relying solely on one lethal preservation method. The microbial stability is achieved by combining these hurdles to increase destruction of the microbial cytoplasmic membrane as well as preventing cell repair of survivors from treatments (e.g. PEF), such as sub-lethally injured cells or bacterial endospores (Galvez, Abriouel, Lopez, & Omar, 2007; Leistner, 2000). Previous studies reported that combining PEF with natural antimicrobials such as bacteriocins, antifungal peptides, essential oils, spices and organic acids can enhance its killing effect on microorganisms in fruit juices (Liang, Mittal, & Griffiths, 2002; Mosqueda-Melgar, Raybaudi-Massilia, & Martin-Belloso, 2008; Nguyen & Mittal, 2007). It has been suggested that the combination of either organic acids or nisin and non-thermal technologies could be effective in the control of undesirable microorganisms in foods (Galvez et al., 2007; Mosqueda-Melgar et al., 2008).

Antimicrobial packaging, which releases antimicrobials into foods from packaging materials, has been widely investigated for various foods. In our previous study (Jin, 2010), glass jars coated with polylactic acid (PLA) containing 250 mg nisin completely inactivated the cells of Listeria monocytogenes in skim milk after 3 days and throughout the 42 day storage period at 4 °C. The same coating treatments rapidly reduced the cell numbers of Listeria in liquid egg white to undetectable

⁎ Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.
level after 1 day, and then remained undetectable throughout the 48 day storage period at 10 °C and the 70 day storage period at 4 °C. In another study, the PLA coating with 500 μL AIT completely inactivated 3 and 7 log CFU/mL of Salmonella after 7 and 21 days of storage, respectively (Jin & Gurtler, 2011). However, to the best of the authors’ knowledge, there are no reported studies combining PEF with antimicrobial bottles. Therefore, the objective of this study was to develop a new approach for pasteurizing and extending shelf-life of juice by combining PEF processing with antimicrobial packaging, using pomegranate juice as a model.

2. Materials and methods

2.1. Juice

Frozen bulk packaged (20 L per bag) and untreated pomegranate juice were provided by the AMC Group, Spain. The frozen juice was shipped at 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.

2.2. Pulsed electric field processing system and treatment conditions

A bench scale PEF continuous processing system (OSU-4H Model) and a commercial scale PEF continuous processing system (OSU-6 Model) located at Eastern Regional Research Center, Agricultural Research Service USDA (Wyndmoor, PA, USA) were used for this study. Both systems provided bipolar square waveform pulses with a maximum peak voltage of ±11 kV and 60 kV, respectively. The high voltage pulse generator operated at a maximum repetition rate of 2000 pulses per second (pps) and pulse width of 1–10 μs. 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). For the benchtop system, 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 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 system, counter flow heat exchangers, controlled by independent PID controllers, maintained the outlet temperature of each chamber at 55 °C. Fig. 1 shows an overview of each system. The treatment conditions are listed in Table 1.

2.3. Antimicrobial bottle coatings

Nine hundred micrograms of potassium sorbate (99%, Fisher Scientific, Fairlawn, NJ) and 1500 mg of sodium benzoate (99%; Fisher Scientific, Fairlawn, NJ) were accurately weighed and added to 100 mL methylene chloride (Fisher Scientific, Fairlawn, NJ), stirring with a magnetic stir bar under chemical hood over night until solid compounds were completely dissolved. Ten microliters of this mixture was taken into a vial and 1 g of PLA resin (NatureWorks, Minnetonka, MN) was added to the mixture, stirring for 6 h. The mixture with PLA was transferred into pre-cleaned 8 oz PET bottle (U.S. Plastic Corp, Lima, OH, USA), which were rolled horizontally on a hot dog roaster machine, allowing antimicrobial mixtures to coat the inside wall of each bottle for 30 min. The methylene chloride was evaporated during the bottle’s rolling and after the coating at room temperature (ca. 22 °C) under a chemical hood for 24 h, sealed with caps and stored until time of use (within 3 days). Fig. 2 shows the bottles before juice packaging.

2.4. Juice packaging and storage

PEF-processed pomegranate juices were collected inside a sanitary laminar hood using connection tubing from PEF outlet to the hood where juices (200 mL) were packaged into regular PET bottles or antimicrobial-coated PET bottles. Untreated juices were packaged in the same way and used as controls. All juice samples were stored at 4 °C.

Table 1

<table>
<thead>
<tr>
<th>Processing parameter</th>
<th>Pilot scale</th>
<th>Lab scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate (L/h)</td>
<td>100</td>
<td>7.2</td>
</tr>
<tr>
<td>Gap distance (cm)</td>
<td>1.27</td>
<td>0.29</td>
</tr>
<tr>
<td>Inner diameter (cm)</td>
<td>0.807</td>
<td>0.23</td>
</tr>
<tr>
<td>Electrical conductivity of juice (s/m)</td>
<td>0.358</td>
<td>0.358</td>
</tr>
<tr>
<td>Maximal outlet temperature (°C)</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>PEF treatment time (μs)</td>
<td>281</td>
<td>72</td>
</tr>
<tr>
<td>Electric field strength (kV/cm)</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Pulse repetition rate (pps*)</td>
<td>2000</td>
<td>2000</td>
</tr>
<tr>
<td>Pulse duration rate (μs)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Number of PEF treatment chambers</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Initial temperature of pomegranate juice (°C)</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

* pps = pulse per second.
2.5. Microbiological analysis

2.5.1. Enumeration of total aerobic bacteria, yeasts and molds

Total aerobic plate counts (TPCs) and yeast and mold counts (YMCs) were determined on plate count agar (PCA, BBL/Difco Laboratories, Sparks, MD, USA) and dichlorane rose bengal chloramphenicol agar (DRBC, Merck, Germany), respectively, using 100 μL surface plating method. PCA plates were incubated at 37 °C for 24 h, and DRBC plates at 25 °C for 5 days. Results were expressed as colony forming units (CFU)/mL.

2.5.2. Enumeration of injury cells

To validate the inactivation and injury by PEF treatments, pomegranate juice inoculated with _E. coli_ or without _E. coli_ was treated at the bench scale PEF system. The non-selective agar medium used for enumeration of viable cells was tryptic soy agar (TSA, BBL/Difco). TSA was supplemented with 3% NaCl (TSA3, selective agar medium) to enumerate injured cells (Saldana et al., 2009). Preliminary experiments (data not shown) confirmed that 100% of healthy _E. coli_ 35218 cells recovered on TSA3, thus this medium was used to assess the level of bacterial injury for _E. coli_. The non-pathogenic _E. coli_ strain 35218 was selected for this study because _E. coli_ strain 35218 is more resistant to PEF treatment than _E. coli_ O157:H7 and may be a more suitable surrogate bacterium in PEF challenge studies with acidic beverages than other non-pathogenic strains of _E. coli_ (Gurtler, Rivera, Zhang, & Geveke, 2010). Non-inoculated juice samples were also plated onto non-selective TSA as well as selective TSA3 to determine injury of natural microflora in pomegranate juice. All plates were incubated at 37 °C for 24 h and counted with an automated colony counting system (Synbiosis aCOLyteR Supercount, Microbiology International, Frederick, MD). Injury (log CFU/mL) was determined by subtracting raw number of cells recovered on non-selective TSA from raw number of cells recovered on selective TSA3 and transforming to log_{10} CFU/mL. Percent injury (reported as a percent of the raw number of cells injured) determined by the formula: PI = (1 − N_s / N_ns) × 100%, where PI is percent injury, N_s is raw number of cells recovered on selective TSA3, and N_ns is raw number of cells recovered on non-selective TSA. Each experiment was performed three times at the bench scale PEF system and the average results are presented.

2.6. Physical and chemical analysis

2.6.1. _pH_, and total soluble solid (TSS) measurements

The _pH_ and total soluble solid content of the juice were measured using a _pH_ meter (Thermo Electron Corp., Beverly, MA) and a digital refractometer (Reichert, Inc., Depew, New York, USA), respectively. The refractometer was calibrated using distilled water and the measurement was done using the temperature compensated mode. The soluble solid content was expressed as °Brix.

2.6.2. Antioxidant activity analysis

Antioxidant activity of pomegranate juice was determined using the automated oxygen radical absorbance capacity (ORAC) method according to Ronald, Wu, and Schaich (2005) and Wang, Cao, and Ronald (1996). Juice samples were centrifuged at 8000 rpm for 10 min and supernatants were used for ORAC assay. The automated ORAC assay was carried out on a BioTek Synergy HT Multi-mode Microplate Reader (Winooski, VT, USA) with a fluorescence attachment. Fluorescent filters were set to pass light with an excitation wavelength of 485 nm and an emission wavelength of 528 nm. In a final assay, 150 μL 0.08 pH sodium fluorescein (fluorescent probe) was used as a target of free radical damage. 25 μL 2,2’-azobis(2-amidino-propane) dihydrochloride (AAPH) (4 × 10^{-3} M) as a peroxyl radical generator, 25 μL diluted supernatants as sample, and Trolox as a control standard. The analyzer was programmed to record the fluorescence every min after AAPH addition. Final results were calculated using the differences of areas under the curves between a blank (25 μL 75 mM phosphate buffer [pH 7.4]) and a sample and are expressed as sample, fresh weight basis (Ronald et al., 2005; Wang et al., 1996). There were four measurements for each sample from one replicate, and there were a total of 12 measurements for each treatment.

2.6.3. Total phenolic content analysis

Total phenolic content was measured using the Folin–Ciochette colorimetric method (Fan, 2005). Juice sample (100 μL) used for the antioxidant activity assay was mixed with 200 μL of Folin–Ciochette reagent (Sigma Chemical Co, St. Louis, MO, USA), and incubated for 5 min at 25 °C. Then 3 mL of 50 g/kg sodium carbonate was added. Absorbances at 760 nm were recorded for the mixtures after 2 h incubation at 25 °C. Total phenolic content was expressed as gallic acid (GA) equivalents in g/mL fresh samples. There were four measurements for each sample from one replicate, and there were a total of 12 measurements for each treatment.

2.7. Statistical analysis

Data were analyzed using analysis of variance with SAS version 9.1 software (SAS Institute, Cary, NC). Duncan’s multiple range test was used to determine the significant differences of mean values. Significance was defined at _p < 0.05_.

3. Results and discussion

Fig. 3 shows the survival of total aerobic bacteria (TPC) and yeasts and molds (YMC) in pomegranate juice after bench scale PEF treatments and during storage at 4 °C. For TPC (Fig. 3A), untreated juice reached 6 log CFU/mL after 21 days, while untreated juice in antimicrobial bottles remained at less than 3 log CFU/mL for 56 days then increased to 5.3 log CFU/mL at day 84. PEF processing reduced ca 0.7 log CFU/mL of bacteria initially after the treatment and remained less than 3 log CFU/mL for 21 days, and then increased to 7 log CFU/mL at day...
35, while PEF processed juice in antimicrobial bottles had less than 3 log of TPC through the 84-day storage. For YMC (Fig. 3B), untreated juice reached 6 log CFU/mL after 14 days, while untreated juice in antimicrobial bottles remained less than 3 log CFU/mL for 84 days. PEF processing reduced more than 2 log CFU/mL of molds and yeasts initially after the treatment and remained less than 3 log CFU/mL for 21 days, and then increased to 7 log CFU/mL at day 35, while PEF processed juice in antimicrobial bottles had undetectable cells (<0.69 log CFU/mL) through the whole storage period.

Fig. 4 shows the survival of total aerobic bacteria (TPC) and yeasts and molds (YMC) in pomegranate juices after pilot scale PEF treatments and during storage at 4 °C. Untreated juice in regular bottles and untreated juice in antimicrobial bottles had similar TPC as in the bench scale tests, while PEF processing significantly inactivated total aerobic bacteria as TPC didn’t increase during the 84-day storage. PEF processing at pilot scale tests totally inactivated yeasts and molds for 56 days, after then, PEF treated samples had 1.3 log CFU/mL at day 70 and 2.2 log CFU/mL at day 84, respectively. The combination of PEF processing with antimicrobial bottle treatment reduced yeasts and molds to undetectable level (<0.69 log CFU/mL) after the treatments and during the whole storage period.

The commercial system has more power than the benchtop one and the treatment chambers used in the commercial system have larger inner diameter and gap distance than those in the benchtop system, consequently, the PEF treatment time was almost three times longer of the former than for the latter (Table 1). Therefore, juice samples processed at the commercial scale system were expected to have longer shelf-life (less TPC and YMC) than their corresponding samples from the bench scale system when the same PEF parameters (field strength, pulse width and frequency) were used for both systems. In a previous study on cranberry juice, PEF treatments for 150 μs achieved more than 4 log reduction of aerobic bacteria and yeasts and molds while PEF treatments for 50 μs only reduced ca. 2.2 log CFU/mL of those microorganisms (Jin & Zhang, 1999).

Plate counts of less than 3 log CFU/mL of TPC or YMC may be considered as acceptable microbiological shelf-life (Mosqueda-Melgar, Raybaudi-Massilia, & Martin-Belloso, 2012). Untreated juice in a conventional bottle had less than one week of shelf-life, while the antimicrobial bottle extended this to 56 days. PEF treated juices in a conventional
McLellan, & Churey, 1996). Zhao, Doyle, and Besser (1993) reported that sorbate and benzoate were found to be effective against yeasts in fruit products in the past (Chipley, 1993; Sofos & Boyd, 2010). Other studies also reported that controlled releases of antimicrobials exhibited more antimicrobial effectiveness than direct addition (Jin, Liu, Zhang, & Hicks, 2009; Salmaso, Elvassore, Bertucco, Lante, & Calicetti, 2004; Zhang, Yam, & Chikindas, 2004). Therefore, antimicrobial packaging provides an additional hurdle and barrier defense against human pathogens and spoilage microorganisms that may survive after PEF processing and grow during shipping, storage and/or display.

It has been reported that PEF inhibits microorganisms by causing temporary or permanent loss of the integrity of the microbial cell membrane, and damaged microorganism in fruits and vegetables have been used as an alternative to PEF for microbial inactivation. The authors thank Anita Parameswaran for excellent technical assistance.

### 4. Conclusion

This is the first report for the combination of PEF processing with antimicrobial bottles. This study demonstrated that the combination significantly improved microbiological shelf-life stability of pomegranate juice and provides juice manufacturers an innovative approach for enhancing the safety and extending the shelf-life of juice products.

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### References


