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Microbial and physico-chemical changes in tomato juice subjected to pulsed electric field treatment

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The possibility of microbial reduction by external pulsed electric field (PEF) offers a new application for pulsed power technology. Applying PEF technology to food pasteurization is a promising non-thermal method. One of such application is prevention of microbial growth, an effect that is based on irreversible permiabilization of cell membranes. The objective of this study was to increase the shelf life of tomato juice using PEF method. It is reported that the microbes present in tomato juice were inactivated by PEF process using co-axial and co-field continuous treatment chambers. The samples were subjected to different electric field intensities (30 and 50 kVcm⁻¹) with the same flow rate (100 mL min⁻¹) and the same pulse number (150 pulses). After treatment, in both co-axial and co-linear chambers, greater reduction in microbial counts were seen at higher intensity of 50 kVcm⁻¹. The co-axial treatment chamber at 150 pulses was more effective than co-linear treatment chamber (1.16 and 0.77 log reduction).

Key words: Pulsed electric field, co-field continuous flow treatment chamber, co-axial continuous flow treatment chamber, tomato juice, microbial growth reduction.

INTRODUCTION

Pulsed electric field (PEF) application is the basic principle of operation in electroporation. Electroporation in cells is the process of destroying the cell membranes through application of high voltage short duration pulses across a liquid (Kishore et al., 2007). Li et al. (2009) have stated that the level of microbial inactivation by high pulsed electric fields is a function of process parameters (electric field strength and electrode-type, total treatment time and temperature, pulse duration and waveform/shapes), media factors (pH, antimicrobial and ionic compounds, conductivity and ionic strength) and microbial entity factors (strain-type and shape, concentration and growth phase of microbes). Previous research has demonstrated that the PEF microbial inactivation depends on two factors, electric field intensity and pulse number. With higher electric field intensity and more pulse number, a better pasteurization effect can be obtained (El-Hag et al., 2009). This paper studies PEF microbial inactivation in tomato juice by using co-field and co-axial continuous treatment chambers to provide a technical basis for industrial application (Qin et al., 1998).

The mechanisms of microbial inactivation in liquid

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foods (Raso and Heinz, 2006; Huang and Wang, 2009; U.S FDA,2009), and the various treatment chambers available (Gaouda et al., 2010; Min et al., 2007) have been discussed. In our earlier study, we reported reduction of microbes in tomato juice using static and co-axial PEF treatment at same intensities (Sathyanathan et al., 2012). The present simulation study was carried out on samples using two different continuous treatment chamber (co-axial and co-linear). Continuous treatment chambers have the advantage of treating larger volumes. Major components in the prototype include a high voltage repetitive pulse generator 220 V/100 kV with maximum stored energy of 490 J, charging capacitor of 50 nF and the wave forming network that has resistance of 22 Ω and tail forming resistance of 1200 Ω.

Microbial inactivation mechanisms

The two theories that explain the mechanism of breakdown of cell membrane are Dielectric Rupture Theory and Electroporation Theory.

Dielectric rupture theory

The cell membrane is considered as a capacitor filled with dielectric material whose dielectric constant of the order of two. Most foods have a dielectric constant in the range of 60 to 80 (Raso and Heinz, 2006). As a result, free charges accumulate at both membrane surfaces which is shown in Figure 1 (Zimmermann, 1986). Exposure of the cell membrane to an electric field leads to an increase in transmembrane potential (TMP) (Huang and Wang, 2009). The increase in TMP leads to a reduction in the membrane thickness. The TMP of the cell membrane is given as:

\[ U(t) = 1.5rE \cos \theta \]  \hspace{1cm} (1)

Where, \( U(t) \) Transmembrane potential (V); \( r \) Radius of the cell (mm); \( E \) Applied electric field strength (Vmm\(^{-1}\)); \( \theta \) Angle between a given membrane site and the field direction (degrees).

Electroporation theory

Electroporation occurs in a cell which when exposed to the high-voltage electric fields temporarily destabilizes the lipid bi-layer and proteins of the cell membrane. In a cell membrane, protein channels, pores and pumps are present. The opening and closing of many channels constituted by proteins is dependent on TMP. When PEF is applied, many voltage sensitive protein channels will open. Protein channels, once open, will experience current much larger than the current normally experienced by the protein channels during metabolic activities. As a result, protein channels are irreversibly denatured by joule heating. Thus, electroporation in the cell membrane occurs both in the protein channels and in the lipid bi-layer resulting in the inactivation of the cell (Min et al., 2007).

During electroporation, lipid bi-layer suffers from any one of the following possible fates on the membrane namely (Figure 2):

1. A slight increase in membrane conductance.
2. Mechanical rupture of the cell wall or any structural changes in the membrane.
3. Reversible electrical breakdown resulting in complete discharge of the membrane.
4. Irreversible electrical breakdown when the intensity of the electric field is raised (Sundarrajan et al., 2008).

PEF microbial inactivation system

The block diagram of PEF bacteria inactivation system is shown in Figure 3. The continuous treatment chamber adopts co-linear electrode and co-axial electrode respectively and the sample is exposed to exponentially
Fig. 2. Electroporation of cell membrane (Wu et al., 2004).

Fig. 3. Block diagram of PEF bacteria inactivation system

decaying electric field.

MATERIALS AND METHODS

Design of treatment chamber and its electric field analysis

In this study, co-axial treatment chamber and the co-linear treatment chamber models were taken and simulation studies were carried out. The treatment chamber was made with aluminum and teflon as electrode material and insulation material. The relative permittivity of aluminum is 1. The inner high voltage electrode has the radius of 4 cm and the outer ground electrodes has the radius of 4 cm; in between these electrodes, a gap of 2 cm is used as the flow path of liquid food. This insulator gives the support to the positioning of the electrodes and gives the smooth flow of the liquid food inside the treatment chamber. The electrical current flows perpendicular to food flow in co-axial treatment chamber and parallel to food flow in co-linear chamber (Min et al., 2007). The modified co-axial and co-linear treatment chambers widely used in industrial applications is shown in Figure 4 (Sale and Hamilton, 1967).

Simulation of co-axial and co-linear treatment chamber

Here co-axial and co-linear continuous flow treatment chambers have been modeled for simulation studies using a software package Maxwell 2D simulator (ANSOFT). The ANSOFT model for co-axial and co-linear treatment chamber is as shown in Figure 5. The primary objective of the simulation is to ensure uniform field distribution across the effective treatment region. Finite element method analysis is used to measure field distribution inside the treatment chamber’s effective treatment region. The problem definition of simulation studies on co-axial and co-linear treatment chambers are given below.

Problem definition

Problem statement: Analysis of electrostatic field distribution in the effective treatment region.

Domain: The field domain is enclosed in a boundary. All space outside this boundary is excluded from field problem domain. This is an interior problem.

Type of problem: Electrostatic problem as liquid foods are usually negligibly magnetizable.

Governing equation: This electrostatic field simulator solves for the electric potential ϕ, in the Laplace equation, \( \nabla^2 \Phi = 0 \).

Plane: Axis symmetry (RZ Plane) has been used for this simulation.

Boundary condition: Balloon boundary condition has been used for this simulation.

The following design aspects have been considered during the design of PEF treatment chambers.
Figure 4. Diagram of the co-axial treatment chamber (Min et al. 2007).

Figure 5. ANSOFT model for co-axial and co-field treatment chambers.

Electrode material

During the PEF treatment, electrochemical reactions can occur and they may result in partial electrolysis of the solution, corrosion of the electrode and introduction of particles of the electrode material in the liquid. These phenomena should be minimized because they could provoke microbial inactivation and over estimate microbial inactivation by PEF. The electrodes can be made up of stainless steel and electro-chemically inert materials such as gold, platinum, carbon and metal oxides.

Electric field strength

Dielectric breakdown of food occurs, when the strength of applied electric fields exceeds the electric field strength of the food product treated in the treatment chamber. This breakdown mechanism of the food is generally characterized as causing damage on the electrode surfaces in the form of pits, a result of arcing and increased pressure, leading to treatment chamber explosions and evolution of gas bubbles.

Electrode gap

Electric field strength in a treatment zone of a PEF chamber is inversely proportional to the cross sectional area of the fluid flow and to the gap distance between the two electrodes. The electrode gap is chosen such that the treatment area has uniform field to ensure all fluids have undergone the field.

Insulator

In continuous PEF treatment system, insulators are used to ensure the separation of electrodes and to provide the smooth flow of liquid between the electrodes. Plexi glass is the most commonly used insulator in the PEF treatment chamber system.

Uniform field distribution

Distribution of the field strength depends on the characteristics of the treatment chamber, basically shape of electrodes, gap of the chamber, gas impurities in electric materials. The treatment chamber should be designed to provide uniform electric field strength, such that the actual applied field strength does not exceed the dielectric strength of the fluid foods under the treatment. The probability of dielectric breakdown in foods can be reduced by the use of a smooth electrode surface to minimize electron emission and the use of round electrode edges to prevent electric field enhancement near sharp edges.

PEF treatment

The Marx’s generator circuit and the complete experimental setup for the continuous flow PEF treatment of tomato juice processing is shown in Figures 8 and 9. The tomato juice was prepared fresh as described in the earlier study (Sathyarathan et al., 2012). Marx’s generator circuit was used to produce impulse voltages of 1.3/45 µs.
Table 1. Applied field and pulses for PEF treatment of tomato juice,

<table>
<thead>
<tr>
<th>Liquid food</th>
<th>Type of treatment chamber</th>
<th>Applied field (kV/cm)</th>
<th>Pulse number</th>
<th>Duration (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato juice</td>
<td>Co-axial</td>
<td>30</td>
<td>150</td>
<td>110</td>
</tr>
<tr>
<td>Tomato juice</td>
<td>Co-axial</td>
<td>50</td>
<td>150</td>
<td>240</td>
</tr>
<tr>
<td>Tomato juice</td>
<td>Co-linear</td>
<td>30</td>
<td>300</td>
<td>240</td>
</tr>
<tr>
<td>Tomato juice</td>
<td>Co-linear</td>
<td>50</td>
<td>150</td>
<td>110</td>
</tr>
</tbody>
</table>

as shown in the Figure 10. Tomato juice was treated with optimized test voltages and pulse rate as shown in the Table 1. The control and treated samples were stored in autoclaved, wide-mouthed, screw-capped glass bottles 75% fill, at room temperature (25±3°C). The experiment was repeated twice.

Analysis of shelf life

Chemical and microbial tests on control and treated samples were carried out in the Food Safety Laboratory of National Agro Foundation, Anna University Campus, Taramani to analyze shelf life of the tomato juice. These tests were carried on the first day and seventh day of PEF treatment. The PEF treatments were carried out on two separate batches of tomato juice on two different days and all chemical, nutritional and microbial analyses were done in duplicates. All chemical and reagents used were of analytical grade.

Chemical and nutritional analysis

The chemical tests were done to analyze whether the treated food had tolerable levels of chemical properties for human consumption as given by U.S FDA (2009). pH, °Brix and acidity were tested. pH value of tomato juice was measured by using AP-1plus pH meter (Susima Technologies, Chennai) and the °Brix value of tomato juice was measured by °Brix meter (Arico India hand refractometer). pH and acidity value of liquid food for human consumption should be less than 5 and 0.4%, respectively. The titratable acidity was estimated in tomato juice filtrate by titration with 0.1 N sodium hydroxide to the end point with phenolphthalein indicator. Further, vitamin C content of the juice was tested by the reduction of the blue dye 2, 6 dichlorophenolindophenol by ascorbic acid (AOAC, 2000).

Microbial analysis

Both control and treated samples were analyzed for microbes present by suitable serial dilution and pour plate method using standard plate count agar (Hi-Media Ltd, Mumbai). Plates were incubated at 37°C for 48 h. The colony counts were carried out using a digital colony counter (Lapiz colony counter). The total bacterial counts were carried out on the same day and seventh day (Harrigan and McCance, 1998).

RESULTS AND DISCUSSION

Simulation results

From the simulation study, it was clearly seen that there were no flux lines inside the conductors (field inside the conductor is always zero) and the electricfield lines start from the electrode with positive charge and terminate at the electrode with zero potential. The electric field is not constant due to fringing effect and it gets equally distributed in between the electrodes. Equal stress distribution between the contacts is due to equal contact gap distance between the electrodes in the effective treatment region as shown in Figure 6. The intensity of the electric field is maximum near the high voltage electrode and it decreases when the distance increases as shown in Figure 7. The uniform electric field distribution is achieved across the effective treatment region of the chamber (Schroder et al., 2009)

Chemical test results

There was no significant difference (p < 0.05) in the chemical properties of tomato juice with respect to pH, acidity, °Brix values in both control and treated samples in co-axial and co-linear continuous treatment chambers (Table 2). The PEF treatment and ionic strength are responsible for electroporation and compression of the cell membrane, where as pH of the medium affects the cytoplasm when the electroporation is complete. U.S FDA reported that, depending on the microorganism present in the liquid medium, acidic pH enhanced microbial inactivation. No mention has been made on what microorganisms were affected or what range of pH was used (U.S FDA, 2009). Vitamin C content also did not change significantly in all the treated samples. In both treatment chambers, only 0.9 and 1.0% reduction of vitamin C for the applied field of 30kVcm⁻¹ and only 2.0 and 4.0% reduction for the applied field of 50 kVcm⁻¹ was observed, which is in accordance with the reports of Zhang et al. (2003). Thus, the chemical properties and vitamin C content did not vary much when fresh tomato juice was subjected to PEF in both co-axial and co-linear continuous treatment chambers.

Microbial test results

Both PEF treatments in co-axial and co-linear chambers
Figure 6. Phi Plot for the co-axial and co-field treatment chambers.

Figure 7. Field plot for the co-axial and co-field treatment chambers.
caused significant reduction in microbial content (Table 3). While 30 kVcm\(^{-1}\) 150 pulses/110 s resulted in 0.82 and 0.35 log cfu mL\(^{-1}\) reduction of microbes for first day and there was 0.87 and 0.37 log cfu mL\(^{-1}\) reduction for seventh day in co-axial and co-linear chamber, respectively (Figure 11). Higher field strength of 50 kVcm\(^{-1}\), 150 pulses/110 s showed 1.09 and 0.66 log cfu mL\(^{-1}\) microbial reduction for first day and 1.16 and 0.77 log cfu mL\(^{-1}\) reduction for seventh day in co-axial and co-linear chambers, respectively (Figure 12). It is thus clearly seen

**Figure 8.** Circuit for producing impulse voltage wave.

**Figure 9.** Complete experimental setup for single stage impulse generator.
Table 2. Chemical changes in tomato juice after PEF treatment in co-axial and co-linear continuous chambers.

<table>
<thead>
<tr>
<th>Treatment chamber</th>
<th>Sample</th>
<th>pH</th>
<th>Acidity (%)</th>
<th>°Brix</th>
<th>Vitamin C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st Day</td>
<td>7th Day</td>
<td>1st Day</td>
<td>7th Day</td>
</tr>
<tr>
<td>Untreated co-axial</td>
<td>Control</td>
<td>4.47±0.33</td>
<td>4.47±0.13</td>
<td>0.19±0.1</td>
<td>0.16±0.4</td>
</tr>
<tr>
<td></td>
<td>30kv, 150 Pulses</td>
<td>4.22±0.02</td>
<td>4.47±0.33</td>
<td>0.19±0.7</td>
<td>0.16±0.2</td>
</tr>
<tr>
<td></td>
<td>50kv, 150 Pulses</td>
<td>4.20±0.02</td>
<td>4.71±0.03</td>
<td>0.19±0.2</td>
<td>0.17±0.2</td>
</tr>
<tr>
<td></td>
<td>50kv, 300 Pulses</td>
<td>4.17±0.03</td>
<td>4.47±0.35</td>
<td>0.19±0.1</td>
<td>0.16±0.1</td>
</tr>
<tr>
<td>Co-linear</td>
<td>30kv, 150 Pulses</td>
<td>4.36±0.08</td>
<td>4.39±0.07</td>
<td>0.28±0.3</td>
<td>0.28±0.3</td>
</tr>
<tr>
<td></td>
<td>50kv, 150 Pulses</td>
<td>4.33±0.09</td>
<td>4.41±0.94</td>
<td>0.28±0.1</td>
<td>0.28±0.2</td>
</tr>
</tbody>
</table>

Acidity - Equivalent to % citric acid; Vitamin C - Equivalent to % ascorbic acid.

Table 3. Microbial counts on first day after PEF treatment in co-axial and co-linear continuous treatment chambers.

<table>
<thead>
<tr>
<th>Type of treatment chamber</th>
<th>Applied field (kV/cm)</th>
<th>Pulse number</th>
<th>Microbial count reduction Log cfu mL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Untreated</td>
<td>Nil</td>
<td>4.09</td>
</tr>
<tr>
<td>Co-axial</td>
<td>30</td>
<td>150</td>
<td>3.27</td>
</tr>
<tr>
<td>Co-axial</td>
<td>50</td>
<td>150</td>
<td>3.00</td>
</tr>
<tr>
<td>Co-linear</td>
<td>30</td>
<td>150</td>
<td>3.74</td>
</tr>
<tr>
<td>Co-linear</td>
<td>50</td>
<td>150</td>
<td>3.43</td>
</tr>
</tbody>
</table>

that higher microbial reduction was achieved by using co-axial treatment chamber at 50 kVcm⁻¹, 150 pulses. Inhibition of microbial growth and better shelf life extension can also be achieved at 50 kVcm⁻¹, 300 pulses in co-axial treatment chamber without much affecting the nutritional value of tomato juice (Table 4).
Figure 11. Microbial reduction in tomato juice on first day PEF treatment.

Figure 12. Microbial reduction in tomato juice on seventh day of PEF treatment.
Table 4. Microbial reduction on the seventh day after PEF treatment in co-axial and co-linear continuous treatment chamber.

<table>
<thead>
<tr>
<th>Type of treatment chamber</th>
<th>Applied field (kV/cm)</th>
<th>Pulse number</th>
<th>Microbial count Log cfu mL(^{-1})</th>
<th>Microbial count reduction Log cfu mL(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Untreated</td>
<td>Nil</td>
<td>4.26</td>
<td>0.00</td>
</tr>
<tr>
<td>Co-axial</td>
<td>30</td>
<td>150</td>
<td>3.39</td>
<td>0.87</td>
</tr>
<tr>
<td>Co-axial</td>
<td>50</td>
<td>150</td>
<td>3.10</td>
<td>1.16</td>
</tr>
<tr>
<td>Co-linear</td>
<td>30</td>
<td>150</td>
<td>3.89</td>
<td>0.37</td>
</tr>
<tr>
<td>Co-linear</td>
<td>50</td>
<td>150</td>
<td>3.49</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Conclusion

PEF treatments of tomato juice samples in co-axial and co-linear continuous treatment chambers at 30 kV/cm\(^{-1}\),150 pulses and 50 kV/cm,150 pulses were performed. Chemical properties- pH, acidity, °Brix value maintained were almost the same in both co-axial and co-linear treatment chambers and vitamin C showed little change for all treated samples. Our results indicate that shelf life extension of the tomato juice at room temperature (25±3°C) for seven days is possible in both co-axial and co-linear continuous treatment chamber. However, better shelf life was achieved by higher electric field with higher number of pulses (50 kV/cm, 150) in co-axial treatment chamber. Further extension of shelf life will be possible by storage under refrigerated conditions after PEF treatment.

Conflict of Interests

The authors have not declared any conflict of interests.

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