Effect of High Intensity Electric Field Pulses on Inactivation of Lactobacillus to Preserve Liquid food

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Objectives

- Provide optimal pulse parameters to inactivate half of the population of Lactobacillus present in an suspension that only contains this type of bacteria. Log reduction $= 0.3$.
- Extend the pulse treatment for fresh orange juice to evaluate the effectiveness of the preservation method.
Introduction

- Electroporation mechanisms
- Pasteurization
- Microbial study
Electroporation mechanisms

- Electrical breakdown
- Osmotic imbalances
Electrical breakdown

Cell membrane

Figure 1: Lipid bilayer in an aqueous electrolyte solution. Reference: Kotnik et al. (2012)
Figure 2: Electrical circuit representing a membrane. Reference: Hodgkin & Huxley (1952)
Figure 3: Transmembrane voltage
The membrane potential $V_m$ is given as Ellappan & Sundararajan (2005)

$$V_m = 1.5ER\cos\delta/[(1 + RG_m(\rho_i + 0.5\rho_a))(1/(1 + jwT))]$$  \hspace{1cm} (1)

$$V_m = 1.5ER\cos\delta/(1 + jwT)$$  \hspace{1cm} (2)

$$V_m = 1.5ER\cos\delta$$  \hspace{1cm} (3)

Where $\rho_i$ and $\rho_a$ are the resistivities inside and outside the cell, $R$ is the cell radius, and $\delta$ is the angle between the electric field $E$ and the radius vector, $w$ is the radian frequency $= 2\pi f$, where $f$ is the frequency, $T$ is the time constant, and $G_m$ is the membrane conductance.

Normal activity: $V_m = 10 \text{ mV}$

Pore formation: $V_m = 0.5 - 1 \text{ V}$
Osmotic imbalances

Figure 4: Aqueous pore forming. Reference: Yarmush et al. (2014)
Figure 5: Aqueous pore forming
Figure 6: Scanning electron micrographs before and after electroporation of Lactobacillus casei. Reference: Kotnik et al. (2015)
Figure 7: Applications
Pasteurization

- Thermal pasteurization
- Non-thermal pasteurization
Non-thermal pasteurization

- High pressure processing (HPP)
- Gamma radiations and electron beams
- Pulsed Electric field (PEF)
Experimental procedure

- Sterilization
- Inoculation
- Dilution
- Electroporation
- Cultivation
Figure 8: Chamber to sterilize samples
Inoculation

Figure 9: Incubator
Dilution

**Figure 10: Serial dilutions**

Each step = $10^2$ dilution or 1:100

10 µl → Mix well → 990 µl

"Unknown" → Mix well → 990 µl

Total dilution = $10^2$ → $10^4$ → $10^6$
Electroporation

Figure 11: ECM 830 Square Wave for electroporation
Cultivation

Figure 12: Cultivation chamber
## Pulse parameters

**Table 1: Pulse parameters applied**

<table>
<thead>
<tr>
<th># Pulses</th>
<th>Pulse Length</th>
<th>Pulse Interval (s)</th>
<th>Voltage (V)</th>
<th>Electric Field (V/cm)</th>
<th>Cuvette Gap Size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTX01.1</td>
<td>10µs</td>
<td>1</td>
<td>100</td>
<td>250</td>
<td>4</td>
</tr>
<tr>
<td>BTX02.1</td>
<td>10µs</td>
<td>1</td>
<td>250</td>
<td>650</td>
<td>4</td>
</tr>
<tr>
<td>BTX03.1</td>
<td>10µs</td>
<td>1</td>
<td>500</td>
<td>1250</td>
<td>4</td>
</tr>
<tr>
<td>BTX04.1</td>
<td>10µs</td>
<td>1</td>
<td>1000</td>
<td>2500</td>
<td>4</td>
</tr>
<tr>
<td>BTX05.1</td>
<td>10µs</td>
<td>1</td>
<td>2000</td>
<td>5000</td>
<td>4</td>
</tr>
<tr>
<td>BTX06.1</td>
<td>10µs</td>
<td>1</td>
<td>3000</td>
<td>7500</td>
<td>4</td>
</tr>
<tr>
<td>BTX07.1</td>
<td>10µs</td>
<td>1</td>
<td>3000</td>
<td>15000</td>
<td>2</td>
</tr>
<tr>
<td>BTX08.1</td>
<td>10µs</td>
<td>1</td>
<td>3000</td>
<td>30000</td>
<td>1</td>
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</table>
pH and temperature measurements before and after electroporation

**Table 2:** Electroporated orange juice bought from a supermarked

<table>
<thead>
<tr>
<th>Sample</th>
<th>Temp. before (°C)</th>
<th>Temp. after (°C)</th>
<th>pH before</th>
<th>pH after</th>
<th>Voltage (V)</th>
<th>Electric Field (kV/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTX04.1</td>
<td>23.2</td>
<td>23.8</td>
<td>4.18</td>
<td>4.16</td>
<td>979.8</td>
<td>2449.6</td>
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<tr>
<td>BTX04.2</td>
<td>23.4</td>
<td>24.2</td>
<td>4.18</td>
<td>4.01</td>
<td>979.5</td>
<td>2448.8</td>
</tr>
<tr>
<td>BTX04.3</td>
<td>23.3</td>
<td>24</td>
<td>4.5</td>
<td>4.13</td>
<td>980.2</td>
<td>2450.4</td>
</tr>
<tr>
<td>BTX04.Av</td>
<td>23.3</td>
<td>24</td>
<td>4.28</td>
<td>4.1</td>
<td>979.8</td>
<td>2449.6</td>
</tr>
</tbody>
</table>
Microbial study results

Lactobacillus plantarum (more likely)

Figure 13: Cell culture of sterilized juice
Figure 14: Cell culture of sterilized orange juice
Figure 15: Cell culture of sterilized orange juice with 3 dilutions
Table 3: Cell counting for figure 15 and log reduction with respect to an applied electric field equal to 7.5 kV/cm

<table>
<thead>
<tr>
<th>Electric Field (kV/cm)</th>
<th>Number of Colony-Forming Units (CFUs)</th>
<th>Log Reduction $\log N_0 - \log N$</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>232</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>Not determined</td>
</tr>
<tr>
<td>30</td>
<td>1</td>
<td>2.3</td>
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</table>

Table 4: Log reduction for some references

<table>
<thead>
<tr>
<th>Electric Field (kV/cm)</th>
<th>Log Reduction $\log N_0 - \log N$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>2.57</td>
<td>Gurtler et al. (2010)</td>
</tr>
<tr>
<td>35.8</td>
<td>2.5</td>
<td>Rodrigo et al. (2001)</td>
</tr>
</tbody>
</table>
Extension for fresh orange juice

Figure 16: Positive control for the tests
Figure 17: Negative control for fresh orange juice cell culture with one dilution
Figure 18: Cell culture for $E = 4.8 \text{ kV/cm}$ application with one dilution
Figure 19: Cell culture for $E = 6.8 \text{ kV/cm}$ application with obe dilution
Figure 20: Cell culture for $E = 9.4$ kV/cm application with one dilution
Table 5: Cell counting for fresh orange juice and log reduction for different electric field applied

<table>
<thead>
<tr>
<th>Electric Field (kV/cm)</th>
<th>Number of Colony - Forming Units (CFUs)</th>
<th>Log Reduction $\log N_0 - \log N$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>167</td>
<td>0</td>
</tr>
<tr>
<td>4.8</td>
<td>166</td>
<td>0</td>
</tr>
<tr>
<td>6.8</td>
<td>91</td>
<td>0.26</td>
</tr>
<tr>
<td>9.4</td>
<td>21</td>
<td>0.9</td>
</tr>
</tbody>
</table>
Future work

- Combine the pulse treatment with different sample temperatures (below and above environment temperature)
- Perform a numerical analysis to extend the treatment for different volumes.


Rodrigo, D., Martínez, A., Harte, F., Barbosa-Cánovas, G. & Rodrigo, M. (2001), ‘Study of inactivation of lactobacillus plantarum in orange-carrot juice by means of pulsed electric fields: Comparison of inactivation kinetics models’, *Journal of Food Protection* **64**(2), 259–263.