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การพัฒนาและการทดสอบเบื้องต้นระบบสนามไฟฟ้าพัลส์ระดับห้องปฏิบัติการสำหรับการยับยั้งเชื้อจุลินทรีย์ในน้ำส้ม

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บทความรับเชิญ

บทคัดย่อ

ระบบสนามไฟฟ้าพัลส์ระดับห้องปฏิบัติการสำหรับการยับยั้งเชื้อจุลินทรีย์ในน้ำส้มได้ถูกพัฒนาและทดสอบเบื้องต้นในการศึกษานี้ ระบบที่พัฒนาประกอบด้วยแหล่งกำเนิดไฟฟ้าแรงดันสูง ตัวเก็บประจุสำหรับเก็บพลังงาน ตัวต้านทานจำกัดกระแสอัดประจุ สวิตช์สำหรับดิสชาร์จพลังงานจากตัวเก็บประจุผ่านอาหารและห้องฆ่าเชื้อแบบแผ่นระนาบ ในระบบนี้ห้องฆ่าเชื้อจะถูกจ่ายแรงดันไฟฟ้าพัลส์กระแสตรงเพื่อสร้างสนามไฟฟ้าพัลส์ความเข้มสูงภายในห้องฆ่าเชื้อสำหรับการยับยั้งจุลินทรีย์ในอาหารเหลวภายในห้องฆ่าเชื้อด้วยกระบวนการอิเล็กโทรโพเลชั่น ในการศึกษานี้ระบบที่พัฒนาขึ้นได้ทำการทดสอบเบื้องต้นกับน้ำส้มสดที่ผสมเชื้อ *E. coli.* และเปรียบเทียบกับการพาสเจอไรซ์ด้วยความร้อน ซึ่งพบว่าทั้งสองเทคโนโลยีสามารถยับยั้งเชื้อ *E. coli.* ในน้ำส้มสดได้โดยไม่มีการปรากฏของเซลล์ใดหลังผ่านกระบวนการพาสเจอไรซ์ด้วยความร้อนหรือสนามไฟฟ้าพัลส์ โดยระบบที่พัฒนาขึ้นสามารถยับยั้งเชื้อ *E. coli.* ลงได้ถึง 6 log ของจุลินทรีย์ที่มีชีวิตได้ที่สนามไฟฟ้าความเข้มที่ 25 kV/cm และจำนวนพัลส์เท่ากับ 500 พัลส์

คำสำคัญ : สนามไฟฟ้าพัลส์ จุลินทรีย์ พาสเจอไรซ์ ระดับห้องปฏิบัติการ น้ำส้ม

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Development and Preliminary Test of a Laboratory-Scale Pulsed Electric Field System for Inactivating Microorganisms in Orange Juice

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Invited Article

Abstract

A laboratory-scale pulsed electric field system for inactivating microorganisms in an orange juice was developed and preliminary tested in this study. The developed system consists of a high-voltage power source, an energy storage capacitor bank, a charging current limiting resistor, a switch to discharge energy from the capacitor across the food and a parallel-plate treatment chamber. In this system, the parallel-plate treatment chamber was applied with the DC pulsed voltage in order to create the high pulsed electric field strength inside the chamber for inactivating a microorganisms in the liquid food inside the chamber by electroporation process. In this study, the developed system was preliminary tested with an orange juice inoculated with *E. coli*. and compared with thermal pasteurization. It was shown that both technologies reduced the population of the *E. coli.* inoculated in orange juice. No viable cells were observed after thermal processing of orange juice whereas PEF treatment achieved 6 logarithmic reductions of the microbial viability at the electric field strength up to 25 kV/cm and the pulse number of about 500 pulses.

Keywords: Pulsed Electric Field, Microorganisms, Pasteurization, Laboratory-Scale, Orange Juice

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1. Introduction

At the desired quality level, conventional preservation methods such as thermal pasteurization often fail to produce microbiologically stable food [1]. It has already been demonstrated that a non-thermal treatment by pulsed electric field (PEF) processing can alternatively be applied to deliver safe and shelf-stable products such as juices, milk, yogurt, soups, and liquid eggs with fresh- like character and high nutritional value [2].

PEF process applies short, high voltage pulses, producing high electric fields between two electrodes. Pulses can be applied to foods at temperatures below pasteurization and can kill contaminating microorganisms without significantly affecting the quality of the product [2 – 3]. This process is known as electroporation and is effective in the inactivation of microorganisms [2 – 3]. Generally, PEF system consists of a high- voltage power source, an energy storage capacitor bank, a charging current limiting resistor, a switch to discharge energy from the capacitor across the food and a treatment chamber. Energy from the power source is stored in the capacitor and is discharged through the treatment chamber to generate an electric field in the food material. The level of microbial inactivation by PEF depends on process parameters including electric field strength, total treatment time, pulse width and pulse waveform and conductivity of food. There have been numerous studies and developments on the PEF processing system [4 – 10]. However, commercial exploitation of PEF as an alternative to traditional preservation techniques requires a detailed analysis of process safety, cost-effectiveness, and consumer benefits.

The aim of this study is to develop and test a laboratory- scale pulsed electric field system for inactivating microorganisms in an orange juice. The developed system was preliminary tested with an orange juice inoculated with *E. coli*. and compared with thermal pasteurization. A detailed description of the laboratory- scale pulsed electric field system design was also presented in this paper.

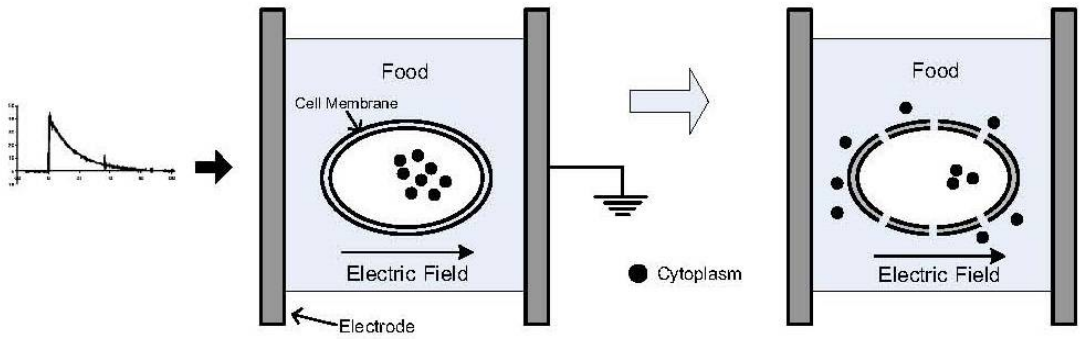


Fig. 1 Electroporation Phenomenon [3]

2. Electroporation Phenomenon

Electroporation, or electropermeabilization, is the phenomenon by which the permeability of the cell membrane to ions and macromolecules is increased by exposing the cell to short high electric field pulses, allowing chemicals, drugs, or DNA to be introduced into the cell (Fig. 1). Electric field strength, E, can be defined as electric potential difference (V) between two given points in space divided by the distance, d, between them [3]:

$$E = \frac{V}{d} \tag{1}$$

The energy stored or the energy density (u) in the electric field can be calculated using the following equation:

$$u = \frac{1}{2}\varepsilon \left|E_v\right|^2 \tag{2}$$

where ε is the permittivity of the medium.

The total energy stored in a particular volume can be measured by:

$$\int_v \frac{1}{2}\varepsilon \left|E\right|^2 dV \tag{3}$$

Energy stored in a unit volume (u_e) is

$$u_e = \frac{1}{2}\varepsilon_0 E^2 \tag{4}$$

where ε_0 is the permittivity of vacuum.

Electroporation is a dynamic phenomenon that depends on the local transmembrane voltage. It is generally accepted that, for a given pulse duration and shape, a specific transmembrane voltage threshold exists for the manifestation of the electroporation phenomenon is ranging from 0.2 V to 2.6 V as shown in Table 1. The electric field in cytoplasm is given as

$$E(t) = E_o \exp\left(-\frac{t}{\tau_m}\right) \tag{5}$$

Table 1 Electrical properties of microorganism cells [3].

Microorganisms	Diameter (μm)	Length (μm)	Electric field (kV/cm)	Treatment time (μs)	Maximum voltage (V)
E. coli.	1.15	6.9	10-20	0.7-1.1	0.26
K. pseudomona	0.83	3.2	8-20	0.7-1.1	1.26
P. aeruginosa	0.73	3.9	8-20	0.7-1.1	1.25
S. aureus	1.03	-	14-20	0.7-1.1	1.00
L. momocytogenesl	0.76	1.7	10-20	0.7-1.1	0.99
C. albicans	4.15	-	10-20	0.14-1.1	2.63

where E_o is the applied electric field strength, t is the pulse duration time and τ_m is the charging time constant. The pulse duration required to reach a critical voltage across the cell membrane is determine by the charging of the outer cell membrane. The charging time constant of the cell membrane is given by

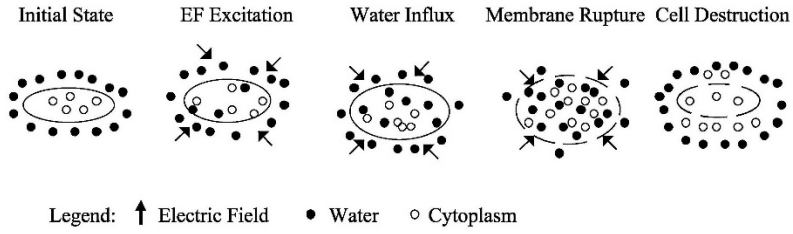
$$\tau_m = C_c r_c \left(\frac{\rho_1}{2} + \rho_2 \right) \quad (6)$$

where ρ_1 is the resistivity of suspending medium, ρ_2 is the resistivity of the cytoplasm, C_c is the capacitance of the cell membrane per unit area and r_c is the radius of the cell membrane. The electric field required to charge the cell membrane therefore as to reach a critical electric field, E_c, can be expressed as

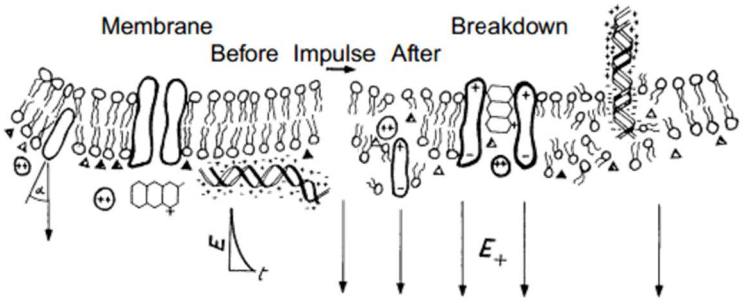
$$E_c = \frac{V_c}{f r_c} \quad (7)$$

where V_c is the critical membrane voltage. A typical value of the critical membrane voltage and f is the spherical cells form factor, typically about 1.5.

This leads to the definition of an electric field magnitude threshold for electroporation; only the cells within areas where the electric field magnitude is larger than threshold electric field are electroporated. If a second threshold is reached or surpassed, the electroporation phenomenon will be too intense and cell homeostasis will be altered to the point of compromising cell viability which can finally result in cell death, either necrotic or apoptotic.



(a) Electroporation of a cell membrane



(b) Mechanism of cell inactivation

Fig. 2 Electroporation processing of a cell [3]

Fig. 2 shows the mechanism of cell inactivation. In general, an increase in the electric field intensity and number of pulses led to an increase in the inactivation of microorganisms. Other factors that influence microbial inactivation by PEFs are the treatment temperature, pH, ionic strength, and conductivity of the medium containing the microorganisms.

3. Description of the Laboratory-Scale Pulsed Electric Field System

The basic principle of the pulsed electric field (PEF) technology is the application of short pulses of high electric fields with duration of microseconds micro- to milliseconds and intensity in the order of 10 – 80 kV/cm. Fig. 3 shows the schematic diagram of the laboratory-scale pulsed electric field system was developed in this study. It consists of a high-voltage power source, an energy storage capacitor bank, a charging current limiting resistor, a switch to discharge energy from the capacitor across the food and a treatment chamber. Fig. 4 shows the laboratory-scale pulsed electric field system was developed in this study. High voltage pulses are supplied to the system via a high voltage power source at required electric field intensity, pulse waveform and pulse width. Generally, the high voltage power source was used to charge the high voltage capacitor bank and store the energy to the high voltage capacitor bank. The high-voltage power source, a high voltage DC generator, was converted the low voltage AC from an utility line of about 220 V into the high voltage AC of about 15 kV by the high voltage transformer (400 W) and was then full-wave rectified to a high voltage DC by the four 40 kV/2 A high voltage diodes. Fig. 5 shows the developed high voltage power supply. The energy from the high voltage power source was stored in the 0.25 μF/30 kV high voltage capacitor and was discharged by the discharge switch (spark gap) through the treatment chamber to generate a high electric field in the food material with exponentially decaying 1500 μs pulse. The maximum voltage across the capacitor is equal to the voltage across the power source, typically about 21 kVrms. The capacitance (F) of the energy storage capacitor is given by

$$C_o = \frac{\tau}{R} = \frac{\tau dA}{d} \tag{8}$$

where τ is the pulse duration, R is the resistance, σ is the conductivity of the food, d is the treatment chamber gap between electrodes and A is the area of the electrode surface. The energy stored in a capacitor is defined by the mathematical expression as

$$Q = \frac{1}{2} C_o V^2 \tag{9}$$

where Q is the stored energy, C_o is the capacitance of the treatment chamber, and V is the charge voltage. The pulse waveforms were monitored using a GwInstek GDS-1052-U oscilloscope, Fig. 6 shows commonly used pulse waveform. In this study, the parallel plate treatment chamber was used and consisted of 2 stainless-steel plate electrodes and an insulator made of Derlin. The distance between the plate electrodes was about 0.8 cm with the volume of the treatment

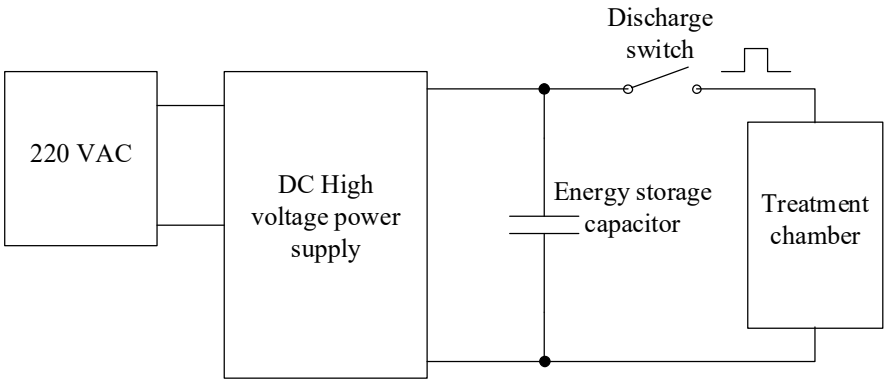


Fig. 3 Schematic diagram of the laboratory-scale pulsed electric field system.

Fig. 4 Developed laboratory-scale pulsed electric field system.

chamber was about 75 cm^3. Fig. 7 shows the treatment chambers. In the treatment chamber, the applied high voltage results in an electric field that causes microbial inactivation by the electroporation phenomena. Electric field strength inside the treatment chamber, E , can be calculated from Equation (1) , At voltage of 20 kV, the electric field strength inside the treatment chamber was about 25 kV/cm

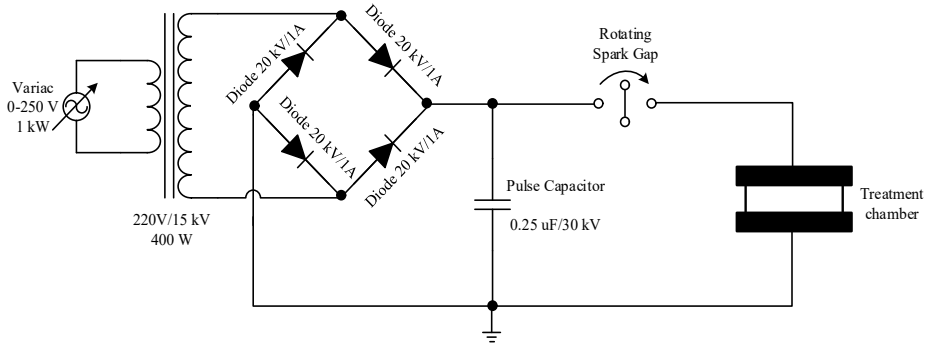


Fig. 5 High voltage power supply.

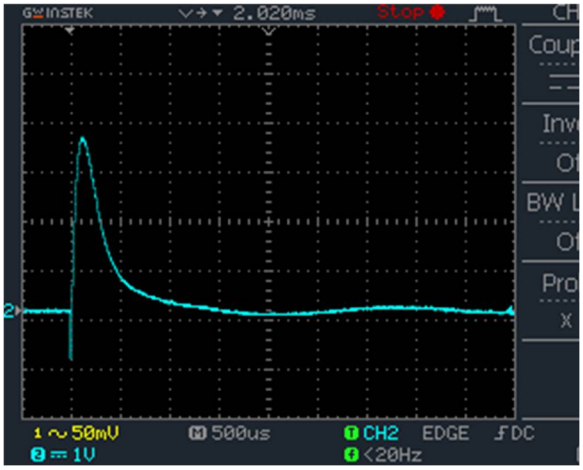


Fig. 6 Commonly used pulse wave shapes.

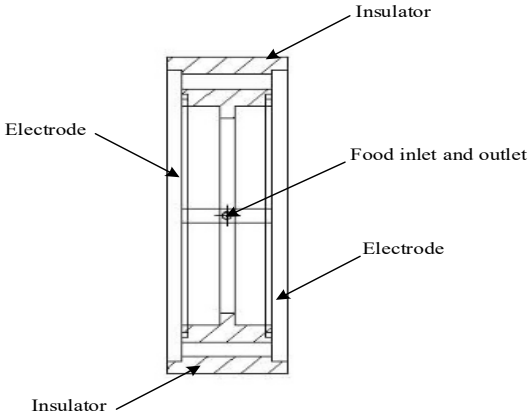


Fig. 7 treatment chambers

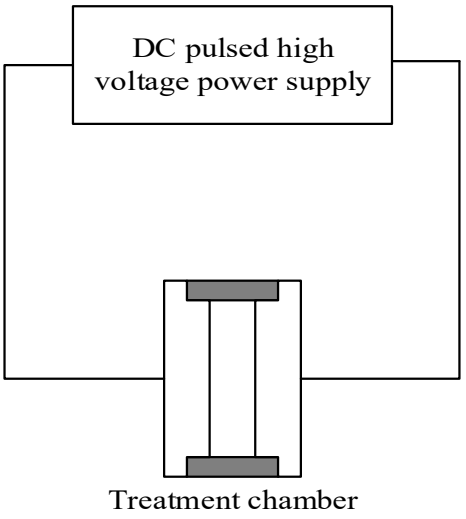
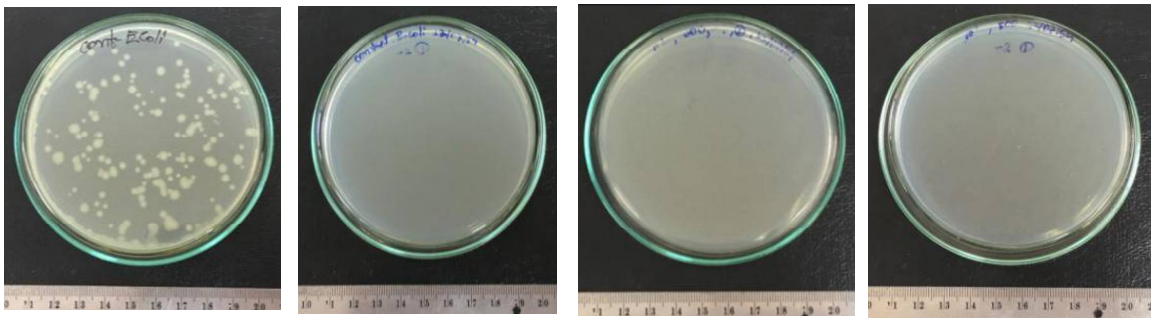


Fig. 8 Schematic diagram of the preliminary test.

3. Preliminary Test and Results

In this study, the developed system was preliminary tested with a fresh orange juice inoculated with *E. coli*. and was compared with thermal pasteurization. *E. coli* was used as the target microorganism. The fresh oranges were purchased at local supermarket. The oranges were squeezed with a juice extractor and the juice was filtered with cheese cloth and stored at 4° C prior to treatment. Based on preliminary test, 18 ml of each incubated culture was inoculated into



(a) (b) (c) (d)

Fig. 9 *E. coli.* on nutrient agar: (a) Control sample (b) Thermal pasteurization (c) PEF Treatment (15 kV, 500 pulses) and (d) PEF Treatment (20 kV, 500 pulses)

1,000 ml of oranges juice for a final concentration of approximately 10^6 CFU/ml and held at 37°C for 12 hr in order to acclimate cells prior to PEF treatment. For number of viable cells before and after PEF treatment, the total plate count method using nonselective growth medium (nutrient agar) was performed at 37°C for 24 hr to count the initial and surviving number of viable cells. Fig. 8 shows the schematic diagram of the preliminary test. The sample was treated with 500 pulses (exponentially decaying) at the difference peak pulse voltage of about 15 and 20 kV corresponding to peak pulse electric field of about 18. 75 and 25 kV/ cm, respectively. For conventional thermal pasteurization, a pasteurization temperature of about 72° C was tested for a holding time of 30 s Each PEF treatment was duplicated. Fig. 9 shows *E. coli.* on nutrient agar compared to control sample not exposed to PEF, thermal pasteurization and PEF treatment at the pulsed voltage of about 15 and 20 kV with 500 pulses. Both technologies reduced the population of the *E. coli.* inoculated in orange juice. No viable cells were observed after thermal processing of orange juice whereas PEF treatment achieved 6 logarithmic reductions of the microbial viability at the electric field strength up to 25 kV/cm and the pulse number of about 500 pulses. Generally, an increase in the electric field intensity and number of pulses led to an increase in the inactivation of microorganisms. However, other factors that influence microbial inactivation by PEFs are the treatment temperature, pH, ionic strength, and conductivity of the medium containing the microorganisms [2].

4. Conclusion

In this study, a laboratory- scale pulsed electric field system for inactivating microorganisms in an orange juice was developed and preliminary tested. The developed system consists of a high-voltage power source, an energy storage capacitor bank, a charging current limiting resistor, a switch to discharge energy from the capacitor across the food and a parallel-plate treatment chamber.

In this system, the parallel-plate treatment chamber was applied with the DC pulsed voltage in order to create the high pulsed electric field strength inside the chamber for inactivating a microorganisms in the liquid food inside the chamber by electroporation process. The high-voltage power source, a high voltage DC generator, was converted the low voltage AC from an utility line of about 220 V into the high voltage AC of about 15 kV by the high voltage transformer (400 W) and was then full-wave rectified to a high voltage DC by the four 40 kV/2 A high voltage diodes. The energy from the high voltage power source was stored in the 0.25 μF/30 kV high voltage capacitor and was discharged by the discharge switch (spark gap) through the treatment chamber to generate a high electric field in the food material with exponentially decaying 1500 μs pulse. The maximum voltage across the capacitor is equal to the voltage across the power source, typically about 21 kVrms In this study, the developed system was preliminary tested with an orange juice inoculated with *E.coli* for 500 pulses (exponentially decaying) at the difference pulse voltage of about 15 and 20 kV and compared with thermal pasteurization. Both technologies reduced the population of the *E. coli.* inoculated in orange juice. No viable cells were observed after thermal processing of orange juice whereas PEF treatment achieved 6 logarithmic reductions of the microbial viability at the electric field strength up to 25 kV/cm and the pulse number of about 500 pulses.

5. Acknowledgements

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