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INACTIVATION OF STAPHYLOCOCCUS AUREUS IN ORANGE JUICE BY PULSED ELECTRIC FIELD TECHNOLOGY

[3]PAPOL SARDYOUNG, [2]CHATCHAWAN KANTALA, [1]PANICH INTRA

[1]Research Unit of Applied Electric Field in Engineering (RUEE), College of Integrated Science and Technology, Rajamangala University of Technology Lanna, Chiang Mai 50220, Thailand
[2]Center of Excellence in Electromagnetic Energy Utilization in Engineering (CEEE), Department of Mechanical Engineering, Faculty of Engineering, Thammasat University, Pathum Thani 12121, Thailand
[3]Department Electrical Technology Industrial, Faculty Technology Industrial, Thepsatri Rajabhat University, Naraymarharaj road, Talachupchorn, Lopburi 15000, Thailand

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Abstract

Abstract: The inactivation of Staphylococcus aureus (S. aureus) in orange juice by pulsed electric field processing was experimentally investigated and compared with orange juice treated by thermal processing in this paper. The experiments were carried out by the total plate count method for the electric field strengths between 20 and 40 kV/cm and number of pulses between 10 and 50. In this study, the spectrophotometric colorimeter was used to evaluate the quality of orange juice. It was showed that both PEF and thermal treatments reduced the population of the S. aureus inoculated in orange juice. No viable cells were observed after thermal processing of orange juice whereas PEF treatment achieved 5 logarithmic cycle reductions of the microbial viability at the electric field strength up to 30 kV/cm and the pulse numbers of about 20 pulses. Finally, the CIE, DE, pH, Viscosity and TSS (oBrix) values of untreated orange juice (control) and orange juices treated by PEF and thermal processes was also not an important difference regarding quality.

Keywords: Pulsed electric field, Microorganisms, Pasteurization, Orange Juice.

*E-mail: [1]panich.intra@rmutl.ac.th, [2]chatchawan.kantala@gmail.com, [3]papolaj@gmail.com

1. INTRODUCTION

Orange juice is the dominant juice manufactured in the beverage processing industry worldwide. Conventional preservation methods such as thermal pasteurization ensures safety and extends the shelf life of orange juice, but it often leads to detrimental changes in the sensory qualities of the food product. High quality foods that are nutritious, with freshly prepared flavour, texture and color, with minimal or no chemical preservatives, and above all safe, were required by consumers [1-2]. Therefore, newly developed food technologies usually focus on preservation while keeping food quality attributes. A non-thermal pasteurization by pulsed electric field (PEF) processing has already been demonstrated. It can alternatively be applied to deliver safe and shelf-stable products such as juices, milk, yogurt, soups and liquid eggs with fresh-like character, high nutritional value and minimal or no chemical preservatives [3].

PEF pasteurization process applies micro-second (s) high voltage pulses, producing high electric field strength (> 20 kV/cm) between two electrodes. Pulses can be applied to food products at temperatures below thermal pasteurization and can inactivate contaminating microorganisms without significantly affecting the quality of the food product [3-4]. This process is known as electroporation phenomena and is effective in the inactivation of microorganisms [5-6]. In the past several decades, there have been numerous research studies and developments on the PEF processing system for microorganisms inactivation of the food juice products [5-8].

Generally, PEF processing system consists of a high-voltage power source, an energy storage capacitor bank, a charging resistor, a discharge switch, a pulse controller, and a treatment chamber. Electrical energy from the power supply was collected in the capacitor and is then discharged through the treatment chamber to generate the electric field strengths in the food product. The survival rate of number of microorganisms in the food product treated by PEF processing depends on process parameters including electric field strength, total treatment time, pulse width and pulse waveform, and conductivity of food [6]. It is known that electric field strength and number of pulses (treatment time) were the major factors determining microorganisms inactivation in PEF processing [9]. In this paper, we demonstrate the inactivation of endogenous microorganisms (*Staphylo- coccus aureus*) in orange juice was experimentally investigated.

Staphylococcus aureus is well known that a gram positive bacteria of chief concern as a food contaminant because of its ability to grow in a wide variety of foods and produce an exotoxin. The exotoxin induces gastroenteritis on the ingestion of food contaminated with *S. aureus*. Also, the quality of orange juice by pulsed electric field processing was compared with that of orange juice by thermally processing.

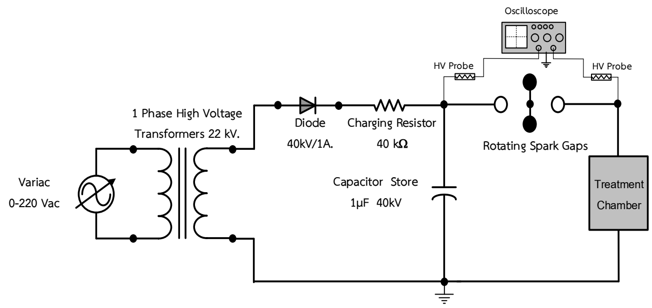


Fig.1. The PEF processing system developed by Panyamuangjai *et al.* [8]

2. MATERIALS AND METHODS

2.1. Mechanisms of Inactivation by PEF

Electroporation, or electro permeabilization, 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. Under the electroporation phenomena, a transmembrane potential, $U(t)$, is developed across the cell membrane in the direction of an applied electric field strength, E , is given as follows [3]

$$U(t) = 1.5rE \tag{1}$$

where r is the radius of the cell, and E is the applied electric field strength. When the trans-membrane potential reached around 1 V, The lysis of the cell resulting from loss of membrane integrity was occurred. This value was termed the breakdown transmembrane potential.

A mathematical model for the survival rate, s , ratio of number of microorganisms present in the food after treatment and initial number of microorganisms present before the treatment, as a function of electric field strength, E , and treatment time, t , is given by [4]

$$s = \left(\frac{t}{t_c}\right)^{(-(E-E_c)/k)} \tag{2}$$

where t is the treatment time, which is the product of number of pulses and pulse width, t_c is the critical treatment time, which is a threshold value above which inactivation occurs, E is the electric field strength, E_c is the critical electric field strength, which is a threshold value above which inactivation occurs (kV/cm), and k is the specific constant for a microorganism. Taking the logarithms to base 10 on both sides of Equation 2 gives us the following:

$$-\log(s) = \frac{(E-E_c)}{k}\log\left(\frac{t}{t_c}\right) \tag{3}$$

The left- hand side of the above equation is commonly referred to as the inactivation ratio or log reduction.

Table1: Ranges and values of variables investigated.

Variable	Range
Pulse width	10 s
Pulse frequency	1 Hz
Number of pulses	10, 20, 30, 40 and 50
Pulse voltage	10, 15 and 20 kV
Electric field strength	20, 30 and 40 kV/cm

2.2. Description of a PEF Processing System

The major components of the PEF pro- cessing system for microorganisms inactivation of orange juice developed in this study. It consists of an AC power input, a rectifier circuit, a DC high voltage power, an energy storage capacitor, a pulse controller to control number and frequency of pulse, a discharge switch to discharge energy from the energy storage capacitor across the food juice, and a treatment chamber. The schematic diagram of the PEF processing system developed by Panyamuangjai *et al.* [8] is shown in Fig. 1. A DC high voltage power in this study, step- up AC voltage from a utility line, 220 V, into 22 kV by the 22 kV, 33 kW high voltage power transformer, and then rectifies AC high voltage into DC high voltage of about 22 kV by a simple- half wave rectifier, a 40 kV, 1 A power diode was used. The energy from the DC high voltage power is stored in the energy storage capacitor, 1 °F/ 40 kV, via the charging resistor and is discharged through the treatment chamber by the discharge switch for generating an electric field strength of about 20 to 40 kV/ cm in the food material with monopolar exponential decaying pulse that causes microbial inactivation by the electroporation phenomena.

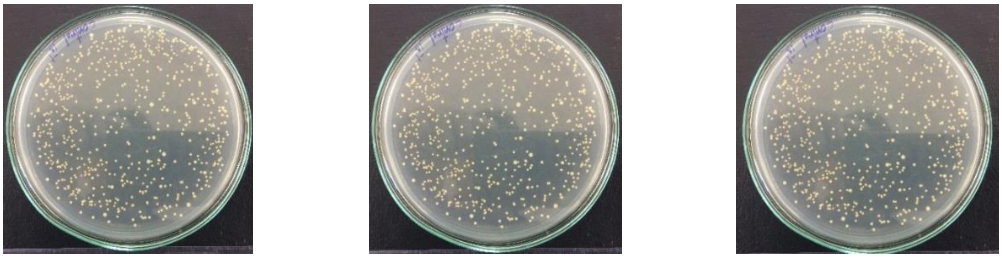
2.2. Experimental

The purpose of the microorganisms inactivation and quality of orange juice studies was to examine how many of microorganisms of orange juice could be inactivated by PEF and thermal treatments. For microorganisms inactivation study, a hight microorganisms load was required in orange juice. In this study, Staphylococcus aureus (S. aureus, TISTR 2329) were purchased from the Thailand Institute of Scientific and Technological Research (TISTR). The fresh oranges were purchased at local supermarket.

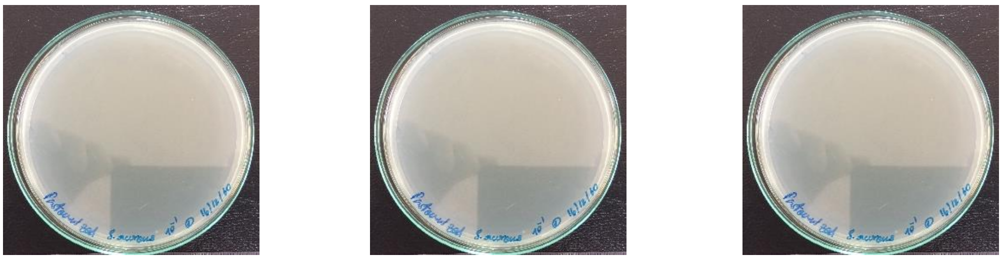
The oranges were orange honey with a screw-type juice extractor and the juice was filtered with cheese cloth and stored at 4 °C prior to treatment. Before PEF treatment, 18 mL of each incubated culture was inoculated into 1,000 mL of oranges juice for a final concentration of approximately 10^5 colony- forming units (CFU) / mL of microorganisms at 4 °C 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 in PEF treatment, thermal treatment and control orange juices. For PEF treatment, electric field strengths of about 20, 30 and 40 kV/ cm corresponding to the pulse voltage of about 10, 15 and 20 kV, respectively, and pulse numbers of 10, 20, 30, 40 and 50 pulses were used. Pulse duration time and frequency (number of pulses per s) were set at 10 s and 1 Hz. For thermal treatment, orange juice was held at 70 °C for 30 s and then was cooled with cold water from a cooler to 25 °C. Table 1 shows the ranges and values of variables investigated. In this study, Hunter Lab Scan Spectrophotometric colorimeter (HunterLab model ColorQuest XE, USA) controlled by a computer that calculated color ordinates from the reflectance spectrum was used to measure the CIE (Commission Internationale l'Eclairage) values, classifies color in three dimensions; L^*, brightness, a^*, red to green color and b^*, yellow to blue color. Wavelength range of this spectrophotometer was about 400 – 700 nm. Three replications of this experiment were performed.

3. RESULTS AND DISCUSSION

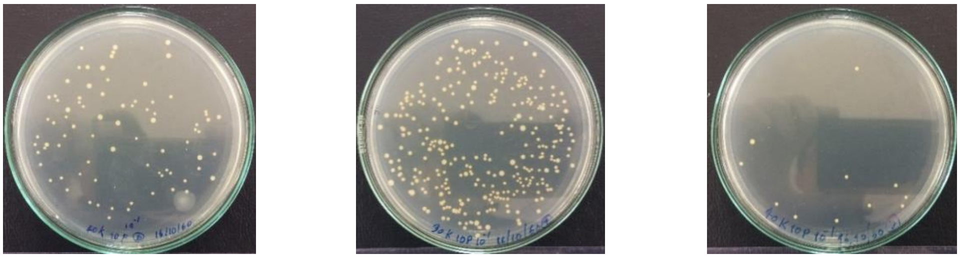
The experimental study of the micro-organisms inactivation by PEF treatment was carried out for the electric field strengths between 20 and 40 kV/cm and the pulse numbers between 10 and 50. Fig.2 shows *S. aureus* on nutrient agar after PEF treatment compared to control sample at electric field strengths of about 20, 30 and 40 kV/cm for pulse numbers of 10, 20, 30, 40 and 50 pulses, respectively. As shown in Fig. 5, an increase in the electric field strengths and the number of pulses rise to an increase in the inactivation of microorganisms. It was observed that no viable cells after PEF treatment of orange juice at the electric field strength up to about 30 kV/cm and the pulse numbers up to about 20 pulses.



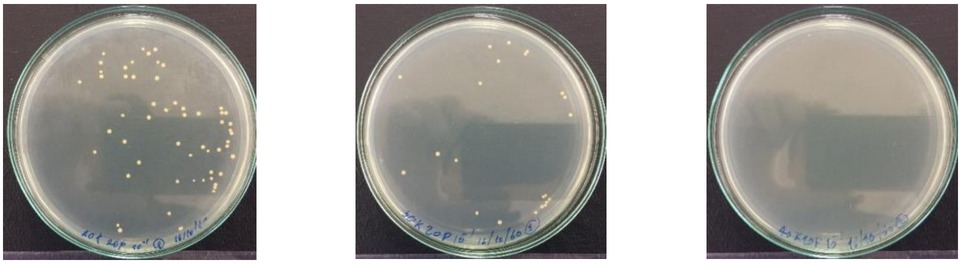
Control



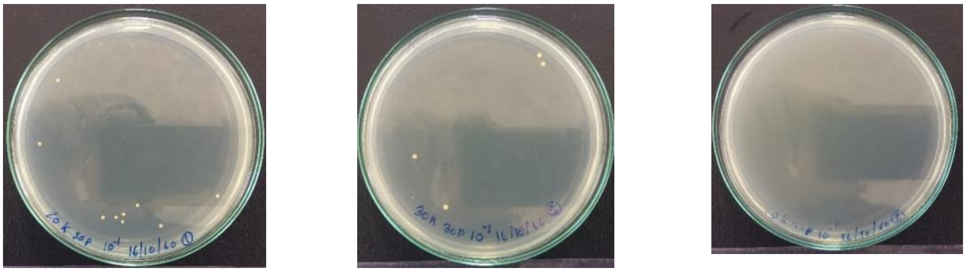
Thermal treatment



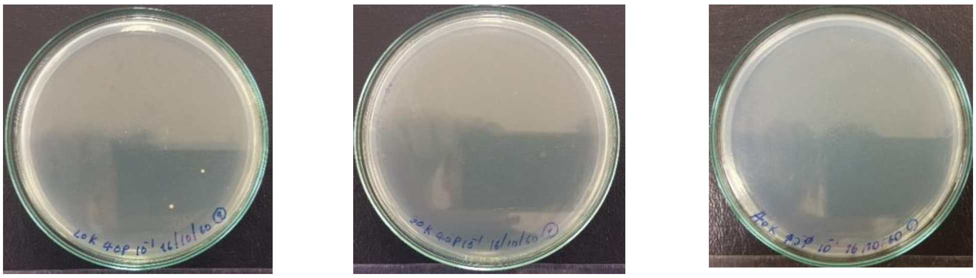
10 Pulses



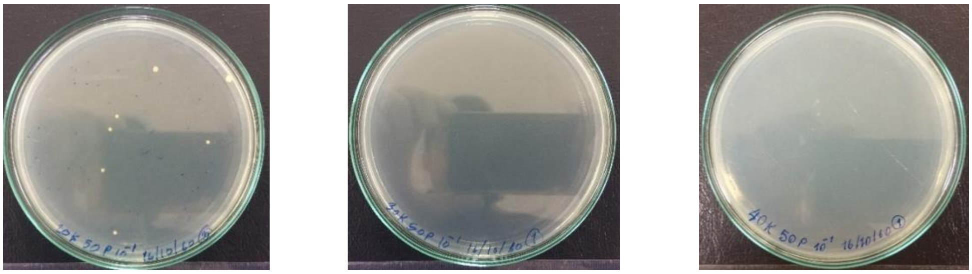
20 Pulses



30 Pulses



40 Pulses



50 Pulses

(a) 20 kV/cm (b) 30 kV/cm (c) 40 kV/cm

Fig.2. *S. aureus* on nutrient agar after thermal and PEF treatments compared to control sample at electric field strengths of about 20, 30 and 40 kV/cm for pulse numbers of 10, 20, 30, 40 and 50 pulses.

Table2: Variations in log cycle reduction of *S. aureus* with number of pulses at different electric field strengths.

Number of pulses	Log cycle reduction, CFU/mL		
	20 kV/cm	30 kV/cm	40 kV/cm
10	5.924	5.924	5.923
20	5.921	5.923	5.924
30	5.923	5.924	5.924
40	5.924	5.924	5.924
50	5.923	5.924	5.924

Table3: CIE, DE, pH, Viscosity and TSS values of orange juices treated by PEF and thermal.

		*L**	*a**	*b**	DE Value	pH	Viscosity (cP)	TSS (Brix)
Control		38.66	6.87	18.80	64.33	4.25	13.3	10
Thermal treatment		39.21	6.09	17.02	62.32	3.49	13.3	10
PEF treatment	20 kV/cm	38.67	7.06	16.28	62.01	3.45	13.3	10
	30 kV/cm	38.87	5.96	16.63	61.46	3.41	13.3	10
	40 kV/cm	38.90	6.92	16.14	61.96	3.44	13.3	10

Table 2 shows the variations in log cycle reduction with number of pulses at different electric field strengths. Higher electric field strengths were found to have higher log cycle reductions of microorganisms. At the same electric field strength, the log cycle reductions increased with increasing the number of pulses. At electric field strength of 20 kV/cm, the log cycle reductions of the micro-organisms were achieved about 3.7, 4.0, 4.1, 4.5 and 4.3 CFU/mL for number of pulses of 10, 20, 30, 40 and 50 pulses, respectively. At electric field strength of 30 kV/cm, the log cycle reductions of the microorganisms wereachieved about 4.9, 4.9, 5.0, 5.0 and 5.0 CFU/mL for number of pulses of 10, 20, 30, 40 and 50 pulses, respectively. At electric field strength of 40 kV/cm, the log cycle reductions of the microorganisms were achieved about 4.9, 5.0, 5.0, 5.0 and 5.0 CFU/mL for number of pulses of 10, 20, 30, 40 and 50 pulses, respectively. This study showed that the PEF treatment achieved 5 log cycle reductions of the microbial viability at the electric field strength larger than 30 kV/cm and the pulse numbers larger than 20 pulses.

Table 3 shows the CIE, DE, pH, Viscosity and TSS (°Brix) values of untreated orange juice (control) and orange juices treated by PEF and thermal processes. In the orange juice treated by PEF and thermal processes, *L** value is higher than the untreated orange juice, showing statistically significant differences. It was showed that *a**and *b** values decrease on the juice treated by PEF and thermal treatments with regard to *a** and *b** values of the untreated juice. There is a statistically significant decrease of the DE and pH values with regard to the DE and pH values of the untreated juice after PEF and thermal treatments, although it is not an important difference regarding quality. The viscosity and TSS values of the PEF and thermal treatments of orange juice do not present significant differences in the viscosity and TSS values in untreated juice. These results agree the ones described by Corts *et al.*[2] after applying PEF treatments for the orange juice.

CONCLUSIONS

Inthis study, the inactivation ofmicroorganisms (*S. aureus*) in orange juice was experimentally investigated by the total plate count method for the electric field strengths between 20 and 40 kV/ cm and the pulse numbers between 10 and 50,respectively. The quality of PEF processed orange juice and thermally processed orange juice was also compared. Spectrophotometric colorimeter was used to measure the CIE values in this study. Both PEF and thermal treatments reduced the population of the *S. Aureus*s inoculated in orange juice. No viable cells were observed after thermal processing of orange juice whereas PEF treatment achieved 5 logarithmic reductions of the microbial viability at the electric field strength up to 30 kV/ cm and the pulse numbers of about 20 pulses. Finally, an important difference regarding quality was not found in the CIE, DE, pH, Viscosity and TSS (°Brix) values of untreated orange juice (control) and orange juices treated by PEF and thermal processes.

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