P-ISSN 2586-9000
E-ISSN 2586-9027
Homepage : https://tci-thaijo.org/index.php/SciTechAsia
Science & Technology Asia
Vol. 27 No.4 October-December 2022
Page: [281-290]
Original research article

Design and Analysis of Pulsed Electric Field Processing for Microbial Inactivation (Case Study: Coconut Juice)

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Received 12 November 2021; Received in revised form 12 December 2021;
Accepted 26 December 2021; Available online 31 December 2022

ABSTRACT

This study aimed to design and analyze the pulsed electric field (PEF) system and examined the performance of the PEF system on microbial inactivation in coconut juice (CJ). The results showed that the PEF system can generate high voltage of the mono-polar exponential decaying at 0-22 kV and a pulse width of 10 µs. The CJ treated by the PEF at an electric field strength of 40 kV/cm and the number of pulses between 20-100 was compared with conventional thermal pasteurization (CTP) at 68.2 °C for 30 minutes and then cooled to < 7 °C. The CJ when PEF-treated generated a higher amount of vitamin C than when CTP-treated, with microbial inactivation by PEF and CTP <1 CFU/ml, and control 6.5 CFU/ml. The electric field strength and pulse number did not significantly affect the physicochemical (sucrose, glucose, fructose, sodium, potassium, magnesium, and calcium) and microbial inactivation (total plate count, *Yield*, and *Mold*) in CJ.

Keywords: Coconut juice; Microbial inactivation; Pasteurization; Pulsed electric field

1. Introduction

At present, the preservation of liquid or fruit juices uses a pasteurization process to inactivate the bacteria so as to prolong the shelf life as long as possible. In the pasteurization process, heat is used to inactivate microorganisms, ranging from 70-100 °C for 15-30 minutes [1]. The disadvantage of using heat to inactivate microorganisms is that vitamins or substances that are sensitive to heat are lost, along with taste, odour, and colour. Therefore, after the heat treatment, it is necessary to add vitamins and various

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nutrients to provide benefits to consumers, but this also results in increased costs. So, the process of inhibiting microorganisms without using heat, such as through high-pressure cold sterilization or a pulsed electric field, is another option for heat-sensitive liquid foods or juices with vitamins or nutritional value, also being able to preserve the flavour, smell, and colour, as nowadays, consumers are drinking more fruit juices such as orange juice, coconut water, apple juice, grape juice, fruit mixtures and others [2-6].

The PEF technology, with short duration, high voltage pulses and a high electric field, can be applied for food and beverages at temperatures below CTP and decrease the contaminant microorganisms without affecting the food's quality [7-11]. The process for eliminating microbes by PEF is the so-called electroporation phenomena [12]. The PEF processing procedure is composed of an energy storage capacitor bank, a high-voltage power source, a treatment chamber, a pulse controller, charging resistor, and a discharge switch [11-22]. In the past decade, many countries have been using commercial-scale PEF processing to inactivate the microorganisms in juices [23]. The commercial-scale PEF systems tend to be relatively numerous, but they are also expensive, with typical starting prices of more than 10,000 US dollars. In Thailand, commercial-scale PEF processing systems are not available in micro-, small- and medium-sized enterprises, and there is also a high cost for operation, such as capital costs [24]. In liquid foods processing, PEF is used for many applications such as food preservation and microbial inactivation. Microbial inactivation by PEF is applied in juice beverages including apple juice, mixed fruit juice and orange juice. Thailand had a total fruit juice export value of 572 million dollars to the United States, Europe, Japan, China and ASEAN.

Coconut water is rich in minerals and vitamins such as potassium, sodium, magnesium, and vitamins B1, B6, C, and sugar. Cold sterilization of Dalam Pangandaran, Genjah Salak and Hybrid PB121 coconut juice in Indonesia will be able to preserve different minerals and vitamins [25] using the high-pressure carbon dioxide process for coconut water pasteurization at a pressure of 120 bar and temperature of 40 °C, for a duration of 30 minutes, and can provide inactivation results up to 5 Log [26].

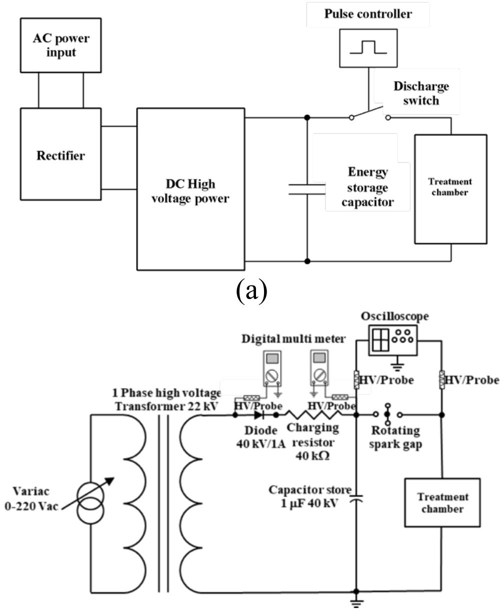
Currently, the PEF is mainly developed in foreign countries, resulting in high prices (millions of baht), and requiring import to Thailand for research on the inactivation of microorganisms in liquid foods. So, we designed and operated a PEF system using Thai materials to solve this problem.

This study aimed to operate the Thai-designed PEF system and investigate a PEF treatment including the electric field strength, treatment time and pulse number for inactivation of microbes in Thai fruit juice. The microbial inactivation (case study: CJ) quality was investigated and the CTP and PEF techniques were compared for food processing application. This study will demonstrate the novelty of the machine for microbial inactivation in Thailand, which may also be applied in other countries.

2. Materials and Methods

2.1. Designing the PEF system

The PEF processing system was adopted from [20]. We designed the PEF schematic diagram for microbial inactivation in CJ (Fig. 1). The PEF processing system includes a rectifier circuit, an alternating current (AC) power input, an energy storage capacitor, direct current (DC) high voltage power, the pulse frequency setting, and a treatment chamber with an electrode (stainless 316L) and insulator (Teflon). The PEF components and photograph are shown in Fig. 2b. A digital oscilloscope (TDS 210, Tektronix, USA) was applied to determine the pulse waveform. A digital multimeter (289 True-RMS, Fluke, USA) and a high-voltage probe (80K-40, Fluke, USA) were used to measure the input and output voltages. The treatment chamber was operated as shown in the schematic diagram (Fig. 2a) and was composed of two substantially parallel stainless-steel electrodes with a gap of 5 mm and a spacer (Teflon). The chamber volume was about 75 cm^3.

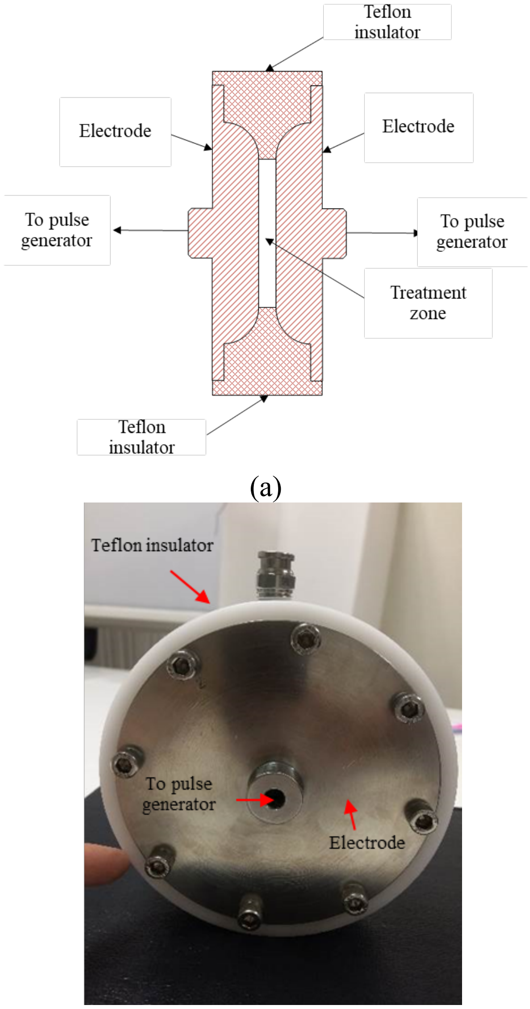


(b)

Fig. 1. The designed PEF system: (a) PEF operation, (b) schematic diagram.

2.2. Materials

The experimental materials contained fresh CJ (approximately 6-7 months), which had a conductivity of 5.736 S/m, obtained from local supermarkets. The specimen was stored at 4 °C before PEF and CTP were applied. All operations were conducted at an ambient room temperature of 26±2 °C.



(b)

Fig. 2. Treatment chamber for the study: (a) schematic diagram of chamber, (b) photograph of treatment chamber.

2.3. Overview of the processing and analysis of CJ

Fig. 3 presents an overview of the processing and analysis of the CJ samples and dissemination of paper, from brushing and drilling the coconut and bottling of the CJ. For CTP, the fresh CJ is then kept at 4-6 °C, pasteurized and tested at 68.2 °C for 30 minutes, then quenched with cold water from a cooler to <7.2 °C. Next, PEF treatment is conducted, after which the CJ's microbiological, physical, and High Performance Liquid Chromatography

(HPLC) properties are tested [26]. The parameters of the PEF and CTP are shown in Table 1.

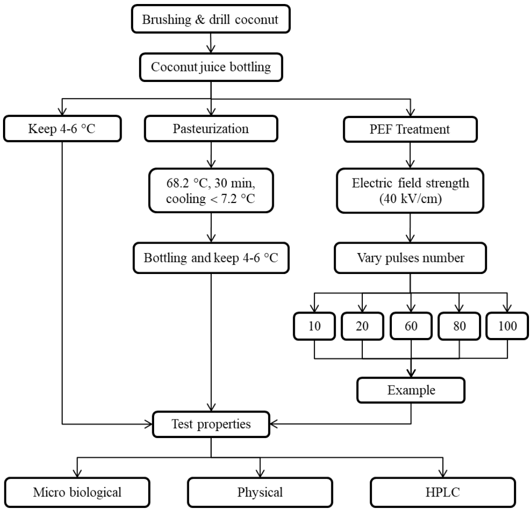


Fig. 3. Overview of the processing and analysis of CJ samples and dissemination of paper.

Table 1. Parameters of the PEF, CTP and control.

Parameters	PEF	CTP	Control
Electric field strength (kV/cm)	40	-	-
Pulse number (n)	20 - 100	-	-
Pulse width (µs)	10 µs	-	-
Pulse wave form	Exponential decay	-	-
Frequency (Hz)	1	-	-
Treatment time	20-100 sec	30 min	-
Temperature (°C)	Ambient temperature	68.2	Ambient temperature

2.4. Microbial inactivation analysis

CJ samples before and after PEF and CTP treatments were evaluated for the microbial inactivation according to the total plate count (FDA BAM online, 2001-chapter 3) and *Yeast* and *Molds* (FDA BAM online, 2001-chapter 18) by Central Laboratory (Thailand) Co., Ltd.

2.5. HPLC analysis

The HPLC was performed using a Shimadzu Prominence 20A diode array detector for the vitamin C conditions, mobile phase: 25 mM KH_2PO_4 in water +1mL H_3PO_4, flow rate: 1.0 mL/min, Shimpack GIST column: 6x150 mm 5 µm C18, column oven: 40 °C, PDA detector: 243 nm, injection volume: 5 µL, for the minerals, including lithium sodium potassium magnesium and calcium condition, mobile phase 3.5 mM H_2SO_4, flow rate 1.0 mL/min, Ionpac CS12A column: 4x250 mm, column oven: 40 °C, conductivity detector, and injection volume: 50 µL, for the sugars, including sucrose, glucose and fructose condition, mobile phase: water, flow rate: 0.6 mL/min, Aminex HPX-87N column: 7.8x300 mm, column oven: 60 °C, reflective index detector, and injection volume: 20 µL.

2.6. The CJ qualification analysis

The quality of the untreated and treated CJ was determined by a thermometer (51-2, Fluke, USA), pH meter (LAQUA twin pH 33, HORIBA, Japan), total soluble solids (TSS) hand refractometer (MASTER-20M, ATAGO, Japan) and viscometer (LVDV-E, Brookfield, Australia). The colours of the untreated and treated CJ were measured using the CIE (Commission Internationale de l'Eclairage) values, comprising brightness (L^*), red colour (a^*) and yellow colour (b^*) using a colour meter (A60-1011-610, ColorQuest XE, USA) [27].

3. Results and Discussion

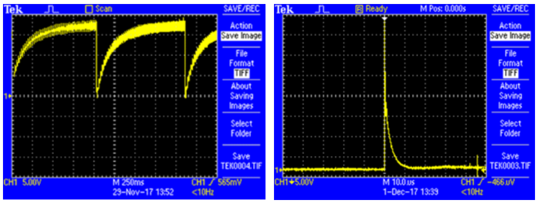
The research results indicate the inactivation of the microorganisms in CJ. These present the effect of the electric field strength and pulse number on inactivation (in terms of total plate count, *Yield*, and *Mold*) in the CJ, temperature, energy, pH, TSS, colour, vitamin C, and minerals before and after the treatments.

3.1. Evaluation of PEF

This study successfully designed and operated the PEF system. The PEF system was composed of a control system, treatment chamber room, spark gap, variac of 0–200 Vac, transformers with 1 phase high voltage of 22 kV, diode of 40 kV 1A, charging resistor of 40 kΩ, and pulse capacitor 1 µF of 40 kV. There are two elements containing the high-voltage pulse generator (Fig. 4) (with adjustable electric field voltage) and the treatment chamber room for the microbial inactivation treatment of the CJ studied.

Fig. 4. The PEF system for CJ treatment.

The typical pulse waveforms were measured, with charging and discharging (exponentials decay waveforms) at 1,000 ms and 10 µs, respectively (Fig. 5).



(a) (b)

Fig. 5. Typical pulse waveforms: (a) charging, (b) discharging.

The AC for the PEF operation comprising primary input and secondary output were 0-200 V and 0.11-20.12 kV, respectively. Moreover, the high voltage DC output was 0.06-23.25 kV, as shown in Table 2.

Table 2. Relationship of AC primary voltage, AC secondary voltage and DC output voltage.

AC primary voltage (V)	AC secondary voltage (kV)	DC output voltage (kV)
0.00	0.11	0.06
20.00	1.96	2.01
40.00	4.03	3.98
60.00	5.82	5.64
80.00	8.07	7.92
100.00	10.23	10.06
120.00	12.29	11.83
140.00	14.42	14.14
160.00	16.35	16.12
180.00	18.29	18.06
200.00	20.12	23.25

These results suggest that this Thai PEF design is a potentially useful technique and similar to the PEF produced in other countries. Another key variable in PEF treatment is temperature drift, which presents variations with the pulse number at different electric field strengths. An increase in the pulse number produced an increase in temperature (Table 3), compared to CTP at 68.2 °C.

Table 3 presents the variations in energy of the CTP and PEF treatments, showing that the energy of PEF is greater than the CTP treatment. The energy (Q) can be calculated by the following Eq. (3.1) [28], and depends on the capacitance of the storage capacitor, C, the initial charge voltage, *Vc*, the number of pulses, *n*, and the volume of the treatment chamber, *v*:

$$Q = \frac{1}{2v} C V_C^2 n. \qquad (3.1)$$

Table 3. Temperature drift and energy of PEF and CTP treatment.

Parameters	Pulse number	PEF	CTP
Temperature drift (°C)	20	8.2 ± 0.52	43.2 ± 0.42
	40	10.1 ± 0.71	
	60	12.3 ± 0.54	
	80	14.9 ± 0.96	
	100	18.6 ± 1.24	
Energy (kJ/L)	20	213.2 ± 5.65	160.0 ± 6.36
	40	426.4 ± 8.51	
	60	640.6 ± 7.12	
	80	853.1 ± 9.40	
	100	1066.3 ± 10.07	

However, the researchers tested the preliminary sterilization in CJ at an electric field strength of 40 kV/cm and found that for every pulse number PEF had more energy than CTP (PEF used a high electric field strength and long treatment times), so in the future the researchers will continue to experiment with reducing the electric field strength (20-30 kV/cm) and the pulse number to find the best parameters for inhibiting microorganisms in CJ. In fact, the pulse field should use less power than CTP, a result similar to a previous study [29].

3.2. Microbial inactivation in CJ

Table 4 shows the comparison of the CTP and PEF treatment inactivation in terms of the total plate count and *Yield* and *Mold* in CJ before and after treatment. It was found that before treatment the total plate count was 6.5 CFU/ml and *Yield* and *Mold* were <1 CFU/ml, while after treatment of the CJ, the total plate count and *Yield* and *Mold* were <1 CFU/ml, respectively. So, the electric field strength and pulse number in the PEF treatment can determine the inactivation, as shown by the total plate count and *Yield* and *Mold* in the CJ. Because of the heat applied in the CTP treatment, the *Yield* and *Mold* values showed the resulting destruction of microbes. In the PEF treatment, the *Yield* and *Mold* were inactivated by electroporation in the cell membrane, where the strength of the electric field applied to the cell membrane was more than the maximum electric field strength of the membrane, and resulted in the destruction or death of the microbes in the cell [12].

Table 4. Comparison of CTP and PEF treatment inactivation: total plate count and *Yield* and *Mold* in CJ.

Treatment	Electric field strength (kV/cm)	Pulse number	Total plate count (CFU/ml)	*Yield* and *Mold* (CFU/ml)
Control	-	-	6.5	<1
CTP	-	-	<1	<1
PEF	40	20	<1	<1
		40	<1	<1
		60	<1	<1
		80	<1	<1
		100	<1	<1

3.3. The nutrition of CJ

The nutritional content of the CJ was investigated before and after CTP and PEF treatments, in terms of various vitamins and minerals. After treatment by CTP, it was found that the vitamins and minerals had decreased. Particularly vitamin C had greatly decreased, being heat-sensitive. However, after PEF treatment, it was found that the increasing number of pulses resulted in the quality of the vitamins and minerals decreasing slightly because between PEF processing heating and treatment times was lower, as shown in Table 5. However, it was surprising to observe that, for sodium after PEF treatment, the increasing number of pulses resulted in a major decrease, by about 50% (with 100 pulses). This may be due to the electrochemical effect present. However, the lower sodium content in the CJ may be suitable for people who need to avoid sodium, such as those with kidney disease, heart disease, hypertension, etc.

Table 5. Nutritional content of CJ before and after CTP and PEF treatment.

Ingredients (ppm)	Control	CTP	PEF 40 kV/cm and pulse number				
			20	40	60	80	100
Vitamin C	8.8 ± 0.52	2.5 ± 0.13	6.9 ± 0.41	6.6 ± 0.33	6.6 ± 0.24	6.6 ± 0.41	6.5 ± 0.39
Sucrose x10^3	9.9 ± 0.83	10.2 ± 0.52	10.5 ± 0.65	9.9 ± 0.29	10.6 ± 0.74	10.4 ± 0.61	11.1 ± 0.47
Glucose x10^3	27.4 ± 0.18	25.7 ± 0.58	27.7 ± 1.05	26.3 ± 0.93	28.1 ± 0.85	27.3 ± 0.49	29.2 ± 0.71
Fructose x10^3	23.8 ± 1.22	21.6 ± 0.75	23.6 ± 0.81	22.5 ± 0.49	23.9 ± 0.57	23.6 ± 1.08	24.9 ± 0.92
Sodium	81.4 ± 2.72	68.4 ± 1.44	59.4 ± 2.25	75.4 ± 2.84	63.5 ± 2.47	51.2 ± 1.89	38.1 ± 1.31
Potassium x10^3	2.3 ± 0.12	2.3 ± 0.09	2.4 ± 0.22	2.3 ± 0.13	2.4 ± 0.21	2.4 ± 0.18	2.5 ± 0.32
Magnesium	73.4 ± 3.16	73.0 ± 2.94	72.6 ± 2.51	76.6 ± 2.82	81.2 ± 2.55	79.7 ± 2.48	74.1 ± 2.35
Calcium x10^2	2.3 ± 12.06	2.2 ± 13.42	2.5 ± 10.15	2.3 ± 11.17	2.6 ± 9.75	2.3 ± 15.11	2.5 ± 12.58

3.3. Effect of the PEF and CTP treatment on CIE, DE, pH, viscosity and TSS in CJ

Table 6 shows the variations in the properties of colour, DE value, pH, and TSS compared with the control, before and after CTP and PEF treatment of the CJ. It was found that the colour *L** *a** *b** and DE values are the sum of the *L** *a** and *b** values [30] and pH, TSS, although there is no significant difference [31].

Table 6. Variations in the properties of colour, DE value, pH and TSS in control and after CTP and PEF treatment.

Treatment	*L**	*a**	*b**	DE Value	pH	TSS (°Brix)
Control	35.53 ± 2.83	-0.72 ± 0.02	2.65 ± 0.21	37.46 ± 1.02	5.15 ± 0.51	7.00 ± 1.95
CTP	34.35 ± 2.45	-0.65 ± 0.05	2.53 ± 0.27	36.23 ± 0.92	5.18 ± 0.64	7.00 ± 1.57
PEF	36.22 ± 2.58	-0.75 ± 0.03	2.42 ± 0.25	37.89 ± 0.95	5.20 ± 0.24	7.00 ± 1.33

The effects of the PEF and CTP of CJ do not present significant differences, which may be due to the tested values being measured instantly after treatment. However, the researcher expects that after the treatments (CTP and PEF) the colour, pH and TSS should not change, So the shelf-life should be tested and the nutritional values checked for CJ in future work.

4. Conclusion

This study showed the design and operation of the PEF system for food processing. Both the PEF and CTP techniques can be applied for inactivation of microbes in CJ. The results confirmed that this PEF system had a potential similar to that shown in previous studies, with the inactivation of microorganisms in CJ, total plate count and *Yield* and *Mold* <1 CFU/ml. No important differences regarding the quality were found in the values of the CIE, DE, pH, viscosity, TSS (°Brix), all sugars and all minerals between the untreated CJ (control) and the CJ treated by PEF and CTP. The quality of vitamin C before and after PEF treatment showed a small decrease, but a noticeable decrease compared with CTP, and the microbial inactivation by the PEF treatment resulted from the electroporation more than the temperature. The researchers suggest that the design analysis of the PEF can effectively inactivate the microorganisms in CJ, and an electric field strength of 40 kV/cm and more than 20 pulses are the best conditions. As future work, there should be additional shelf-life tests on juices or liquid foods.

Acknowledgements

The authors gratefully acknowledge the Royal Golden Jubilee (RGJ) Ph.D. Program (PHD/0091/2561), Thailand Science Research and Innovation Fundamental Fund, the Program Management Unit for Human Resources & Institutional Development, Research and Innovation, NXPO and the National Science and Technology Development Agency (NSTDA) for financial support, research contract no. P-14-50757 that contributed financial support for this study.

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