

Pulsed Electric-field Processing of Orange Juice Containing *Paecilomyces variotii* Spores: Comparisons to Power Ultrasound and Thermal Treatments

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Abstract

Background and Objective: Presence of heat-resistant mold ascospores in fruit juices includes a significant concern for the food industry. Pulsed electric field and power ultrasound technology, as an innovative non-thermal food preservation technology, were used for the decrease of *Paecilomyces variotii* spores in orange juice.

Material and Methods: In the current study, pulsed electric-field method (65 kV cm^{-1}) was compared to ultrasound ($< 5 \text{ W ml}^{-1}$) and thermosonication (combined ultrasound and heat, $< 5 \text{ W ml}^{-1}$ - 75°C) methods for the inactivation of *Paecilomyces variotii* spores in orange juice. The major objectives were to investigate log reductions of *Paecilomyces variotii* spores and effects of juice soluble-solid contents under pulsed electric-field treatments and to compare pulsed electric-field followed by thermal processing and conventional thermal processing alone. Standard deviations and statistical analyses of the results were reported to highlight differences between the treatments. In addition, the log survival curves after pulsed electric field were modelled and the spore morphology was investigated.

Results and Conclusion: For a 30-min process (corresponding to a 288-s effective time), pulsed electric field was more effective in decreasing *Paecilomyces variotii* spores than that ultrasound and thermosonication were, showing three and up to one logs, respectively. The soluble solid content of orange juices affected the spore decreases after pulsed electric field, lesser in higher soluble solids (more than four logs in 10° Brix and equal or less than two logs in 30° Brix juices). The Weibull model could well demonstrate the log survival curves after pulsed electric-field treatments. Pulsed electric field followed by thermal process (144 s at 52, 72 and 92°C for 10 min) was the best method, compared to that pulsed electric field or thermal processing alone was, providing more than two-log reductions in *Paecilomyces variotii* spores. Scanning electron microscopy showed a severe damage to *Paecilomyces variotii* spores after using a combination of pulsed electric field and heat process. In general, pulsed electric field assisted thermal technology, included better prevention of juice spoilage and enhanced safety of orange juices that were contaminated with *Paecilomyces variotii* spores.

Conflict of interest: The authors declare no conflict of interest.

Article Information

Article history:

- Received 3 July 2024
- Revised 3 Aug 2024
- Accepted 23 Aug 2024

Keywords:

- Mold spores
- Pulsed electric field (PEF)
- PEF-assisted thermal
- Thermosonication
- Sugar protection

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How to cite this article

Evelyn E, Chairul C, Sriningsih S, Aulia Y. Pulsed Electric-field Processing of Orange Juice Containing *Paecilomyces variotii* Spores: Comparisons to Power Ultrasound and Thermal Treatments. *Appl Food Biotechnol*. 2024; 11 (1): e27. <http://dx.doi.org/10.22037/afb.v11i1.45741>

1. Introduction

Spoilage microorganisms in pasteurized fruit juices, pulps and concentrates pose a major economic risk and hence a great concern for the fruit juice processors globally. Most listed spoilage and high-level heat resistance bacteria in these beverages include *Alicyclobacillus acidoterrestris* [1]. Molds such as *Byssoschlamys nivea*, *B. fulva*, *Neosartorya fischeri*, *Talaromyces* spp. and *Eupenicillium* spp. are involved in quality and safety of these foods due to their production of mycotoxins [2]. *Paecilomyces* species is

another heat-resistant mold that can survive heat treatments due to its ability to produce ascospores [3]. According to Dijksterhuis et al. [4], *B. nivea* (also known as *P. niveus*) and *B. spectabilis* (also known as *P. variotii*) have frequently been associated with fruit juice spoilage [4]. The *P. variotii* and *P. lilacinus* are two prevalent molds that contribute to food and beverage spoilages. Moreover, *P. variotii* has shown a capability to grow at low oxygen concentrations under refrigeration conditions in pasteurized

fruit juices, canned fruits and non-carbonized sodas [2, 5]. Thus, little attentions have been dedicated to *Paecilomyces* spp. and their inactivation in foods and beverages. Isolation of *Paecilomyces* spp. in fruits, cereals, grains, meat products, nuts, oils, margarines, processed cheeses [5], Moroccan olives, olive cakes [6], couscous, rice [7], sauces, juices [8], seeds [9] and roasted coffees [10] has been reported. In addition, degradation in food preservatives such as sorbic, benzoic and propionic acids with shifts in the aroma of food offerings has been observed [4].

Several investigators have reported heat resistance indices of *P. variotii* spores. Pieckova and Samson [7] showed that the ascospores of *P. variotii* survived 100 °C for 0.5–1.5 min in juices, whereas Houbaken et al. [11] reported survive of this mold spores after 1 h of treatment at 85 °C. The *D*-value of 3.4 min for *P. variotii* in distilled water (DW) after thermal treatment at 85 °C and *D*-value of 3.5 s for *P. variotii* in deionized water after a combination of hydrogen peroxide (40%) (H₂O₂) and heat (60 °C) were registered [12]. Evelyn et al. [2023] reported Weibull model with tailings (indicating high resistance) for the inactivation of *P. variotii* ascospores using a combination of ultrasound and heat (75 °C) [13]. The thermotolerant nature of this species is associated with its pathogenicity such as hyalohyphomycosis, showing a variety of human infections and hence potential dangers such as cutaneous, endocarditis, endophthalmitis, peritonitis, pneumonia, osteomyelitis, pyelonephritis and sinusitis [3]. Although the relationship of pathogenicity to contaminated foods is still unclear.

Thermal processing is the most common technology used in industrial food production by decreasing harmful microorganisms. However, heating can affect nutritional characteristics and sensory appearance of the processed foods [14]. Pulsed electric field (PEF) technology is one of the creative, contemporary, non-thermal approaches for food preservation that is interested worldwide. Other technologies include high-pressure processing, ultrasound, supercritical carbon dioxide (CO₂) and irradiation, which can avoid significant deterioration to the food characteristics and extend the food shelf life [15, 16]. Less adverse effects of the technologies are due to their uses without or with little direct heats on the food materials, whereas the achievement of the shelf-life extension is due to the inactivation of commonly 5 or 6 logs of the pathogenic or spoilage microorganisms. Food preservation through PEF consists of using electric forces of generally 10–80 kV cm⁻¹ to the food materials for a few micro to milliseconds [17]. Variables such as the intensity of the electric field, pulse width and configuration, number of pulses and temperature of the PEF treatment with the product nature (pH, water activity and electrical conductivity) and the characteristics of microbes (type of microorganisms, species and varieties) determine how much microbial inactivation is achieved. The PEF

technology seems an appropriate pasteurization technique for liquid edibles such as fruit juices and dairy products [18]. Recent literatures have demonstrated its potentials for extending the shelf life of milks while maintaining their quality attributes [19, 20]. Other studies have shown that the PEF technology can preserve bioactive characteristics of the black-chokeberry juices [21].

Numerous researchers have assessed inactivation of disease-causing and deteriorating fungal spores (conidiospores and ascospores) using PEF; however, none was reported with the spoilage and pathogenic of significant importance of *P. variotii* and its associated ascospores. Reported fungal spores were *B. nivea* [22], *B. fulva* [23], *N. fischeri* [23], *Zygosaccharomyces bailii* [24], *Penicillium expansum* [25], *E. javanicum* [26], *Botrytis cinerea* [27] and *Saccharomyces cerevisiae* [28, 29], all in fruit juices and concentrates as well as beers. The simple (first-order and Weibull) kinetic models were only reported for *Z. bailii* spores [24] as well as the current authors' previous studies with *E. javanicum* spores [26]. The magnitude of inactivation is mostly affected by factors such as type of spores, electric field intensity, treatment time, suspending media and total soluble solids in juice. The mechanism of spore inactivation or decrease by PEF technology has been suggested for the bacterial spores, which is generally involves disruption of spore membranes, followed by interference with essential cellular functions and DNA damages, ultimately leading to spore death [30].

Developing effective preservation techniques to lessen risks of heat-resistant molds in fruit juices includes significant importance. Regarding the lack of data regarding the efficacy of PEF in combating spoilage and pathogenic *P. variotii*, as well as the kinetic model and mechanism of fungal spore inactivation by PEF, objectives of this study were (i) to compare and model PEF and ultrasound inactivation of *P. variotii* spores in orange juices; (ii) to investigate and model PEF resistance of *P. variotii* spores in orange juices with various soluble solid contents; (iii) to compare log reductions of *P. variotii* spores after sequential PEF and heat treatments (PEF-assisted thermal), PEF or thermal process alone; and (iv) to investigate morphology of spores after PEF-assisted thermal or PEF process alone using electron microscopy.

2. Materials and Methods

2.1. Microorganism

This study used *P. variotii* mold strain of InaCC F166 sourced from the Indonesian Culture Collection (InaCC). The fungal strain was grown on potato dextrose agar (Oxoid, UK) plates at 27 °C for 3 d following the suppliers' instructions. The *P. variotii* colonies showed a velvety and



tan to olive-brown color on the agar surfaces, which was previously documented in the literature [31].

2.2. Ascospore production and enumeration

Ascospores of *P. variotii* were achieved after a 30-d cultivation at 27 °C on PDA (Oxoid, UK) plates. Spores were collected from the agar surface by lightly scraping the mycelia floating in sterile DW using clean curved glass rods. Then, suspension was passed through sterilized glass wools to eliminate lingering fungal fragments. This suspension was originally prepared from the pellets acquired through spinning at 4,000× g and 4 °C for 15 min (DLAB Scientific, China) and three times of washing with sterile DW. Then, spore suspension was stored at 2 °C until use [32, 33]. Presence of viable colonies of *P. variotii* in juices before and after processing was accomplished through the use of surface-plating onto PDA (Oxoid, UK). Before plating, appropriate decimal dilutions were carried out using sterile 0.1% (w/v) peptone water (Difco, USA) followed by incubation at 27 °C for 3–5 d until visible colonies were formed. Calculation of the average colony count was carried out from two plates with a range of 20–100 colonies [32, 33]. Number of the ascospores was reported as colony-forming units per milliliter (CFU ml⁻¹) in the juice samples.

2.3. Orange juice preparation and inoculation

The PEF technology is seen as a promising technology for the pasteurization of orange juices [34] as well as possible contamination of *P. variotii* in this juices. Therefore, orange juice concentrates (pH 3.8 ±0.1) purchased from a local supermarket in aseptic containers were used as the suspending media for *P. variotii* spores. Depending on the designed experiments, concentrates were diluted to soluble solid concentrations of 30, 20 and 10° Brix. For PEF experiments, 10 ml of the spore suspension in 690 ml orange juice provided an almost 10 times more diluted inocula than that 0.3 ml of spore suspension in 2.7 ml of orange juice did in thermal treatments. A spore concentration of approximately ~10⁶ CFU ml⁻¹ before processing was used. For the two ultrasound treatments, 1.0 ml of *P. variotii* suspensions was added to 49 ml of orange juice to prepare an initial concentration of nearly 10⁵ CFU ml⁻¹.

2.4. Experimental design and statistical data analysis

In the first experiment, effectiveness of *P. variotii* spore decreases was investigated using PEF against ultrasound (US) and thermosonication (TS) (75 °C) processes for 30 min in 10° Brix orange juices. This treatment time corresponded to a maximum effective time of 288 s in PEF, achieved by multiplying a residence time of 30 min by the pulse width in the batch system [35]. The 30-min time was selected to show marked differences between the treatments. Three survival experiments were carried out for each treatment

followed by calculating and plotting the *P. variotii* spore logarithmic reduction ($\log N/N_0$); where, N_0 and N were the microbial count before and after processing, respectively. Results from the first experiment showed various inactivations between the PEF and ultrasonication. Therefore, in the second set of experiments, PEF treatments of orange juice were carried out at various soluble solid contents. At least two survival experiments and duplicate samples were used and the logarithmic number of survivors ($\log N/N_0$) against time was plotted for each soluble solid. Survival curves in the first and the second experiments were used to model and estimate inactivation parameters. The model parameters corresponded to the means ±SD (standard deviation) of the results. In the other survival experiments, PEF-assisted thermal and thermal treatments of the orange juices were carried out at similar temperatures (three times) to compare results from the PEF treatments alone. Equipment and procedures used for PEF, including ultrasound and thermal treatments, were described in detail in other sections. Significant differences in the microbial log reductions or kinetic parameters in treatments/soluble solids were investigated by carrying out one-way analysis of variance (ANOVA) followed by the Tukey's test with a confidence level of 95% ($p < 0.05$) (Systat software v.13, Statsoft, USA). Random residuals, mean square error (MSE) and coefficient of determination (R^2) were used to compare the performance of the various models. A relatively small MSE and R^2 values close to 1 indicated adequacy of the model to describe the survival data [32, 33].

2.4.1 Pulsed electric field and pulsed electric field-assisted thermal processing

Domestically-produced and laboratory-sized PEF system with a layout format by previous studies was used to carry out a series of PEF assays [36]. System was equipped with a cooling system in the outlet. Setup consisted of a high-voltage pulse generator and a treatment enclosure containing 700 ml of the corresponding fruit juice. High-voltage square pulse generator produced an electric field intensity of up to 65 kV cm⁻¹ (with electrodes 1 cm apart) with a width of 160 μs and frequency of 250 Hz, which was the maximum intensity for this equipment. Moreover, PEF with electric field intensities of 30–65 kV cm⁻¹ was used to decrease fungal spores in fruit juices. Before each assessment, the PEF chamber was meticulously cleaned and sterilized with diluted Vircon solution (further washed with sterilized DW). This was repeated after the experiment was carried out. Juice samples at an inlet temperature of 25 °C with various Brix levels were exposed to PEF treatment for 288 s. An intense PEF treatment, such as high voltages and long times, might be necessary to ensure substantial microbial inactivation for food safety and stability [37]. Then, outcomes of spore decreases were analyzed to report



the best model for explaining the inactivation, as discussed in another section. Regarding PEF-assisted thermal processing, PEF treatments were carried out for 144 s followed by heating at 52, 72 and 92 °C for 10 min. This PEF time was selected because it showed satisfactory initial and constant decreases of spores. Further samples of the juice were used for microbial counting using a method previously described.

2.4.2 Ultrasound processing and thermosonication

The UCD-250 disrupter ultrasonic cell processor (20–25 Hz, 250 W; Biobase Biodustry, China) with a 3-mm sonotrode tip and an amplitude of 70% was used for all US and combinations of thermal experiments or TS assessments. This condition (the maximum energy allowed for the equipment) was selected due to high resistant of the fungal ascospores. Ultrasonication procedures for US and TS were generally followed based on the previous studies [32, 33]. Power density of the ultrasound equipment was 5 W ml⁻¹; however, this value could be nearly 50% less than the actual value (< 5 W ml⁻¹), as explained in previous reports [16]. In each process, a 50-ml sample of orange juice was transferred into an Erlenmeyer flask. Then, a sterilized sonotrode was immersed in the orange juice at a depth of approximately 1 cm from the bottom of the flask. Sterilization was carried out using glutaraldehyde (2.4% v/v) followed by rinsing off the residues using sterile DW. Furthermore, US and TS processed samples were removed from the flask at specific intervals (5, 10, 20, 30 and 40 min) for enumeration. A temperature of 75 °C was used for the TS experiments (the maximum temperature for the equipment and similar temperatures for conditions in PEF and thermal processes), whereas US processes were carried out at room temperature (without heat). Then, samples were used to count the rest of spores, as explained in Section 2.2.

2.4.3 Thermal processing

Thermal resistance of *P. variotii* ascospores was assessed at 52, 72 and 92 °C for 10 min to compare the results from PEF and ultrasound treatments, as well as comparing them to those of previous studies. First, thermostatic water bath was heated until the treatment temperature was achieved. Orange juices containing spore samples within plastic pouches were submerged into the preheated thermostatic water bath and incubated for various times. Treated samples were removed at various times and stored in ice water shower until the microbial count.

2.5. Kinetic modeling after pulsed electric-field and ultrasound treatments

Weibull equation expressed in decimal logarithmic form was used to investigate the log survivors of *P. variotii* ascospores once they were subjected to PEF, US and TS processes (Eq. 1). This model was commonly used to

describe the log inactivation under non-thermal methods [15, 16].

$$\log \frac{N}{N_0} = -bt^n \quad \text{Eq. 1}$$

Two parameters from this model included *b* (the rate parameter that affected the speed; at which, the microorganism was inactivated) and *n* (the shape factor, which denoted a concavity with *n* > 1 or a tail with *n* < 1). Conventional first-order kinetic model was used to compare the Weibull models. *D_T*-values in this model included time in minutes at a particular temperature necessary to cause a one-log reduction in the number of microbes from 10ⁿ to 10ⁿ⁻¹ in the microbial concentration in foods. The logarithmic *D*-values could be present about the lethal temperature (or soluble solid content, *D_{SS}*); where, inverse of the incline matched the *z_{SS}*-value (°Brix) (Eq. 2) [26, 37].

$$\log \frac{D_{SS}}{D_{ref}} = \frac{SS_{ref} - SS}{z_{SS}} \quad \text{Eq. 2}$$

Where, *D_{ref}* was *D*-value at the reference soluble solid *SS_{ref}* and *z_{SS}* was the decrease of soluble solids that decreased *D_{SS}* by a factor of 10.

2.6. Scanning electron microscopy

In general, PEF (65 kV cm⁻¹, 144 s) and PEF-assisted thermal (65 kV cm⁻¹, 92°C, for 10 min) treated ascospores in orange juices were selected and transferred to an external certified laboratory to assess effects of these treatments on the spore physical characteristics using scanning electron microscope (SEM). The two technologies demonstrated the best results for spore decreases within the other treatments. Spores were filtered from the liquids before drying using 0.45-µm filter papers and then delivered to the laboratory.

3. Results and Discussion

3.1 Effects of pulsed electric field, ultrasound and thermosonication on *Paecilomyces variotii* spores

Figure 1 compares the 65 kV/cm PEF and < 5 W ml⁻¹-20 kHz ultrasound (US and TS) processes of 10° Brix orange juices containing *P. variotii* spores for up to 45 min. Time in PEF represented the residence time during these treatments. Much higher log reductions were achieved with 65 kV cm⁻¹-PEF than the US alone, indicating the benefit of PEF technology. For example, a spore decrease of 3.0 log was registered after 30 min of PEF treatment (corresponding to an effective treatment time of 288 s), while only a small effect on the spores was observed such as a 0.4-log reduction after 30 min of US process (*p* < 0.05). Increasing lethality of the ultrasound treatment using simultaneous ultrasound and thermal or TS at 75 °C only increased the reduction up to 1.0 log. Other studies compared PEF to the US and reported similar results such as 1.2-log reductions after 32.3 kV cm⁻¹-340 µs-PEF against 4.3-log reductions after high-power ultrasound (1.5 W ml⁻¹-24 kHz, 40 °C, 3 min) for *Aspergillus niger* spores in oil-water emulsions



[36]. Comparing to this study, various results of spore decreases in the highlighted study (lower for PEF and higher for US) might be due to various factors such as various strengths of the PEF and US, fungal species, suspended media and microbial inactivation mechanisms of each treatment.

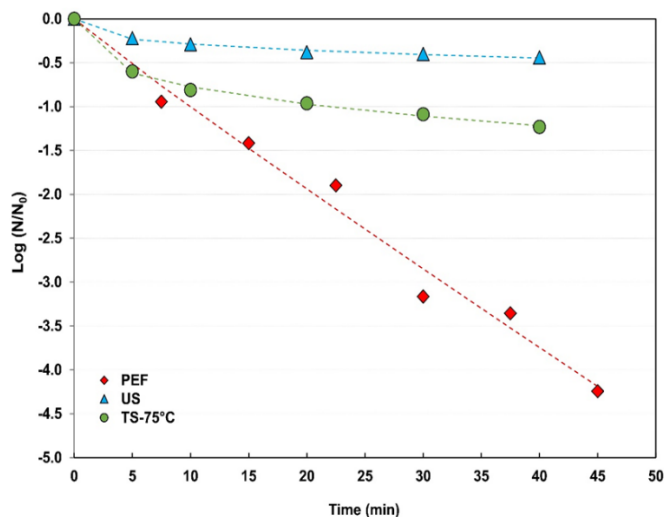


Figure 1. Weibull model fitted to the log reduction of *Paecilomyces variotii* spores using 65 kV cm⁻¹-pulsed electric field, ultrasound and thermosonication (75 °C) in 10° Brix orange juices. Time in pulsed electric field is the residence time of juice in the treatment chamber.

In previous studies, comparisons between the PEF, US and TS processes for the inactivation of microbial spores were limited. Regarding ultrasound, TS (24 kHz, 0.33 W ml⁻¹) treatments for 40 min at 75 °C resulted in nearly 3.5 log for *N. fischeri* spores in apple juices while a 2.5 log was achieved for *B. nivea* spores in strawberry purees following spore activation after similar lethal treatments [32, 33]. Much higher log reductions (> 5 log) were seen for *A. flavus* spores in Sabouraud broth after 20 min of 20 kHz-120 μm-TS at 60 °C [39]. The *S. cerevisiae* yeast ascospores in 4.8% (alcohol/volume) beers exhibited 2.7 log reductions after 16.2 W ml⁻¹-70 °C [395]. All these results verified effects of the highlighted factors. Comparison of log reductions under PEF treatments was explained in further details in other sections.

Table 1 shows Weibull parameters achieved after fitting the survival curves of *P. variotii* spores post PEF and ultrasound uses. Weibull model is a straightforward simple model with two elements (*b* and *n*); in which, *b* stands for the spore inactivation rate and *n* signifies the variance from linearity [15, 16]. This has been used to model numerous thermal and non-thermal food processes. Although the PEF process showed a lower *b*-value (0.11 ± 0.04) than that the TS process did at 75 °C (0.37 ± 0.05) (*p* < 0.05), *n*-value of the PEF method was close to 1.0, indicating a close rate to linearity in contrast to survival curves with concave-

upwards or tailings [15, 16]. These results verified the benefit of PEF technology.

Electric field strengths of 33–100 kV cm⁻¹ and frequencies of 2–466 Hz, either alone or in combination with various temperatures (56–123 °C), have been used to decrease fungal [23, 24, 26] and bacterial [41–44] spores with logarithmic reductions reported from 2.5 to 5.9 logs. Generally, PEF exposure resulted in less spore decreases in bacterial spores than in fungal spores. As previously explained, the mechanism of spore inactivation by PEF has been suggested based on the membrane rupture caused by high-voltage electric pulses that create permanent pores in the cell membranes (irreversible electroporation), leading to the loss of vital cellular contents and ultimately causing cell death [30].

Table 1. Weibull parameters for the decreases of *Paecilomyces variotii* spores using pulsed electric field, ultrasound and thermosonication*

Processes	Weibull	
	<i>b</i> ± SD	<i>n</i> ± SD
PEF (without heat)	0.11 ± 0.04 ^b	0.95 ± 0.17 ^a
US (without heat)	0.14 ± 0.03 ^b	0.32 ± 0.06 ^b
TS (75 °C)	0.37 ± 0.05 ^a	0.33 ± 0.04 ^b

**b* and *n* are the Weibull scale and shape factors, respectively (Eq. 1). The Weibull model presents lower MSE values (0.001–0.063) and a high R² (0.99). Letters (a-b) that differ in a column suggest significant variations (*p* < 0.05).

3.2 Effects of the soluble solid contents on pulsed electric-field decreases of *Paecilomyces variotii* spores

Figure 2 illustrates the log survivors (fitted by the Weibull model) of *P. variotii* spores in 10, 20 and 30° Brix orange juices after 65 kV cm⁻¹-PEF processes for up to 288 s. As can be seen from this figure, increased treatment time generally led to decreases in the spore at all soluble solid contents. The log reduction of *P. variotii* after 288 s-PEF treatments was 4.2 log when spores were suspended in 10° Brix juices. Sensitivity of the mold spores (ascospores and conidiospores) and conidia to PEF treatments leading to inactivation has been reported by several researchers. Spore-producer molds included *B. fulva* [23], *Z. bailii* [24], *E. javanicum* [26] and *S. cerevisiae* [28, 29]; although this behavior was less observed with *N. fischeri* [23]. In other studies, several researchers reported complete inhibition of germination-tube elongation of *P. expansum* and *B. cinerea* spores after increasing the electric field strength without reporting the logarithmic of spore decreases [25, 27].

In a previous study by the current authors, nearly 4.0 logs were achieved for *E. javanicum* ascospores in 10° Brix pineapple juices with similar conditions [26], indicating similarity in the resistance of the two mold spores to the PEF treatments. Other researchers achieved ≈4 log reduction of other mold spores in unadjusted orange juices using milder processing conditions such as 30 kV cm⁻¹-2 pulses



(corresponding to 4 μ s, calculated from the number of pulses and pulse width) with *Z. bailii* ascospores in orange juices and 30 kV cm⁻¹-11 pulses (corresponding to 24.2 μ s, calculated from the number of pulses and pulse width) with *B. fulva* conidiospores in pineapple juices [23, 24], suggesting that the two fungal pathogens of *P. variotii* and *E. javanicum* might include higher resistances to PEF, compared to those *Z. bailii* and *B. fulva* spores.

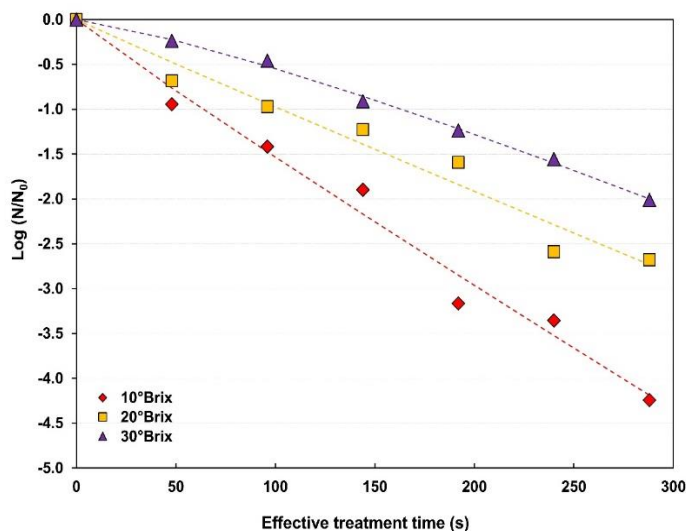


Figure 2. The 65 kV cm⁻¹-pulsed electric-field inactivation of *Paecilomyces variotii* spores (fitted by Weibull model) in 10, 20 and 30° Brix orange juices.

In a previous study [23], less than 1.0 log of *N. fischeri* ascospores was observed in fruit juices when subjected to 40 kV cm⁻¹-40 pulses-PEF (corresponding to maximum 132 μ s, calculated from the number of pulses and pulse width). Similarly, other authors reported a 1.2 log reduction of *A. niger* spores in oil-water emulsions after 32.3 kV cm⁻¹-340 μ s-PEF [36]. Milani et al. [28] achieved a 2.2 log reduction in *S. cerevisiae* ascospores in beers after an exposure of 46 kV cm⁻¹-70 μ s, while another study achieved 2.6-log decreases of *S. cerevisiae* ascospores in orange juices after 50 kV cm⁻¹ accompanied by 50 °C for 2.6 μ s [29]. In conclusion, fungal spore inactivation by PEF depends on the

species, food media, PEF intensity and temperature. The PEF with electric field strength equal or greater than 65 kV cm⁻¹ or in combination with temperature greater than 50 °C should be used to inactivate fungal ascospores to achieve recommended decreases in fruit juices ($\approx$ 5 log).

The 288 s-PEF treatments resulted in 4.2, 2.7 and 2.0 log reductions in the *P. variotii* ascospores when the spores were suspended in 10, 20 and 30° Brix orange juices, respectively ($p < 0.05$). In previous research, the authors reported effects of soluble solids in the processing of pineapple juices containing *E. javanicum* ascospores (4.0 logs at 10° Brix, 3.1 logs at 20° Brix and 1.3 logs at 30° Brix) under similar conditions. Eshtiaghi and Nakthong [45] showed increased resistances of yeasts during PEF treatment when sugar concentration increased from 20 to 50% in apple juices. In addition to previous studies on *E. javanicum* spores by the current authors, this finding (protective effects of soluble solids on the PEF resistance of fungal spores) should add further data to the current literature. However, associated studies used thermal or non-thermal treatments and bacterial and mold spores in fruit juices as well as other non-liquid food items [46-48].

Weibull model compared to the first-order kinetic model was used to investigate effects of sucrose level on the inactivation rate. Based on the MSE and R^2 -values such as $0.043 \leq \text{MSE} \leq 0.218$, $0.96 \leq R^2 \leq 0.99$, Weibull model provides better performance indices than those the linear model does. As seen in Table 2, differences in sucrose level by 20° Brix affected b -values such as 0.066–0.113 at 10-20° Brix against 0.019 at 30° Brix corresponding to D_{ss} -value of 23.8 min $\pm$ 0.97 ($p < 0.05$). A previous study with *E. javanicum* showed decreases in b -values from 0.673 at 30° Brix to 0.01 at 10° Brix juices. As previously stated, limited information were available on the kinetic inactivation of fungal spore after PEF treatments. Various investigations into the microbial cells and spore inactivation by PEF seem to manifest concavity trends in the survival curves or use a non-linear model such as Weibull to explain their inactivation [23, 49, 50]. The n -values describe nonlinearity or concavity of the survival curves.

Table 2. Weibull and first-order model parameters for 65 kV cm⁻¹ pulsed electric-field decreases of *Paecilomyces variotii* spore in orange juices*

SS (°Brix)	First order		Weibull	
	D_{ss} -value $\pm$ SD (s)	z_{ss} -value $\pm$ SE (°Brix)	$b \pm$ SD	$n \pm$ SD
10	10.6 $\pm$ 2.23 ^c	56.8 $\pm$ 0.01 $R^2 = 0.99$	0.113 $\pm$ 0.04 ^b	0.95 $\pm$ 0.17 ^b
20	16.4 $\pm$ 2.18 ^b		0.066 $\pm$ 0.03 ^b	0.98 $\pm$ 0.09 ^b
30	23.8 $\pm$ 0.97 ^a		0.019 $\pm$ 0.004 ^a	1.23 $\pm$ 0.04 ^a

* b and n are the Weibull scale and shape factors, respectively (Eq. 1); D_{ss} is the decimal reduction value that depends on soluble solids content; the Weibull model was more successful than the first-order model (Eq. 2), exhibiting lower MSE values (0.043–0.218) and higher R^2 (0.96–0.99). Letters (a-c) that differ in a column suggest significant variations ($p < 0.05$).



From Table 2, these values vary from 0.95 ± 0.17 to 1.23 ± 0.04 , showing ascending (n less than 1) and descending (n greater than 1) tendencies of the PEF survival curves (Fig. 2). Generally, an upward-curving type demonstrates a combination of the resistances of spore populations to lethal treatments such as kills, whereas a downward-curving type reveals spore resistance to the sublethal treatment such as a number of survivors being physiologically injured [51]. Fruit juices available in the market usually include 10 and 30° Brix; thus, a 65 kV cm^{-1} PEF treatment for 288 s could decrease 2.0 and 4.2 logs of *P. variotii* ascospores in 30 and 10° Brix juices, respectively.

3.3 Pulsed electric-field assisted thermal processing of *Paecilomyces variotii* spores in orange juices

Comparison of PEF-assisted thermal, PEF and heat alone on *P. variotii* spore survivors were investigated (Fig. 3). Thermal processes were carried out at 52, 72 and 92 °C. Results included proofs of disparities in the log reductions achieved after these processes with PEF and 92 °C and generally showed the highest difference to the PEF or heat alone. For the spores suspended in 10° Brix juices, treatments resulted in 1.42 logs for PEF against 3.28 logs for PEF and 52 °C, 3.74 logs for PEF and 72 °C and 4.13 logs for PEF and 92 °C ($p < 0.05$). Although log reductions were lower for 30° Brix juices due to the protective effects of sugar content, differences in the log reductions between PEF and PEF-thermal were observed (0.48 log for PEF against 1.34 logs for PEF and 52 °C, 1.70 logs for PEF and

72 °C and 2.0 logs for PEF and 92 °C ($p < 0.05$). Thermal processing of spores suspended in 10° Brix juices produced 0.72, 1.0 and 1.52 logs at 52, 72 and 92 °C, respectively ($p < 0.05$). In addition, heating the 30° Brix juice inoculated with the spores provided 0.29–0.37 log at 52–72 °C against 0.57 log at 92 °C ($p < 0.05$). Therefore, results showed that the PEF-assisted thermal treatments provided up to 1.3 logs more inactivation in the orange juices, compared to when the individual methods (PEF and thermal) were used in combination, indicating synergistic effects. The PEF-assisted thermal process showed more spore decreases (up to 2.74 logs) than those the thermal process alone did, suggesting that the PEF-assisted thermal process was the best method for spore inactivation, compared to PEF or thermal process alone. To the best of the authors' knowledge, no studies have reported the PEF-assisted thermal treatments of fungal spores. However, other investigators revealed that 50 kV cm^{-1} -PEF treatments with an outlet temperature of 50 °C for 2.6 μs resulted in 2.5-log decreases in the ascospores of *S. cerevisiae* in orange juices [29]. This was lower than the log reduction achieved after 50 kV cm^{-1} -PEF at 52 °C in the present study (3.28 logs). No other information are available on the PEF assisted thermal treatments on fungal spores. However, these results were similar to those by previous studies with bacterial spores [52], showing additional inactivation of up to 3.3 logs for *Geobacillus stearothermophilus* spores in skim milks and *Bacillus subtilis* in Ringer's solutions after PEF-assisted thermal treatments.

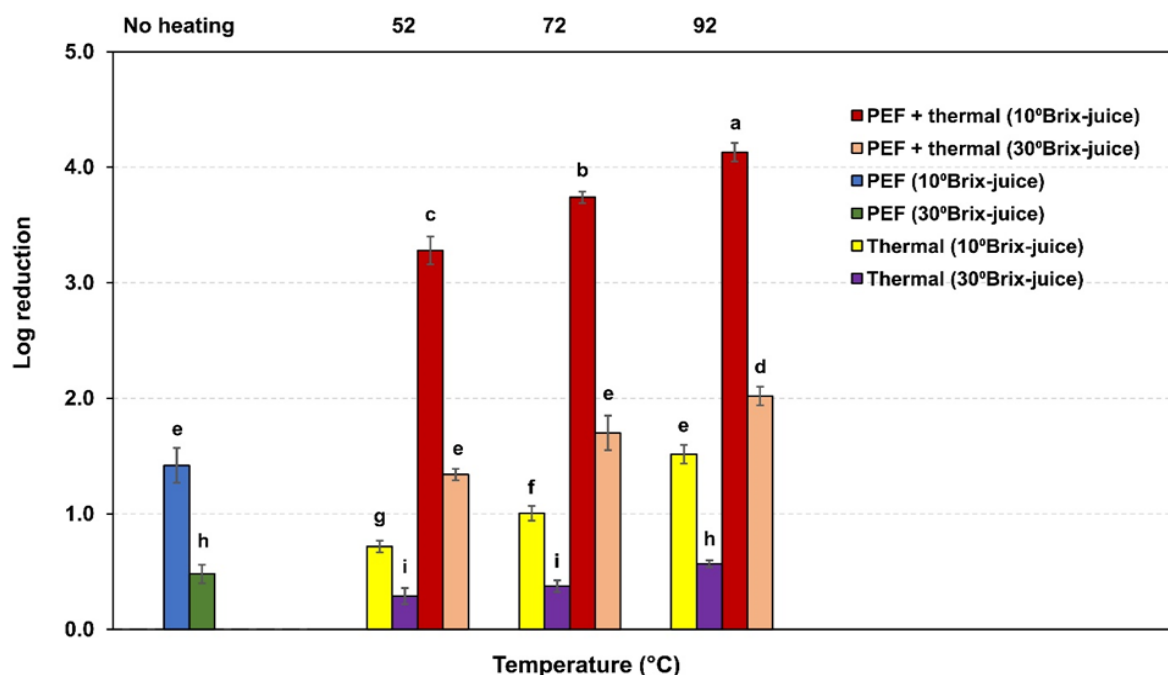


Figure 3. Comparison of *Paecilomyces variotii* spore log reductions in orange juices using pulsed electric field-assisted thermal treatments (144 s and 52/72/92 °C-10 min) to Pulsed electric field (144 s) and thermal processing (52, 72, 92 °C-10 min) alone. Columns with distinct letters are significantly various ($p < 0.05$). Error bars demonstrate the average deviation of the means.



In conclusion, results have suggested that PEF treatment can sensitize fungal spores to heat treatments; although the exact mechanism with fungal spores is still unclear and needs further investigations. Based on previous hypotheses by the current authors on bacterial spores [52], increased inactivation due to PEF-thermal was likely caused by the displacement of ions from the internal spore membrane and their migration through the inner core of the spores, decreasing protective barrier function of the inner membrane and hence making spores further susceptible to further processing (heat). Further studies are needed to describe exact mechanism of the PEF inactivation of heat-resistant fungal spores. Nonetheless, SEM studies of the spores after this process and PEF alone were further carried out.

3.4 Morphological observation of *Paecilomyces variotii* spores after pulsed electric field and pulsed electric field-assisted thermal treatments

Naturally, *P. variotii* produces heat-resistant ascospores and hence spore characteristics resemble *Byssoschlamys* (*Byssoschlamys*-morph) spores or others in the Subkingdom Ascomycetes, including *Neosartorya* and *Talaromyces* spp. Orange juice-suspended spores of *P. variotii*, which were treated by PEF-thermal and PEF alone, were analyzed by SEM (Fig. 4). According to Rozali et al. [53], morphology of the live untreated *N. fischeri* spores presents precisely-defined sharp edges in the external surface of the mold spores with breaks between the pointed edges and cubic forms. The two images in the figure (especially shown by

yellow and red arrows) provide well-visible evidence of cellular destruction around the spores, including significant changes in the spore appearance such as thinning and release of intracellular spore components following the lethal processes. However, lesser destructions and denser populations were seen for the spores treated by the PEF alone (65 kV cm⁻¹-144 s, Fig. 4A), compared to the spores treated by PEF-thermal method (65 kV cm⁻¹-144 s and 92 °C-10 min, Fig. 4B). These results indicated that degrees of the cell membrane damages and electroporation were higher after the PEF-assisted thermal treatments [54]. Not much information are available on the SEM inspections of heat-resistant mold ascospores after thermal and non-thermal processing such as PEF, particularly for *P. variotii*. Using SEM images, Evrendilek et al. observed the morphological alterations such as cytoplasmic coagulation, vacuolation, shrinkage and protoplast leakage of *B. cinerea* spores and *P. expansum* conidia after 17–30 kV cm⁻¹-163 μs PEF treatments, which completely inhibited spore germination and germination-tube elongation [25, 27]. In another study with *S. cerevisiae*, morphology study by SEM showed that the surface of cells treated with PEF-assisted thermal process (25 kV cm⁻¹, 50 °C) was rougher and more incomplete and porous than that of the cells treated by PEF alone [54]. Further extreme conditions were used in this study, which might damage microbial spores (e.g., spore bursts and release intracellular contents) after the lethal treatments.

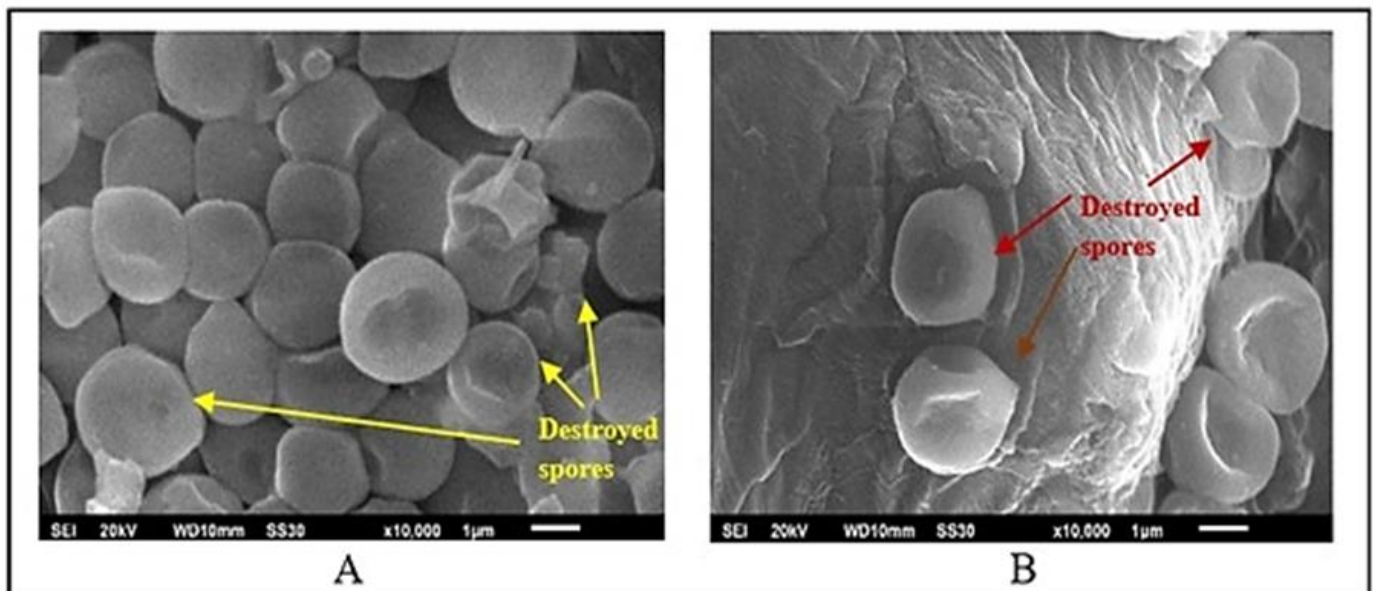


Figure 4. SEM images of *Paecilomyces variotii* spores after (A) 144 s of pulsed electric-field treatment and (B) 144 s pulsed electric field followed by 92 °C-10 min thermal process (10.000×).

4. Conclusion

The 65 kV cm⁻¹-PEF use for an effective time of 288 s was a better method for decreasing *P. variotii* spores than thermosonication of less than 5 W ml⁻¹ at 75 °C. The PEF processing was able to respectively decrease 4.2, 2.7 and 2.0 logs of *P. variotii* ascospores in 10, 20 and 30° Brix orange juices, thus showing protective effects of the soluble solid contents for the inactivation. The log survival curves of these fungal spores after PEF were accurately described by the Weibull model with the achieved *b* and *n* values. However, the PEF-assisted thermal method (144 s PEF followed by 92 °C-10 min thermal process) was the best method to process orange juices, compared to PEF or thermal process alone. Moreover, SEM studies verified these results. Therefore, the hurdle method such as sequential PEF and thermal treatment is a promising technique to achieve pasteurization by the manufacturers of orange juices contaminated by *P. variotii* ascospores. However, further studies are necessary to investigate the quality of juices after this process, suggesting that lower temperature may be needed for a better quality of the processed juices.

5. Acknowledgements

This study was financially supported by The Directorate General of Higher Education, Culture, Research and Technology Republic Indonesia (grant no. 2361/UN19.5.1.3/PT.01.03/2022). The authors thank technicians (Suci Ramadhana and Indra Permana) for their assistance in the laboratory, Chemical Engineering Department, Faculty of Engineering, University of Riau.

6. Conflict of Interest

The authors report no conflict of interest.

7. Authors Contributions

“Conceptualization, E.E. and C.C.; methodology, E.E.; software, E.E. and C.C.; validation, C.C. and S.S.; formal analysis, E.E.; investigation, S.S. and Y.A.; resources, E.E.; data curation, C.C.; writing—original draft preparation, E.E.; writing—review and editing, C.C., S.S. and Y.A.; visualization, S.S.; supervision, C.; project administration, Y.A.; funding acquisition, E.E.”.

8. Using Artificial Intelligent Chatbots

Artificial intelligent chatbots were not used in any section of this manuscript.

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فرایند میدان الکتریکی پالس آب پرتقال حاوی اسپورهای پسیلومایسس واریوتی: مقایسه با فراصوت قدرتی و تیمارهای حرارتی

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تاریخچه مقاله

دریافت ۳ جولای ۲۰۲۴

دوری ۳ آگوست ۲۰۲۴

پذیرش ۲۳ آگوست ۲۰۲۴

واژگان کلیدی

- اسپور کپک
- میدان الکتریکی پالسی (PEF)
- PEF به همراه حرارت
- فراصوت حرارتی
- محافظت قند

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چکیده

سابقه و هدف: وجود آسکوسپورهای کپک مقاوم به حرارت در آب میوه‌ها نگرانی بسیاری برای صنایع غذایی به وجود آورده است. میدان الکتریکی پالسی و فناوری فراصوت قدرتی^۱، به عنوان یک فناوری نوآورانه نگهداری مواد غذایی غیرحرارتی، برای کاهش اسپورهای پسیلومایسس واریوتی در آب پرتقال مورد استفاده قرار گرفت.

مواد و روش‌ها: در مطالعه حاضر، روش میدان الکتریکی پالسی (65 kV cm^{-1}) با روش‌های فراصوت ($W > 5$) و روش‌های حرارتی (فراصوت ترکیبی و گرما، $W > 5$ و 75°C) برای غیرفعال‌سازی اسپورهای پسیلومایسس واریوتی در آب پرتقال مقایسه شد. هدف اصلی بررسی کاهش ورود به سیستم اسپورهای پسیلومایسس واریوتی و اثرات میزان مواد جامد محلول آب میوه تحت تیمارهای میدان الکتریکی پالسی و مقایسه میدان الکتریکی پالسی به دنبال فرایند حرارتی و فرایند حرارتی معمولی به تنهایی بود. انحراف استاندارد و تجزیه و تحلیل آماری نتایج برای برجسته کردن تفاوت بین تیمارها گزارش شد. علاوه بر این، منحنی‌های لگاریتمی زنده‌مانی یا بقا پس از اعمال میدان الکتریکی پالسی مدل‌سازی شد و مورفولوژی اسپور مورد بررسی قرار گرفت.

یافته‌ها و نتیجه‌گیری: فرایند ۳۰ دقیقه‌ای (با زمان موثر ۲۸۸ ثانیه)، میدان الکتریکی پالسی بر کاهش اسپورهای پسیلومایسس واریوتی موثرتر از فراصوت و گرمافراصوت بود که به ترتیب کاهش سه و حداکثر یک log را موجب شدند. میزان مواد جامد محلول آب پرتقال بر کاهش هاگ پس از اعمال میدان الکتریکی پالسی اثر دارد، هنگامی که میزان مواد جامد محلول بیشتر یا کمتر از این میزان باشد (بیش از چهار لگاریتم در درجه بریکس ۱۰ و برابر یا کمتر از دو لگاریتم در آب‌میوه با درجه بریکس ۳۰). مدل ویبال^۲ به خوبی می‌تواند منحنی‌های لگاریتمی بقای سیستم را پس از تیمارهای میدان الکتریکی پالسی نشان دهد. میدان الکتریکی پالسی و به دنبال آن فرایند حرارتی (۱۴۴ ثانیه در ۵۲، ۷۲ و 92°C به مدت ۱۰ دقیقه) بهترین روش بود، در مقایسه با میدان الکتریکی پالسی یا تیمار حرارتی به تنهایی، که باعث کاهش بیش از دو لگاریتم در تعداد اسپورهای پسیلومایسس واریوتی شد. میکروسکوپ الکترونی روبشی پس از استفاده از ترکیب میدان الکتریکی پالسی و فرایند حرارتی، آسیب شدیدی به اسپورهای پسیلومایسس واریوتی را نشان داد. به طور کلی، فناوری حرارتی به همراه میدان الکتریکی پالسی، پیشگیری بهتری برای فساد آب‌میوه و افزایش ایمنی آب پرتقال آلوده به اسپورهای پسیلومایسس واریوتی بود.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافع مرتبط با انتشار این مقاله ندارند.

Weibull^۲

^۱ power ultrasound