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Pulsed Electric Fields as a Cutting-Edge Solution to Ensure the Safety of Pineapple Juice: Targeting *Escherichia coli* O157:H7 and *Listeria monocytogenes* 5672

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ABSTRACT: This study evaluates the effectiveness of pulsed electric field (PEF) technology for pasteurizing pineapple juice to inactivate *Escherichia coli* O157:H7 and *Listeria monocytogenes* 5672. Pineapple juice was treated with PEF pulses of 5 μs at electric field intensities of 15, 20, and 25 kV/cm, with frequencies ranging from 8 to 44 Hz. Treatment durations ranged from 12 to 197 s, corresponding to energy levels between 18 and 192 kJ/kg, while exit temperatures were controlled between 40 and 70 °C. Results showed that higher exit temperatures and electric field strengths significantly enhanced the inactivation of *E. coli* O157:H7, with the most effective treatment being at 70 °C and 25 kV/cm, achieving ≥3-log reductions. For *L. monocytogenes* 5672, reductions of 4−5 log cycles were observed under most conditions, except at 15 kV/cm, where a 2-log reduction was noted. After treatment, all samples were incubated for 72 h under refrigeration; *E. coli* O157:H7 showed an additional lethal effect during storage. This leads to reductions exceeding 5 logs for those juices treated at 20 and 25 kV/cm, while *L. monocytogenes* 5672 showed less significant changes. These findings highlight the potential of PEF technology for enhancing food safety in juice preservation.

KEYWORDS: *pulsed electric fields, pineapple juice processing, poststorage effectiveness*

1. INTRODUCTION

In recent decades, food processing has undergone significant evolution, driven by growing interest in nonthermal technologies that can maintain products' nutritional and sensory quality while improving microbiological safety. One of the most promising technologies in this field is pulsed electric fields (PEFs), which has proven highly effective in destroying pathogenic microorganisms and enhancing the organoleptic properties of various foods. Pulsed electric fields are based on the application of high-intensity electrical pulses to liquid or semiliquid foods, inducing changes in the permeability of cell membranes and generating biological effects that can be leveraged for food preservation.[1−3]

The fundamental principle of PEF involves the application of high-intensity electrical pulses through a food sample, typically within a voltage range of 20 kV to 80 kV. These short-duration pulses (microseconds to milliseconds) are applied at frequencies that vary depending on the product type and the treatment objective. The process triggers phenomena such as electroporation, which temporarily alters the structure of cell membranes, facilitating both the release of bioactive compounds and the inactivation of pathogens. This mechanism is particularly useful in the pasteurization and sterilization of food products without the need for high temperatures, thus better preserving the sensory and nutritional properties of the product.[4−6]

Food pasteurization using PEFs represents an innovative approach for improving microbial inactivation efficiency while preserving food quality. Unlike traditional methods such as thermal pasteurization, which use high temperatures to destroy microorganisms, PEF applies brief pulses of high-intensity electricity to food, allowing for a significant reduction in microbial load without generating the excessive heat that can degrade sensitive components such as vitamins, aromas, colors, and textures.[7]

The application of PEF significantly reduces processing times, enhancing both energy and operational efficiency, while also reducing water and raw material consumption, thus minimizing environmental impact. It also improves production efficiency by increasing yield and lowering operational costs. Moreover, it supports more sustainable and adaptable manufacturing practices that align with evolving market demands, while simultaneously enhancing food quality and safety. PEF technology not only ensures microbial safety but also boosts industry competitiveness by offering a preservation method that avoids the drawbacks of conventional heat treatments, such as the degradation of sensory attributes or the formation of undesirable compounds.[7−9] Additionally, PEF technology easily adapts to different food matrices, such as

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juices, dairy products, and sauces, by adjusting parameters such as electric field intensity, exposure time, and temperature, which allows for optimizing the process according to the specific needs of the product and target microorganisms.[10−12]

PEF has been successfully applied to a wide range of food products, including juices, sauces, dairy products, and meats. In juices and beverages, PEF is primarily used for the inactivation of pathogens such as *Escherichia coli* and *Salmonella*, while in dairy products, improvements in texture and emulsion stability have been observed.[2,13−15]

For instance, Rezaeimotlagh et al.[16] studied the inactivation of *E. coli* in cranberry juice via PEF, varying the electric field (2.2−13.2 kV/cm), treatment stages (1−6), and flow rate (13−25 L/h) at 20, 30, and 40 °C. They reached a reduction of 6.57 ± 0.02 log CFU/ml in *E. coli* at 40 °C across six treatment stages. Their findings suggested that higher temperatures at a constant electric field increased microbial inactivation. Timmermans et al.[17] evaluated PEF's ability to inactivate *Salmonella* Panama, *E. coli*, *Listeria monocytogenes*, and *Saccharomyces cerevisiae* in apple, orange, and watermelon juices using a continuous flow system at 20 kV/cm and variable frequencies. *S. cerevisiae* was most sensitive, followed by *S.* Panama and *E. coli* with similar kinetics. *L. monocytogenes* was the most resistant. A synergistic effect between temperature and electric pulses was noted above 35 °C, reducing the energy needed at higher temperatures. Kantala et al.[18] studied how PEF treatment impacts microbial inactivation and quality parameters in Thai orange juice. Their results demonstrated that PEF effectively inactivates *Staphylococcus aureus* and *E. coli* in orange juice. A 5-log reduction occurred after 10 PEF pulses at 30 kV/cm.

It is important to note that the effectiveness of PEF treatment can be affected by the insoluble content of the food matrix. Suspended solids, such as pulp, fibers, or other insoluble materials, can shield microorganisms from the electric field, reducing treatment uniformity or altering local electroporation dynamics. This behavior is supported by studies showing that the structural characteristics and microheterogeneity of plant tissues affect PEF-induced permeabilization and mass transfer. Additionally, research on electroporation-driven mass transfer shows that heterogeneous food matrices can alter the electric field distribution, which impacts the efficiency of PEF treatments.[3,19,20]

Recent studies on fruit juices highlight PEF's dual role in enhancing both microbial safety and product quality. PEF induces electroporation in both plant tissues and microbial cells, causing irreversible membrane damage in microorganisms while also increasing the release of intracellular bioactive compounds without the harmful thermal effects typically associated with heat treatments.[21] In ginger juice, for instance, treatments between 1.5 and 3.0 kV/cm increased the cell disintegration index, raised soluble solids (°Brix), and significantly improved antioxidant activity (DPPH/ABTS) by increasing phenolics, flavonoids, and gingerol-related compounds, reinforcing the nonthermal nature and functional benefits of the technology.[22] Similarly, in *Phyllanthus emblica* (amla), PEF at 4 kV/cm also improved the nutritional profiles of vitamin C, phenolic acids, and flavonoids, while preserving a greater proportion of aromatic esters compared to thermal processing and increasing antioxidant capacity.[23] Meanwhile, in berry blends processed at 6 kV/cm and temperatures below 25 °C, PEF demonstrated superior color preservation (lower ΔE) compared to thermal treatments, underscoring processing advantages for pigment-rich juices.[24] Concerning nutrients and sensory quality, available evidence consistently reports positive or protective effects of PEF on heat-sensitive compounds. This includes: (i) higher vitamin C, phenolic content, and flavonoids in PEF-treated amla juice, along with superior antioxidant activity compared to both untreated and thermally processed controls; (ii) increased total phenolics, total flavonoids, flavanols, condensed tannins, and antioxidant activity in ginger juice, coupled with enhanced release of gingerols and shogaols; and (iii) preservation or enhancement of aromatic profiles, such as improved retention of esters in amla and increased volatile diversity in ginger.[22,23]

However, the effectiveness of PEF depends greatly on the matrix, requiring optimization of field intensity, pulse number, pulse width, and specific energy based on product characteristics and processing goals. In berry blends, for example, PEF achieved better color preservation than thermal treatments; however, formulations richer in grape showed a reduction in anthocyanins, while strawberry−honeysuckle blends were less affected, emphasizing how composition influences pigment stability. Conversely, in highly acidic products such as amla (pH ≈ 3), PEF improved juice yield and enhanced both nutritional and antioxidant parameters, while also increasing electrical conductivity due to ionic release. This conductivity shift point is related to more efficient energy transfer during PEF treatment, which is important for matrices with moderate conductivity, such as pineapple juice.[24−26]

Pineapple juice is widely consumed due to its refreshing taste and nutritional benefits, including high levels of vitamin C and antioxidants. The global consumption of pineapple juice is particularly high in Asia and the European Union, with countries like Thailand, Indonesia, and the Philippines accounting for 47% of worldwide consumption in 2022.[27] However, traditional thermal processing can negatively affect its sensory and nutritional properties. In this context, pulsed electric fields have emerged as a nonthermal technology that offers a viable alternative for juice pasteurization, more efficiently preserving its original characteristics. Pineapple juice has an average pH of 4.2 ± 0.3, classifying it as an acidic food, and an electrical conductivity of 1.94 ± 0.2 mS/cm, indicating a moderate concentration of dissolved ions. These physicochemical characteristics are crucial for PEF treatment, as conductivity influences the efficiency of energy transfer during the application of electric pulses, while pH affects the permeability of cell membranes, which can impact the effectiveness of electroporation.[28]

Pineapple juice is susceptible to microbial contamination by pathogens such as *E. coli*, *Salmonella* spp., and *L. monocytogenes*, which present a significant challenge to food safety. For this reason, the objective of this research is to evaluate the effectiveness of pulsed electric field technology in the treatment of pineapple juice with physicochemical characteristics. The impact of electrical pulses on the inactivation of common juice pathogens, such as *E. coli* O157:H7 and *L. monocytogenes* 5672, will be analyzed, and the feasibility of PEF as a nonthermal pasteurization alternative will be assessed to maintain the microbiological safety of the final product during storage.

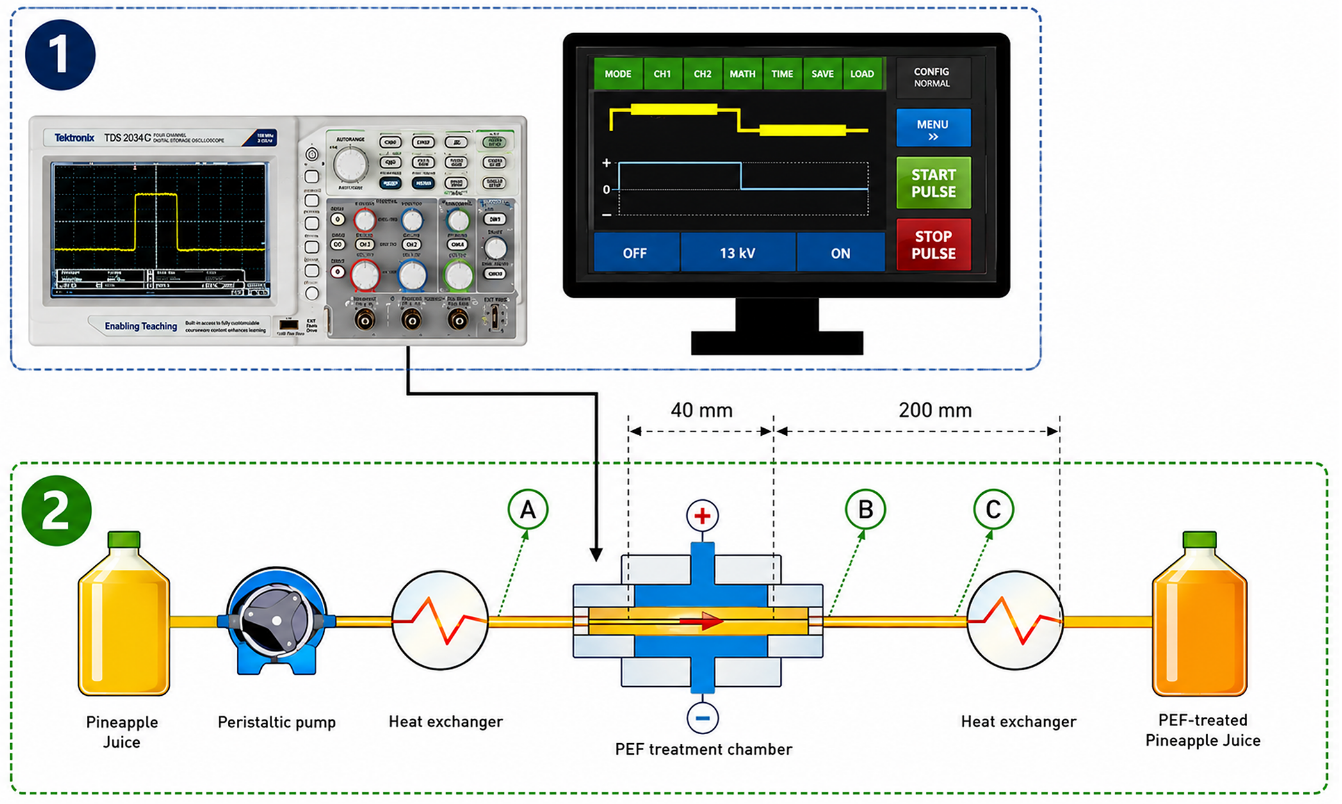

Figure 1. (1) Applied voltage monitored. (2) Schematic representation of the PEF treatment setup: Points A, B, and C indicate the location of each thermocouple. Residence time in the PEF treatment zone: 0.84 s. Residence time in the pipe from the exit of the treatment chamber to the exit of the cooling coil pipe: 2.11 s.

2. MATERIALS AND METHODS

2.1. Preparation of Juice Samples

Commercially pasteurized pineapple juice (pH 4.2 ± 0.3; electrical conductivity 1.94 ± 0.2 mS/cm) was purchased from a local supermarket. The conductivity was measured with a calibrated conductivity meter (COND7+ XS, LabProcess, Barcelona, Spain) using deionized water as the reference. Prior to inoculation, the native microbial load of the juice was evaluated by plating on nonselective agar; counts were below the detection limit (<1 colony-forming unit per milliliter [CFU/mL]). No additional sterilization was performed.

The juice was separately inoculated with *E. coli* O157:H7 and *L. monocytogenes* 5672 (University of Zaragoza, Spain). Both microorganisms were grown to the stationary phase ($\approx 10^9$ CFU/mL) prior to inoculation. *E. coli* O157:H7 was cultured in Tryptic Soy Broth (TSB; Oxoid, Basingstoke, UK) at 37 °C for 24 h, whereas *L. monocytogenes* 5672 was grown in Tryptic Soy Broth supplemented with 0.6% yeast extract (TSB + YE; Oxoid) at 30 °C for 24 h. The bacterial suspensions were then added to the pineapple juice to obtain an initial concentration of approximately 10^7 CFU/mL before PEF treatment.

2.2. PEF Processing and Experimental Design

The PEF treatments were applied in continuous-flow mode using a commercial generator (Vitave, Prague, Czech Republic). The applied voltage was monitored with a high-voltage probe (P6015A, Tektronik, Wilsonville, Oregon, USA), while the current intensity was recorded with a current probe (HCT5514, MeatrolElectrical, Shanghai, China). Both probes were connected to an oscilloscope (TDS 220, Tektronik). Square waveform monopolar pulses were delivered in a parallel titanium electrode chamber with a 0.56 cm gap, 4.0 cm length, and 0.5 cm width. A schematic representation of the experimental setup and process flow is provided in Figure 1. Homogenized pineapple juice was pumped using a peristaltic pump (BVP, Ismatec, Wertheim, Germany) operating at a flow rate of 4.8 L/h, corresponding to a residence time in the treatment chamber of 0.84 s. Juice was tempered at different temperatures (25, 30, and 35 °C) using a heat exchanger (LAUDA Alpha RA 12, USA) positioned before the treatment chamber. After treatment, the juice was cooled to 8 °C in 2.11 s using a second heat exchanger downstream of the chamber.

As shown in Figure 1, the temperature was continuously monitored at three critical points in the system: just before entering the treatment chamber, immediately after exiting it, and at the inlet of the cooling coil. Type K thermocouples were inserted directly into the processing line to ensure that accurate temperature readings were obtained throughout the treatment.

Pulsed electric field (PEF) treatments were conducted using electric field strengths of 15, 20, and 25 kV/cm with a constant pulse width of 5 μs. The pulse repetition frequency was adjusted between 2 and 120 Hz to achieve target outlet temperatures of 40, 50, 60, and 70 °C at the treatment chamber exit. Depending on the selected processing conditions, the total effective treatment time ranged from 10 to 530 μs, corresponding to specific energy inputs of 18−192 kJ/kg.

To properly evaluate the effects and interactions of electric field strength, inlet temperature, and total specific energy on microbial inactivation, a full-factorial experimental design was defined as shown in Table 1. The responses were then evaluated using a response surface methodology. The experimental design includes three levels of electric field strength (voltage gradients applied across the treatment chamber); three inlet temperatures (juice temperature in the PEF chamber entrance); and four levels of outlet temperature (target temperature at the PEF chamber exit, regulated by adjusting the pulse repetition frequency and reflecting the combined electrical and thermal effects of the treatment). These levels were selected on the basis of preliminary experiments and literature reports to cover a range of mild to moderate processing conditions.

Table 1. Factors and Experimental Levels for Evaluating the Inactivation of *E. coli* O157:H7 and *L. monocytogenes* 5672 in Pineapple Juice

factors	experimental uncoded values (levels)			
voltage (kV/cm)	15	20	25	
inlet temperature (°C)	25	30	35	
outlet temperature (°C)	40	50	60	70

2.3. Specific Energy Calculation

The specific energy delivered during pulsed electric field (PEF) treatment was calculated using standard electroporation energy relationships. The specific energy ($kJ \cdot kg^{-1} \cdot pulse^{-1}$) per pulse was determined as follows:

$$W = \frac{\sigma E^2 \tau}{\rho \cdot 10^3} \tag{1}$$

where σ is the electrical conductivity of the juice ($S \cdot m^{-1}$), E is the electric field strength ($V \cdot m^{-1}$), τ is the pulse width (s), and ρ is the density of the liquid ($kg \cdot m^{-3}$).

The total specific energy ($kJ \cdot kg^{-1}$) applied to the juice was calculated as

$$W_T = n \cdot W \tag{2}$$

with n represents the total number of pulses delivered. In continuous flow operation, the number of pulses was obtained from

$$n = f \cdot t_{treat} \tag{3}$$

The electric field strength for parallel-plate electrode geometries was calculated according to

$$E = \frac{U_0}{d} \tag{4}$$

where U_0 is the applied voltage (V) and d is the electrode gap (m). The temperature rise attributable to Joule heating was estimated by

$$\Delta T = \frac{W_T}{C_p} \tag{5}$$

with C_p denotes the specific heat capacity of the liquid ($kJ \cdot kg^{-1} \cdot °C^{-1}$).

Table 2. Inactivation of *E. coli* O157:H7 under Different PEF Parameters: Electric Field Strength, Inlet and Outlet Temperatures, Frequency, Total Energy, and Treatment Time

		E coli O157:H7				
electric field (kV/cm)	T^0 inlet (°C)	T^0 outlet (°C)	frequency (Hz)	total energy (kJ/kg)	total treatment time (μs)	inactivation (log N_t/N_0)
15	25	41.10 ± 1.08	20.67 ± 2.36	67.30 ± 4.51	86.80 ± 9.89	−0.12 ± 0.07
		50.63 ± 0.86	29.67 ± 5.10	106.20 ± 3.74	124.60 ± 21.43	−0.36 ± 0.14
		61.37 ± 0.96	38.00 ± 5.19	152.01 ± 4.02	159.60 ± 21.78	−0.79 ± 0.38
		70.33 ± 0.73	46.67 ± 2.85	189.49 ± 3.04	196.00 ± 11.96	−2.18 ± 0.02
	30	40.77 ± 0.28	15.67 ± 9.22	45.00 ± 1.19	51.80 ± 11.96	−0.14 ± 0.01
		50.67 ± 0.40	27.00 ± 11.81	86.39 ± 1.66	91.56 ± 8.12	−0.27 ± 0.07
		60.63 ± 1.41	36.33 ± 15.45	128.05 ± 5.91	123.48 ± 10.28	−0.68 ± 0.22
		70.30 ± 0.57	46.67 ± 16.02	168.45 ± 2.37	160.72 ± 3.06	−2.19 ± 0.04
	35	40.50 ± 0.78	9.00 ± 6.88	22.99 ± 3.28	28.84 ± 11.5	0.12 ± 0.06
		50.27 ± 0.77	19.67 ± 10.27	63.81 ± 3.22	65.80 ± 11.96	−0.23 ± 0.01
		60.67 ± 0.40	29.67 ± 13.12	107.29 ± 1.66	100.52 ± 9.03	−0.52 ± 0.12
		69.6 ± 0.23	40.67 ± 15.07	144.63 ± 0.95	139.44 ± 5.03	−2.20 ± 0.08
20	25	40.77 ± 0.88	13.00 ± 5.66	65.90 ± 3.67	44.52 ± 11.91	−0.4 ± 0.15
		51.10 ± 0.99	20.00 ± 7.42	109.10 ± 4.12	68.88 ± 11.91	−0.47 ± 0.24
		60.97 ± 0.74	24.33 ± 8.03	150.34 ± 3.08	84.28 ± 12.41	−0.74 ± 0.24
		70.77 ± 1.24	31.67 ± 11.16	191.30 ± 5.19	108.92 ± 4.78	−3.24 ± 0.36
	30	40.43 ± 0.40	8.00 ± 4.93	43.61 ± 1.66	26.32 ± 6.74	−0.29 ± 0.12
		50.73 ± 0.28	14.83 ± 8.01	86.67 ± 1.19	49.42 ± 8.45	−0.44 ± 0.24
		60.10 ± 0.93	19.83 ± 9.02	125.82 ± 3.87	67.06 ± 6.89	−0.89 ± 0.49
		70.07 ± 0.99	27.00 ± 9.8	167.48 ± 4.13	92.68 ± 0.55	−2.80 ± 0.17
	35	40.43 ± 0.28	4.50 ± 3.53	22.71 ± 1.19	14.42 ± 6.66	−0.28 ± 0.14
		51.10 ± 0.69	11.17 ± 5.72	67.30 ± 2.88	37.38 ± 5.48	−0.28 ± 0.24
		60.50 ± 0.97	16.50 ± 8.37	106.59 ± 4.04	55.30 ± 8.35	−1.12 ± 0.45
		70.33 ± 0.69	25.17 ± 10.71	147.69 ± 2.89	85.54 ± 7.81	−2.8 ± 0.39
25	25	41.43 ± 1.62	6.67 ± 2.36	72.87 ± 14.76	28.00 ± 9.89	−0.38 ± 0.09
		51.07 ± 0.94	10.50 ± 2.59	108.96 ± 3.94	44.10 ± 10.89	−0.53 ± 0.16
		60.07 ± 0.95	13.33 ± 1.73	146.58 ± 3.97	56.00 ± 7.26	−0.95 ± 0.42
		69.97 ± 0.47	16.67 ± 0.65	187.96 ± 1.97	70.00 ± 2.74	−2.79 ± 0.11
	30	40.87 ± 1.31	4.33 ± 1.63	45.42 ± 5.46	18.20 ± 6.86	−0.35 ± 0.20
		50.03 ± 0.17	7.83 ± 1.63	83.74 ± 0.72	32.90 ± 6.86	−0.91 ± 0.42
		60.93 ± 0.62	11.00 ± 1.96	129.30 ± 2.61	46.20 ± 8.23	−1.81 ± 0.19
		71.23 ± 0.28	15.33 ± 0.65	172.36 ± 1.19	64.40 ± 2.74	−2.72 ± 0.26
	35	41.27 ± 1.76	2.43 ± 1.05	26.19 ± 7.35	10.22 ± 4.42	−0.28 ± 0.12
		52.20 ± 2.78	6.33 ± 1.73	71.90 ± 11.62	26.60 ± 7.26	−0.70 ± 0.24
		62.07 ± 2.09	9.33 ± 1.73	113.14 ± 8.74	39.20 ± 7.26	−1.91 ± 0.47
		71.63 ± 0.80	13.83 ± 0.33	153.13 ± 3.36	58.10 ± 1.37	−3.44 ± 0.10

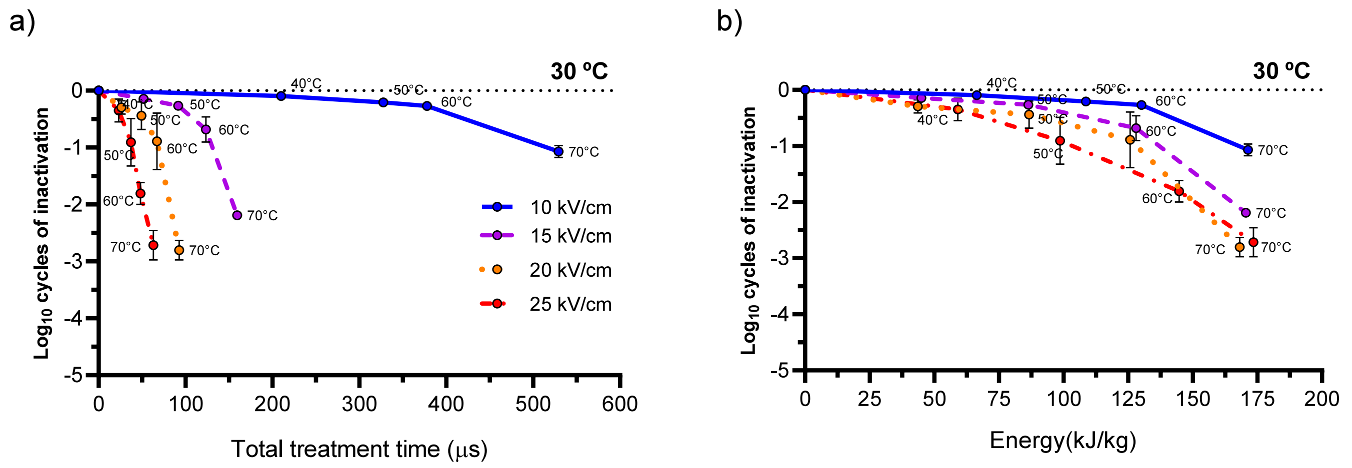

Figure 2. *E. coli* O157:H7 inactivation under pulsed electric fields (PEF). (a) Log_{10} reduction as a function of total treatment time and (b) as a function of total specific energy, for electric field strengths of 10, 15, 20, and 25 kV/cm. Each curve is identified by its corresponding color, and the outlet temperature reached at each condition is indicated next to the data points. All measurements were taken immediately after PEF treatment (0 h). The inlet temperature of the juice was 30 °C.

2.4. Microbial Viability

The inactivation of *E. coli* O157:H7 and *L. monocytogenes* 5672 after PEF treatments was evaluated immediately after treatment (0 h) and after 72 h of incubation at 4 ± 1 °C by plate count. Aliquots of pineapple juice samples, diluted in peptone water (Oxoid, Basingstoke, Hampshire, UK), were plated onto Tryptic Soy Agar (TSA; Oxoid) and incubated at 30 °C for 48 h for *L. monocytogenes* 5672 and incubated at 37 °C for 24 h for *E. coli* O157:H7. The number of colonies counted after incubation corresponded to the number of viable microorganisms expressed as colony-forming units per milliliter (CFU/mL). The survival fraction was calculated as the ratio of the number of microorganisms surviving the treatment (N_t) to the initial number of viable cells (N_0). Survival curves were obtained by plotting Log_{10} of the survival fraction vs treatment time or energy for each electric field strength.

2.5. Mathematical Modeling

In continuous flow PEF systems, microbial cells experience uneven residence times along with simultaneous changes in electric field strength, temperature, and energy input. Under these conditions, the lethal effect cannot be accurately described solely by treatment time, which restricts the use of traditional kinetic models that rely on rate constants. Therefore, an empirical polynomial model based on response surface methodology was employed to assess the primary effects and interactions of electric field strength (15−25 kV/cm), inlet temperature (25−35 °C), and outlet temperature (40−70 °C) on microbial inactivation. The experimental inactivation data were fitted to second-order polynomial equations (eq 6) to identify the relative contribution of each variable and their interactions to the overall microbial reduction of *E. coli* O157:H7 and *L. monocytogenes* 5672 (eq 1). Data analysis was performed with Design-Expert software (version 10.0, Stat-Ease, Inc., Minneapolis, MN, United States).

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^{n} + \sum_{i>1}^{2} \beta_{ii} X_i X_j \tag{6}$$

where Y is the predicted response, and β_0, β_i, β_{ii}, and β_{ij} are the intercept, linear, quadratic, and cross-product coefficients, respectively. X_i and X_j represent the independent factors, and n is the total number of independent factors. To determine the model's significant parameters and to remove the nonsignificant effects ($p > 0.05$), backward regression was applied.

2.6. Statistical Analysis

The samples were analyzed in triplicate, and the data are presented as the mean ± standard deviation. A one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed in GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) to assess differences between mean values. Differences were considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of PEF Treatment on *E. coli* O157:H7 Inactivation

Table 2 presents the combined effect of inlet temperature, outlet temperature, and electric field intensity on the inactivation of *Escherichia coli* O157:H7 in pineapple juice. Three inlet temperatures (25, 30, and 35 °C), four outlet temperatures (40, 50, 60, and 70 °C), and three field intensities (15, 20, and 25 kV/cm) were evaluated, resulting in different specific energy deliveries and treatment times. The highest reductions (≥3 log cycles) were obtained at 70 °C with 25 kV/cm, regardless of inlet temperature. At 60 °C, comparable inactivation required ≥ 20 kV/cm, while 15 kV/cm achieved <2-log reductions. At 40 and 50 °C, reductions remained limited (<1 log at 15 kV/cm and <2 logs at 20 kV/cm), indicating minimal thermal contribution at these conditions. Inlet temperature had a smaller but evident effect, with slightly greater inactivation at 35 °C than at 25 °C, suggesting a modest synergistic effect between preheating and PEF. A strong correlation was observed between outlet temperature and the number of applied pulses, particularly above 35 °C, where temperature-induced phase transitions in cell membrane phospholipids reduce membrane stability and facilitate electropermeation. Increasing frequency generally enhances membrane permeability, facilitating inactivation, although excessively high frequencies may reduce electric field penetration. Likewise, higher specific energy, resulting from stronger fields and longer pulse durations, tends to improve microbial inactivation, but excessive doses may compromise product quality. Longer treatment times complement these effects by increasing the interaction between the electric field and bacterial cells, further enhancing their destruction.

Regarding output temperature, the study demonstrated that as the exit temperature increased from 50 to 60 °C, *E. coli* O157:H7 inactivation improved markedly. At 50 °C, the reduction was minimal, suggesting that this temperature was insufficient to significantly reduce the microbial load. However, at 60 °C, a considerable reduction was noted, with the most pronounced inactivation occurring at a temperature of ≥60 °C, where the highest microbial reduction rates were achieved. These findings suggest that *E. coli* O157:H7 is particularly susceptible to thermal treatments starting at 60 °C, with temperatures above 60 °C proving effective for substantial inactivation.

When comparing the highest exit temperature (70 °C) with voltage and exit temperature, it was observed that in most treatments at 70 °C and voltages of 20 and 25 kV/cm, inactivation followed a similar trend regardless of the juice's entry temperature in the PEF system (Figure 2). This indicates that, under these conditions, the inactivation efficiency is primarily influenced by the exit temperature and voltage, with minimal variation across entry temperatures.

Rezaeimotlagh et al.[16] investigated the microbial inactivation of cranberry juice using PEFs by exploring a temperature range of 20−40 °C, an electric field intensity of 2.2−13.2 kV/cm, and a flow rate of 13−25 L/h. The most effective microbial reduction, 6.57 ± 0.02 log CFU/mL of *E. coli*, was achieved through six treatment cycles at 40 °C. Notably, elevated temperatures facilitated comparable levels of inactivation with fewer treatment steps, highlighting the importance of thermal assistance in PEF processing. Key variables influencing microbial inactivation in liquid matrices include pH, conductivity, temperature, and treatment duration, all of which collectively determine the efficacy of PEF.

Kayalvizhi et al.[29] determined that the most effective processing condition involved treating sugar cane juice, with or without the addition of lemon and ginger, at 4 °C, showing pH and soluble solid characteristics similar to pineapple juice, with 30 kV/cm and 150 pulses, achieving a 2.71 log CFU/mL reduction in aerobic mesophilic bacteria. Interestingly, this lower-intensity protocol produced greater microbial inactivation than more intense treatments, likely due to an optimized balance between electric field strength and cumulative exposure from the number of pulses. Such controlled electroporation at milder conditions can effectively inactivate cells while minimizing sublethal injury that might otherwise allow microbial recovery.

Similarly, Roobab et al.[21] assessed the application of PEF as a nonthermal preservation strategy for various fruit juices, focusing on microbial stability and retention of sensory and nutritional properties. Their findings confirmed PEF's ability to inactivate microorganisms efficiently without the detrimental effects associated with conventional thermal pasteurization. For instance, treatment of watermelon juice at 30 kV/cm and 60 °C led to 99.9% inactivation of *E. coli* and *Salmonella* spp., while orange juice processed at 40 kV/cm and 55 °C achieved a 99.5% reduction of *S. aureus*. Carrot juice showed a 98% reduction in *L. monocytogenes* at 35 kV/cm and 50 °C, apple juice exhibited up to 95% reduction of yeasts and molds at 25 kV/cm and 45 °C, and strawberry juice demonstrated a 97% reduction of *S. cerevisiae* at 30 kV/cm and 50 °C. These results collectively underscore PEF's adaptability and effectiveness across diverse juice matrices, reinforcing its value as a sustainable, consumer-oriented alternative to thermal processing.

Meanwhile, Figure 2 presents the microbial survival of *E. coli* O157:H7 at a PEF inlet temperature of 30 °C, an intermediate value within the range evaluated in this study (25−35 °C), and shows results as a function of both treatment time and specific energy. When inactivation is plotted against treatment time, it is evident that treatment efficacy increases with electric field intensity, with shorter exposure times required to achieve reductions close to 3 log cycles as the applied intensity increases. At high field intensities (20−25 kV/cm), significant reductions are achieved within 100 μs, whereas at low intensities (10 kV/cm), inactivation remains minimal even after prolonged treatment (Figure 2a).

When inactivation is expressed as a function of specific energy, defined as the energy required to generate the electric field and calculated from the applied field strength, treatment time, and chamber resistance, for *E. coli* O157:H7, between 15 and 25 kV/cm, the reduction depends mainly on the applied energy, with little direct influence from field intensity. At energy values above 150 kJ/kg, reductions of up to 3 log cycles are observed, particularly when the final temperature reaches 70 °C.

Another relevant observation is the influence of outlet temperature on microbial inactivation. Above 60 °C, temperature can be lethal to microorganisms, raising the question of whether the observed reductions are due exclusively to the electric field, to heat, or to a combined effect. Assessing thermal resistance at the outlet temperatures achieved is challenging, as the residence time in the treatment chamber is less than 1 s. To isolate the thermal effect, a low field intensity (10 kV/cm) was applied, which alone is insufficient to cause significant inactivation. Under these conditions, when the outlet temperature reached 70 °C, the reduction was less than 1 log cycle, attributable solely to heat. Therefore, any reduction exceeding 1 log cycle at 70 °C with field intensities ≥ 15 kV/cm can be attributed to the PEF treatment.

Consistent with previous reports, Gram-negative bacteria exhibit increased resistance under acidic conditions, which explains *E. coli* O157:H7 higher tolerance in pineapple juice (pH ≈ 3.5). In this study, effective inactivation required either high electric field intensities (≥20 kV/cm) or elevated outlet temperatures (≥60 °C), with the latter showing a pronounced synergistic effect on microbial reduction. This observation aligns with García et al.[30] who demonstrated that sublethal injury and inactivation are strongly influenced by both the treatment medium and applied field intensity, wherein acid-adapted Gram-negative cells require greater energy inputs for effective control. Our findings confirm that *E. coli* O157:H7 inactivation by PEF in acidic matrices is governed by the interplay of electric field intensity, specific energy, and processing temperature, underscoring the need to integrate these parameters into the design of industrial-scale treatments for fruit juices.

A similar effect was observed by Timmermans et al.,[17] who demonstrated a synergistic effect between the juices' entry temperature and the PEF treatment. Additionally, this interaction influenced not only the microorganism's inactivation but also the pH of the treated juice. In general, there is a strong correlation between temperature and the number of pulses applied, especially at temperatures above 35 °C. This is because a temperature increment causes phase transitions in the phospholipids of the cell membrane, reducing membrane stability, and facilitating electropermeation. In general, a synergistic effect between temperature and the number of pulses applied is observed, especially at temperatures above 35 °C.

In parallel, the relationship between treatment energy and antimicrobial activity observed here aligns with the general

Table 3. Inactivation of *L. monocytogenes* 5672 under Different PEF Parameters: Electric Field Strength, Inlet and Outlet Temperatures, Frequency, Total Energy, and Treatment Time

		L. monocytogenes 5672				
electric field (kV/cm)	T^0 inlet (°C)	T^0 outlet (°C)	frequency (Hz)	total energy (kJ/kg)	total treatment time (μs)	inactivation (log N_t/N_0)
15	25	40.57 ± 0.36	24.00 ± 1.96	72.45 ± 15.48	96.60 ± 9.51	−0.31 ± 0.11
		50.43 ± 0.56	33.00 ± 1.96	111.52 ± 9.49	132.72 ± 12.47	−0.58 ± 0.24
		60.70 ± 1.18	41.50 ± 2.94	152.26 ± 5.95	168.28 ± 13.79	−1.06 ± 0.40
		71.00 ± 1.08	48.00 ± 1.96	193.28 ± 3.12	198.24 ± 8.12	−2.28 ± 0.09
	30	40.07 ± 0.73	14.50 ± 2.94	48.03 ± 14.68	61.60 ± 7.26	−0.33 ± 0.17
		50.10 ± 0.34	26.00 ± 1.96	87.09 ± 6.19	103.88 ± 11.46	−0.55 ± 0.26
		60.30 ± 0.3	35.50 ± 0.98	128.82 ± 4.80	143.92 ± 10.43	−0.83 ± 0.30
		70.10 ± 0.41	42.00 ± 1.96	169.84 ± 4.29	175.56 ± 5.03	−1.99 ± 0.21
	35	40.53 ± 0.35	8.50 ± 0.98	24.57 ± 3.40	33.04 ± 5.73	−0.37 ± 0.07
		50.17 ± 0.33	20.50 ± 0.98	65.18 ± 3.05	80.08 ± 12.04	−0.68 ± 0.06
		60.47 ± 0.35	30.50 ± 0.98	107.18 ± 1.83	121.52 ± 13.11	−1.08 ± 0.09
		70.30 ± 0.49	38.00 ± 1.96	144.43 ± 8.18	151.76 ± 16.08	−2.17 ± 0.18
20	25	40.80 ± 1.26	12.50 ± 0.98	66.04 ± 5.27	50.12 ± 5.24	−0.70 ± 0.31
		50.53 ± 0.28	18.50 ± 0.98	106.73 ± 1.19	74.48 ± 6.74	−1.23 ± 0.26
		60.03 ± 0.28	22.50 ± 0.98	146.44 ± 1.19	89.88 ± 9.36	−1.81 ± 0.27
		70.03 ± 0.99	26.50 ± 0.98	188.24 ± 4.13	108.64 ± 5.73	−4.61 ± 0.34
	30	40.57 ± 0.43	8.50 ± 0.98	44.17 ± 1.79	34.72 ± 3.06	−0.67 ± 0.18
		50.97 ± 0.92	14.75 ± 0.49	87.64 ± 3.85	59.78 ± 4.42	−1.22 ± 0.24
		60.63 ± 1.4	19.00 ± 1.96	128.05 ± 5.84	77.56 ± 6.47	−1.98 ± 0.09
		70.67 ± 0.86	22.75 ± 0.49	169.99 ± 3.58	96.46 ± 2.14	−4.44 ± 0.44
	35	40.97 ± 0.75	5.25 ± 0.49	24.94 ± 3.15	20.58 ± 3.12	−0.66 ± 0.07
		50.63 ± 1.14	11.10 ± 0.20	65.35 ± 4.78	44.24 ± 3.84	−1.32 ± 0.28
		60.57 ± 0.28	16.50 ± 0.98	106.87 ± 1.19	66.36 ± 6.23	−1.93 ± 0.33
		70.53 ± 0.51	20.50 ± 0.98	148.53 ± 2.13	85.96 ± 2.39	−4.06 ± 0.47
25	25	40.63 ± 0.46	9.10 ± 0.20	52.46 ± 1.91	34.44 ± 6.59	−0.96 ± 0.30
		50.47 ± 0.52	12.50 ± 0.98	106.45 ± 2.18	48.44 ± 8.30	−1.58 ± 0.30
		61.07 ± 0.83	15.50 ± 0.98	150.76 ± 3.49	61.88 ± 6.74	−2.12 ± 0.35
		71.10 ± 1.08	17.50 ± 0.98	192.70 ± 4.51	72.52 ± 3.06	−4.70 ± 0.24
	30	40.53 ± 0.26	5.50 ± 0.98	34.82 ± 0.65	21.28 ± 4.29	−1.07 ± 0.28
		49.93 ± 0.17	9.25 ± 0.49	83.32 ± 0.72	36.82 ± 4.15	−1.54 ± 0.37
		61.07 ± 1.9	13.05 ± 0.10	129.86 ± 7.93	52.36 ± 4.39	−2.23 ± 0.36
		70.37 ± 0.62	15.05 ± 0.10	168.73 ± 2.61	63.84 ± 1.65	−4.25 ± 0.15
	35	39.47 ± 2.22	3.25 ± 0.49	18.37 ± 9.30	12.46 ± 2.62	−0.79 ± 0.13
		51.27 ± 0.57	8.05 ± 0.10	67.99 ± 2.38	30.80 ± 5.49	−1.96 ± 0.42
		61.00 ± 1.01	11.05 ± 0.10	108.68 ± 4.20	43.40 ± 5.49	−2.53 ± 0.59
		70.50 ± 0.52	13.25 ± 0.49	148.39 ± 2.17	55.58 ± 1.20	−4.88 ± 0.19

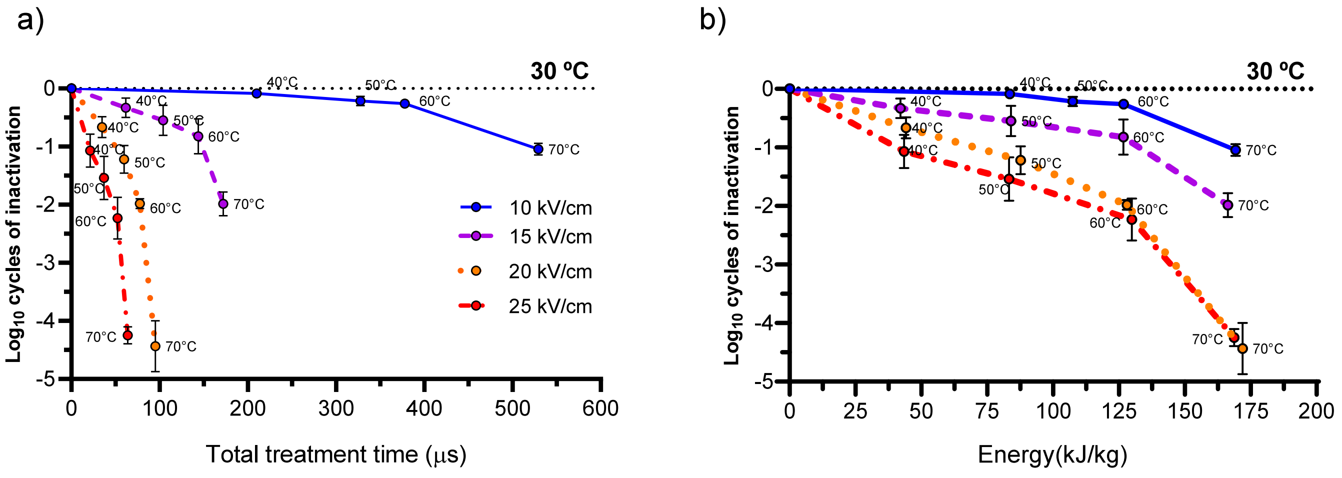

Figure 3. *L. monocytogenes* 5672 inactivation under pulsed electric fields (PEF). (a) Log_{10} reduction as a function of total treatment time and (b) as a function of total specific energy, for electric field strengths of 10, 15, 20, and 25 kV/cm. Each curve is identified by its corresponding color, and the outlet temperature reached at each condition is indicated next to the data points. All measurements were taken immediately after PEF treatment (0 h). The inlet temperature of the juice was 30 °C.

mechanism described by Thamsuaidee et al.[31] for ultrasound-enhanced antimicrobial films, where delivering higher energy to the target system increases the extent of microbial damage. Although their study focused on a solid matrix, the principle of cumulative physical stress—whether mechanical or electrical—leading to increased membrane disruption remains applicable. In our case, higher specific energy, derived from increased field strength and pulse duration, resulted in greater *E. coli* O157:H7 inactivation, particularly when combined with outlet temperatures exceeding 60 °C.

3.2. Effect of PEF Treatment on *L. monocytogenes* 5672 Inactivation

The results in Table 3 indicate that microbial inactivation increased consistently with outlet temperature and electric field strength, demonstrating a synergistic interaction between electrical and thermal effects. At low intensities (10−15 kV/cm), reductions were limited (<2 log cycles), whereas higher fields (20−25 kV/cm) markedly enhanced inactivation, especially at outlet temperatures of 60−70 °C, where reductions ≥3−5 log cycles were achieved. The most pronounced effect occurred at 25 kV/cm and 70 °C, suggesting that *L. monocytogenes* is particularly susceptible to strong electric fields at elevated temperatures.

Compared with *E. coli* O157:H7, *L. monocytogenes* 5672 exhibited greater sensitivity to PEF under equivalent processing conditions. While *E. coli* O157:H7 required higher field strengths and temperatures to achieve comparable reductions, *Listeria* responded more rapidly to increased energy input, showing substantial decreases even under intermediate conditions. This differential response may be attributed to structural variations in their cell envelopes; the thick but less flexible peptidoglycan layer of *L. monocytogenes* facilitates irreversible electroporation when exposed to intense pulsed electric fields, whereas the outer membrane of *E. coli* O157:H7 provides additional protection at moderate intensities.

Figure 3a,b illustrates the inactivation behavior of *L. monocytogenes* 5672 in pineapple juice (pH 4.2 ± 0.3) treated with PEF at an inlet temperature of 30 °C under varying electric field intensities (10, 15, 20, and 25 kV/cm), as a function of total treatment time and specific energy input. In both cases, microbial inactivation increased markedly with higher outlet temperatures and field intensities, evidencing a clear synergistic effect between thermal and electrical factors. At 10 kV/cm, reductions remained below 1 log cycle even after prolonged exposure, indicating limited electroporation at low field strength.

Conversely, increasing the field intensity to 15 and 20 kV/cm substantially enhanced inactivation, particularly at outlet temperatures of 60 and 70 °C, achieving reductions up to 3−5 log. The strongest effect was observed at 25 kV/cm, where ≥5-log reductions were reached within 100 μs at 70 °C. As shown in Figure 3b, a similar trend was observed for specific energy: inactivation remained minimally below 50 kJ/kg but increased sharply beyond 100 kJ/kg, reaching complete or near-complete inactivation at >150 kJ/kg under the highest field intensities and outlet temperatures. Compared to *E. coli* O157:H7 (Figure 2), *L. monocytogenes* 5672 (Figure 3) exhibited greater sensitivity to PEF treatment under equivalent processing conditions. While *E. coli* O157:H7 required higher specific energies and outlet temperatures to achieve comparable reductions, *L. monocytogenes* 5672 displayed more pronounced inactivation at moderate field strengths and energy levels. Overall, these results confirm that *L. monocytogenes* 5672 is more susceptible to PEF-induced damage, particularly when combined with mild thermal effects, reinforcing the importance of optimizing both electric and thermal parameters to ensure microbial safety in acidic juice matrices.

The effectiveness of PEF treatment also depends on the physicochemical characteristics of the medium, particularly pH and conductivity. The juice used in this study had a pH of 4.2 ± 0.3, which favors electroporation efficiency. At low pH, the greater abundance of undissociated organic acids can permeate microbial membranes, leading to intracellular acidification and loss of metabolic function. This effect, combined with electrical stress, explains the greater inactivation observed for *L. monocytogenes* 5672 compared to *E. coli* O157:H7, in agreement with findings by Rezaeimotlagh et al.,[16] who reported a similar trend in cranberry juice at pH 2.49. Likewise, product conductivity influences current flow during treatment; higher conductivity facilitates energy transfer, reinforcing the electroporation process. Therefore, optimizing both pH and conductivity is essential to maximize PEF efficiency in juice matrices.

Conductivity is another crucial factor influencing PEF efficacy as it affects the flow of electric current through the product. High conductivity enhances energy transfer during the PEF treatment, thereby improving microbial inactivation. The interplay between pH and conductivity underscores the need to optimize these parameters to maximize the PEF efficiency in juice processing.

The outlet temperature significantly influences the inactivation of *L. monocytogenes* 5672 (Table 2 and Figure 2). As the outlet temperature increased, a corresponding reduction in microbial load was observed, with the most pronounced change occurring at temperatures above 60 °C. This observation aligns with the findings of Kantala et al.,[18] who investigated the inactivation efficiency of two bacteria (Gram-positive and Gram-negative) in orange juice (pH = 3.5) treated by PEF. In their study, they reported a similar trend, where the Gram-positive bacterium *S. aureus* showed lower resistance to inactivation, while *E. coli* also exhibited a significant reduction after treatment. Additionally, they employed a PEF treatment chamber similar to that used in this study.

Moreover, the same thermoelectric synergistic mechanism also explains the higher susceptibility observed in *L. monocytogenes*. Unlike *E. coli*, whose outer membrane can partially shield the cell from electrical stress in acidic matrices, *L. monocytogenes* possesses a thick but highly ordered peptidoglycan layer that transmits electric field gradients more efficiently, making it more prone to irreversible electroporation once the critical transmembrane potential is exceeded. Mild heating further enhances this effect by increasing membrane fluidity and reducing structural rigidity, thereby lowering the voltage threshold required for pore expansion. As a result, the strong inactivation achieved at ≥60 °C and ≥20−25 kV/cm in this study is consistent with the well-documented sensitivity of Gram-positive bacteria to combined thermal and electric stresses, confirming that *L. monocytogenes* inactivation follows the same synergistic principle observed for *E. coli* but with a lower energetic requirement.[32]

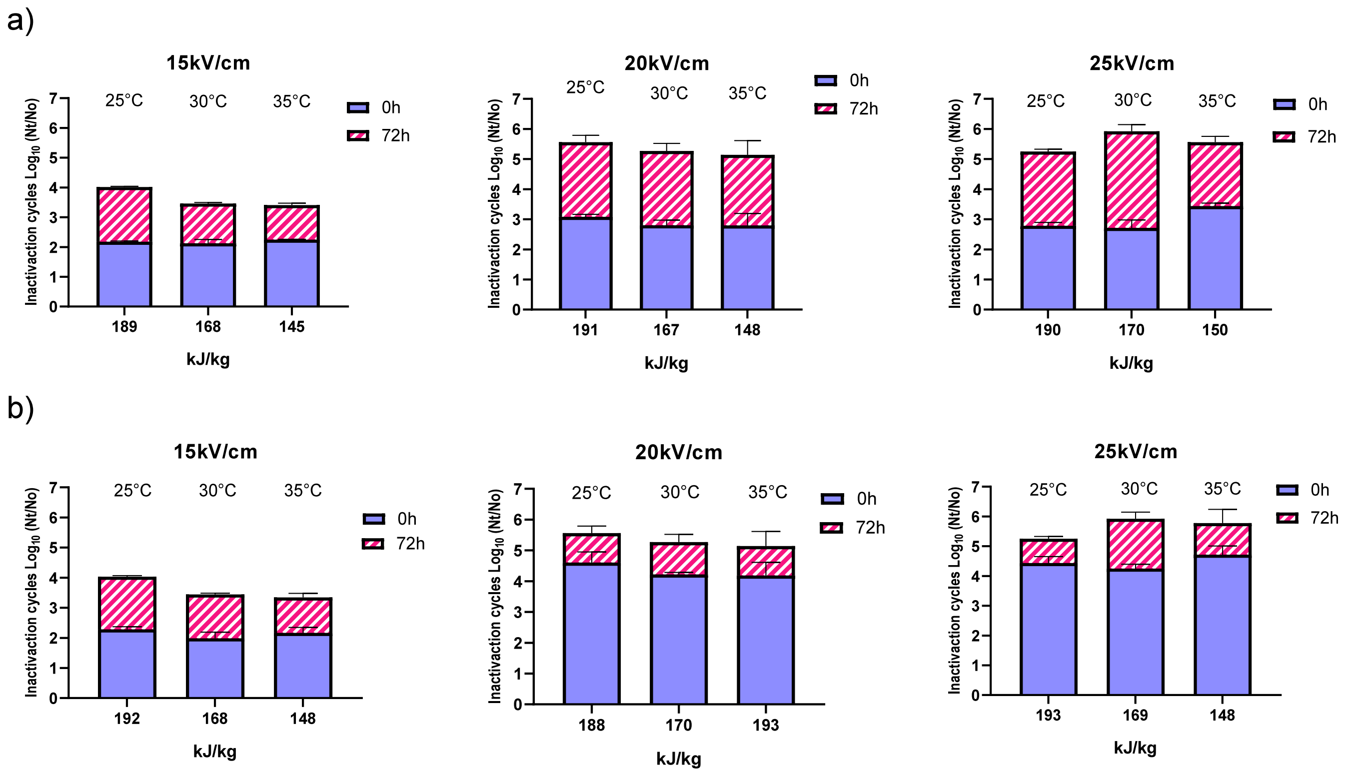

Figure 4. Comparative inactivation of (a) *E. coli* O157:H7 and (b) *L. monocytogenes* 5672 under pulsed electric field (PEF) processing. Bars represent microbial inactivation immediately after PEF treatment (0 h, blue) and after 72 h of refrigerated storage at 4 °C (pink). Results are shown for electric field strengths of 15, 20, and 25 kV/cm at inlet temperatures of 25 °C, 30 °C, and 35 °C. Total specific energy values (kJ/kg) corresponding to each condition are indicated below the bars.

3.3. Comparative Inactivation of *E. coli* O157:H7 and *L. monocytogenes* 5672 Inactivation after 72h of Treatment

As observed in Figures 2 and 3, both microorganisms responded effectively to PEF treatment, although their resistance patterns differed. Figure 4 provides a more detailed view of this effect at 0 and 72 h after the treatment, showing how the inactivation ($\log_{10}$ reduction) varies with electric field intensity (15, 20, and 25 kV/cm), treatment temperature (25, 30, and 35 °C), and the specific energy applied (ranging from 145 to 193 kJ/kg). At all tested conditions, *L. monocytogenes* 5672 exhibited higher immediate inactivation (0 h) than *E. coli* O157:H7, confirming its greater susceptibility to PEF. However, after 72 h of storage, both microorganisms showed additional reductions, evidencing a post-treatment lethal effect. This delayed inactivation was particularly noticeable in *E. coli* O157:H7, whose total reduction increased significantly during storage, especially at higher electric field intensities and temperatures. In contrast, *L. monocytogenes* 5672 displayed a smaller increase in inactivation over time, suggesting lower sensitivity to storage-related lethal effects.

This behavior can be attributed to structural and physiological differences between the microorganisms. The outer membrane of *E. coli* O157:H7 likely provides initial protection against PEF, resulting in lower immediate inactivation, but during storage, the acidic environment of pineapple juice (pH 4,2 ± 0,3) may promote the formation of undissociated organic acids that penetrate the *E. coli* O157:H7 membrane, intensifying sublethal damage and leading to a delayed inactivation effect, commonly referred to as the lethal after-effect. In contrast, *L. monocytogenes* 5672, despite being more susceptible to immediate electroporation, appears less affected by post-treatment sublethal injury due to its intrinsic physiological robustness.

Additionally, based on the total specific energy applied in each condition (145−193 $kJ\cdot kg^{-1}$), the contribution of the electric field to the outlet temperature can be estimated using eq 4. Considering a heat capacity of 4,2 $kJ\cdot kg^{-1}\cdot{}^{\circ}C^{1-}$ for pineapple juice, the expected PEF-induced temperature rise ranged from approximately 34.5 to 46.0 °C. These values align with the outlet temperatures observed in Figures 2 and 3, confirming that the moderate heating observed during PEF treatment is primarily attributable to ohmic (Joule) heating from the applied energy.

Overall, these results demonstrate that increasing the electric field intensity and post-treatment storage time enhances microbial inactivation, with *L. monocytogenes* 5672 showing a faster and more efficient response to PEF than *E. coli* O157:H7 under equivalent processing conditions. This difference reflects the influence of intrinsic microbial factors, such as cell size, shape, and membrane composition, on electroporation dynamics as Gram-positive and Gram-negative bacteria display distinct structural resistance to electric stress.

These findings are further supported by Demir et al.,[33] who highlighted the significant role of pH in modulating the effects of reversible electroporation and microbial metabolism during PEF treatment. Each microorganism presents a distinct electroporation threshold, and parameters such as medium composition, pH, and temperature influence both the extent of membrane permeabilization and the capacity for metabolic recovery. Although some cells may survive reversible electroporation, sublethal membrane damage can impair their shortterm viability and physiological functions, as noted by Wang et al.[34]

Complementary strategies to enhance PEF efficacy have been explored by Li et al.,[35] who assessed the combined effect of PEF, low pH (4,0), refrigeration (4 °C), and natural preservatives (e.g., tea polyphenols and natamycin) on the microbial and physicochemical stability of cantaloupe juice. This multifactorial approach resulted in significantly improved microbial inactivation and inhibition of recovery in sublethally damaged cells. The interaction between low pH and temperature creates a hostile environment that limits microbial repair mechanisms, while the addition of natural antimicrobials enhances membrane disruption. Tea polyphenols, known for their antioxidant and antimicrobial properties, and natamycin, an antifungal compound, synergized with PEF to reinforce irreversible damage to microbial cells.[36]

Additional evidence of electroporation stages was provided by Wu et al.,[36] who found that 34.3% of *E. coli* O157:H7 cells remained in a sublethal state after exposure to intense electric fields (800 V, 400 Hz, 50 °C), retaining enzymatic activity despite membrane damage. Their study demonstrated a progressive transition from intact cells to fully inactivated ones, with a 98.1% increase in cells entering the Q1 phase (dead cells) at 600 V and 400 Hz. This supports the model where cells pass through distinct states: viable, sublethally injured, and nonviable, depending on PEF intensity and environmental stressors.

Interestingly, both *E. coli* O157:H7 and *L. monocytogenes* 5672 displayed comparable inactivation immediately post-treatment at 15 kV/cm; however, during storage, *E. coli* O157:H7 exhibited greater sensitivity to increased field strengths (≥20 kV/cm), suggesting that post-treatment conditions can potentiate the antimicrobial effects of PEF. These observations reinforce the need to optimize PEF parameters not only at the point of application but also to account for storage conditions and the intrinsic resistance profile of the target microorganisms.

3.4. Estimation of Treatment Conditions to Achieve the Microbial Inactivation Target Using the Developed Polynomial Models

Finally, all of the experimental data for *E. coli* O157:H7 and *L. monocytogenes* 5672 inactivation treated with PEF were fitted to a quadratic polynomial model at 0 and 72 h after treatment. The model exhibited a high correlation coefficient ($R^2 > 0.9$), demonstrating a good agreement between the predicted and experimental data. A backward regression was used to eliminate the nonsignificant terms. Table 4 shows the coefficient models for each case.

Figure 5 illustrates the relationship between electric field intensity (kV/cm) and the specific energy (kJ/kg) required to achieve a 3-log reduction in *E. coli* O157:H7 and *L. monocytogenes* 5672 in pineapple juice for an inlet temperature of 30 °C, evaluated immediately after treatment (0 h) and after 72 h of storage. Across all conditions, *L. monocytogenes* 5672 (orange lines) consistently requires lower energy inputs than *E. coli* O157:H7 (purple lines), confirming its greater susceptibility to PEF treatment. After 72 h of storage, the energy required to achieve the same level of inactivation decreased further for both bacteria, suggesting a post-treatment lethality effect in which sublethally injured cells gradually lost viability. This phenomenon was particularly evident in *E. coli* O157:H7, where the energy difference between 0 and 72 h was more pronounced, implying a delayed inactivation response during storage.

Table 4. Coefficients (β_n) for the Polynomial Model (eq 1) That Describes Microbial Inactivation as a Function of Treatment Conditions Immediately after Treatment (0 h) and after 72 h at 4 °C

	E. coli O157:H7		*L. monocytogenes* 5672	
polynomial term	0 h	72 h	0 h	72 h
intercept	−12.4586	−8.4373	−8.056	−13.6106
X_1-electric field (V)	−0.1199	−0.2655	−0.5601	−0.6289
X_2-inlet temperature (°C)	0.6992	0.0339	0.6776	1.0704
X_3-energy (W)	0.097	0.1302	0.1089	0.1045
X_1*X_2	−0.0053	−0.0089	−0.0058	[a]
X_1*X_3	−0.0006	−0.001	−0.0014	0.0004
X_2*X_3	−0.002	−0.0023	−0.0019	−0.0027
X_1^2	0.0076	0.0145	0.0192	0.0129
X_2^2	−0.0078	[a]	−0.0076	−0.015
X_3^2	−0.0002	−0.0003	−0.0002	−0.0003
R^2	0.96	0.903	0.927	0.957
adjusted R^2	0.956	0.892	0.92	0.952

[a] Nonsignificant parameters ($p > 0.05$).

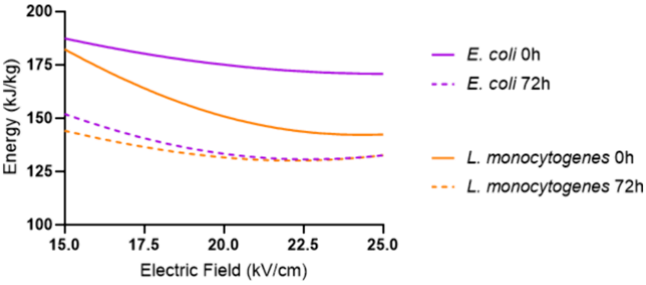

Figure 5. Relationship between electric field strength and the total specific energy required to achieve a 3-log reduction of *E. coli* O157:H7 and *L. monocytogenes* 5672 in pineapple juice. Curves show inactivation measured immediately after PEF treatment (0 h) and after 72 h of refrigerated storage at 4 °C, illustrating that both microorganisms require progressively lower energy inputs as the electric field intensity increases.

Figure 6 shows the relationship between electric field strength and energy needed to reduce 5 log cycles *E. coli* O157:H7 (Figure 6a) and *L. monocytogenes* 5672 (Figure 6b) in pineapple juice treated by pulsed electric fields (PEF) at inlet temperatures of 25, 30, and 35 °C, before treatment (0 h) and after 72 h storage.

For *E. coli* O157:H7 (Figure 6a), energy demand decreases as electric field strength increases, showing more efficient inactivation at higher fields. After treatment (0 h), the 5-log reduction occurred only at 35 °C, suggesting that higher temperatures aid cell membrane permeabilization and PEF effectiveness. After 72 h, the energy needed for microbial reduction decreased at all temperatures, especially at 35 °C, indicating cell weakening during storage. For *L. monocytogenes* 5672 (Figure 6b), the energy−field relationship showed a similar decline, with energy needs decreasing as the electric field increased. After treatment (0 h), a 5-log reduction was possible at all inlet temperatures, but energy consumption was higher at 25 °C, highlighting the synergistic effect of moderate temperatures on PEF cell disruption. After 72 h, the energy required decreased at all temperatures, especially at 35 °C,

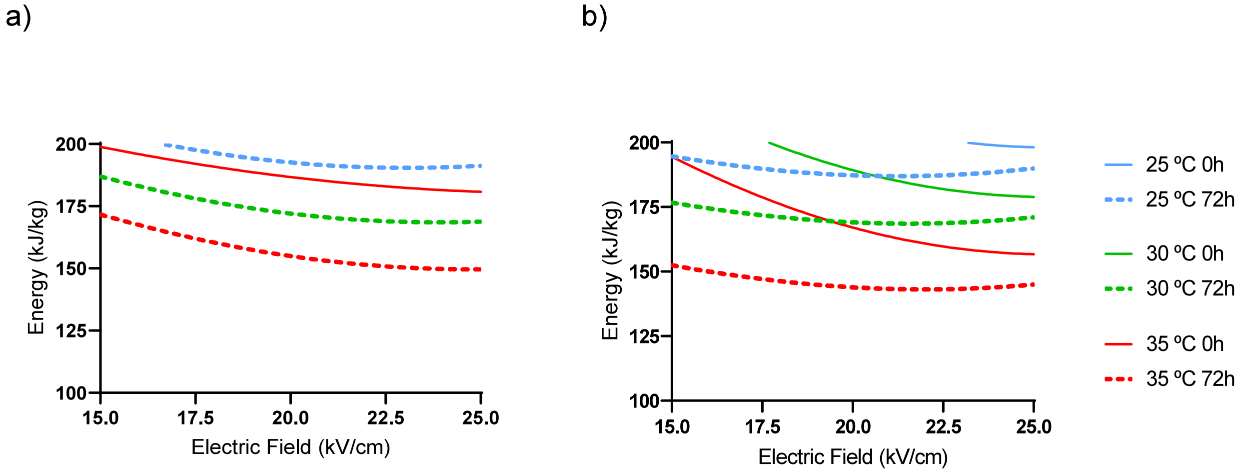

Figure 6. Total specific energy required to achieve a 5-log reduction of (a) *E. coli* O157:H7 and (b) *L. monocytogenes* 5672 in pineapple juice as a function of electric field strength. Solid lines represent inactivation measured immediately after PEF treatment (0 h), while dashed lines show the energy required after 72 h of refrigerated storage at 4 °C. Curves correspond to inlet temperatures of 25 °C (blue), 30 °C (green), and 35 °C (red), highlighting the combined influence of thermal preconditioning and electric field intensity on the energy needed to meet the 5-log reduction target.

suggesting that storage altered cell susceptibility, likely due to reduced membrane repair or changes in the cell wall over time.

FDA's Juice HACCP[37] regulation requires processors to demonstrate at least a 5-log reduction of the pertinent pathogen within a single facility and to maintain that reduction over shelf life. Figure 6 illustrates the estimation of operating conditions that allow achieving this standard for this acidic matrix, with a particular focus on *E. coli* O157:H7. In industrial deployment, validation will follow 21 CFR 120[38] by (i) selecting the pertinent organism (*E. coli* O157:H7 or a validated surrogate cocktail); (ii) operating within the effective region indicated by our models and prior literature—≥20–25 kV/cm, outlet temperature ≥60–70 °C, and specific energy ≥150–200 kJ/kg; and (iii) verifying *a* ≥5-log reduction sustained throughout shelf life, including moderate abuse conditions. Thus, Figure 6 may help determine the appropriate conditions to guide Juice HACCP validation runs.[32]

The selected operating region exploits PEF's thermoelectric properties: moderate heating lowers the critical electric field required for irreversible electroporation by increasing membrane fluidity and promoting phospholipid phase transitions, thereby accelerating pore formation and molecular transport at a given pulse regime. These effects have been demonstrated experimentally and corroborated via finite-element simulations and are consistent with the steeper inactivation observed at ≥60 °C and ≥20–25 kV/cm in our data.[39]

Thermal preconditioning at 30–50 °C before PEF enhances microbial inactivation in dairy products.[10] Yan et al.[40] reported that higher temperatures notably reduced the electric field required to inactivate microorganisms such as *S. cerevisiae* and *E. coli* suspended in culture media. Landi et al.[41] found that the energy required for a 5-log reduction of *E. coli* decreased from 102 kJ/kg to 38 kJ/kg as the inlet temperature rose from 35 to 55 °C during PEF orange juice pasteurization.

The gradual decrease in energy requirements after storage also supports the idea that sublethal stress during storage can alter membrane integrity, thereby increasing susceptibility to electroporation. The present findings align well with current literature, confirming that combining moderate inlet temperatures (30–35 °C) with sufficiently high electric fields (>17.5 kV/cm) maximizes microbial inactivation efficiency while minimizing energy consumption.

Regarding microbial growth after PEF treatment, Delso et al.[2] evaluated the microbiological stability of red grape juice stored at 4 and 10 °C following a two-step PEF treatment. Juices that did not undergo PEF decontamination exhibited substantial yeast growth (>10^6 CFU/mL) within 10–15 days, together with a decrease in pH and soluble solids (°Brix), indicative of spoilage. In contrast, juices treated with the two-step PEF process maintained microbial populations, including aerobic mesophilic bacteria, yeasts, and molds, below the quantification limit (<30 CFU/mL) throughout the entire storage period.

Overall, the findings emphasize the importance of customizing PEF parameters to the target pathogen and matrix, considering pH, conductivity, field strength, specific energy, and thermal aspects profile. Under industrially relevant conditions for acidic pineapple juice (pH ≈ 4,2), PEF provides robust, pathogen-specific lethality: immediately after treatment, 25 kV/cm with an outlet temperature of 70 °C yielded ≈3-log reduction of *E. coli* O157:H7 and ≈5-log reduction of *L. monocytogenes* 5672, indicating that Gram-positive organisms are more susceptible to thermo-assisted irreversible electroporation. After 72 h at 4 °C, a clear post-treatment lethal effect further increased *E. coli* inactivation, enabling ≥5-log reductions within a practical operating window of 20–25 kV/cm, ≥ 60–70 °C, and ≈150–190 kJ/kg, while *L. monocytogenes* consistently maintained ≥5 log without substantial additional decline.

Future process design should incorporate electric field strength, temperature, and the energy–time profile to ensure microbial safety and product quality. Temperature and electric field have independent effects but synergize, with higher temperatures increasing membrane fluidity and lowering the electric threshold for electroporation. Finally, coupling PEF with complementary hurdles (e.g., mild heat or natural antimicrobials) offers a promising route to sustainable, cost-effective, and product-specific preservation strategies.

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Notes

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