Research Article

Pulsed Electric Field Processing to Reduce Oxalate Content in Walur Tuber: Effects of Electric Field Strength, Soaking Time, and Sequential Treatments

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Abstract
Walur tubers contain high levels of oxalates, which raise nutritional concerns and necessitate their reduction. Pulsed Electric Field (PEF) treatment is a non-thermal technology that applies high-voltage pulses to enhance mass transfer in plant tissues, thereby facilitating the release of oxalates from intracellular matrices. This study aimed to determine the optimal PEF conditions for reducing oxalate levels by evaluating the effect of electric field intensity, soaking time, and sequential treatments. Tuber samples were subjected to various PEF treatments, after which total oxalate content was quantified using HPLC, while structural changes were examined using SEM and TEM. The results showed that an electric field of 3.32 kV/cm effectively reduced oxalate content, while a 5-minute soaking significantly enhanced oxalate diffusion. Two consecutive PEF treatments also resulted in a reduction of oxalate levels, although this decrease was not as significant as that achieved through the combination of PEF and soaking. SEM analysis revealed fractures in calcium oxalate crystals and the formation of debris, while TEM analysis showed the cellular damage after PEF treatment at 1.08 kV/cm, indicating irreversible electroporation. The optimal conditions for oxalate reduction in walur tubers were achieved through a PEF treatment at 1.08 kV/cm combined with a 5-minute soaking. This condition yielded the lowest oxalate levels, recognized as the most energy-efficient option, resulting in statistically similar reduction of oxalate (p-value > 0.05) to that achieved with higher field strengths. These findings suggest that PEF is a promising and sustainable method to enhance the quality and safety of walur tubers.

Keywords: Calcium oxalate crystals, Oxalate reduction, Pulsed electric field, Sequential PEF, Walur tuber

1 Introduction

Walur (*Amorphophallus campanulatus* or *A. paeoniifolius*) is a tuberous plant belonging to the Araceae family. Tubers from this family often face limitations in utilization due to the high oxalate content. In Indonesia, there are two varieties of *A. paeoniifolius*, namely *A. paeoniifolius* var. hortensis, known as Suweg, and *A. paeoniifolius* var. sylvestris, referred to as walur [1].

Unlike suweg, which has long been utilized as a traditional food and cultural plant in rural communities of West Java [2], walur tubers remain underexplored. They are often considered weeds due to their high oxalate concentration. Fresh walur tubers contain a total oxalate content of 3.6059 g/100 g [3], which is approximately six times higher than that found in taro (*Colocasia esculenta* L. Schoot), with a total content of 0.6940 g/100 g [4]. Oxalate can cause itching and irritation upon consumption, reduce

calcium bioavailability in the body, and contribute to the formation of kidney stones. Therefore, the high oxalate content in walur tubers greatly limits their potential as a safe and nutritious food source [5].

There are various methods for reducing oxalate content. Boiling and soaking can reduce soluble oxalates in leafy vegetables by 30–67%, with the extent of reduction depending on the duration of treatment [6]. Similarly, [7] discovered that blanching and cooking diminished both total and soluble oxalate levels in taro tubers from North Sumatra by 18–52%. A recent study on taro tubers (*Colocasia esculenta*) using 6% NaCl soaking for 120 min achieved about 58.7% reduction [8]. Various strategies for managing oxalic acid in foods have been reviewed. The result showed that there is none of these processing interventions such as soaking, boiling, blanching, and fermentation can simultaneously reduce soluble oxalate levels while maintaining mineral bioavailability and nutritional quality [9]. Their review emphasizes the importance of combining traditional and emerging processing approaches to minimize oxalate-related health risks.

In addition to these traditional methods, non–thermal processing techniques, such as the Ultrasound–assisted method, can reduce oxalate content in elephant foot yam (*Amorphophallus paeoniifolius*) up to 43.2% [10]. Pulsed Electric Field (PEF) can minimize oxalate content in Oca tubers by 25–45% while maintaining tissue integrity [11]. These results highlight the importance of developing mild and energy–efficient processing methods for tropical tubers to improve food safety and expand their industrial applications.

PEF preserves nutrients and tissue quality more effectively than thermal methods, such as boiling or blanching [12]. Research has also indicated that PEF improves extraction efficiency and mass transfer more effectively than methods based on mechanical or thermal processes [13]. Moreover, consecutive PEF applications can enhance tissue permeability over time, providing improved control and adaptability in optimizing the process [12]. These benefits emphasize PEF as an effective and low–impact method for lowering oxalate levels in plant-derived materials.

A PEF is a non–thermal food processing technology that employs high–voltage electric pulses to treat biological materials positioned between two electrodes for a short time. The application of an electric field to biomaterial cells induces structural changes and increases the size of the membrane pores. This phenomenon, known as electroporation or electropermeabilization, enables both the entry of molecules into cells and the release of biomolecules from within the cell [14].

Cell electroporation is divided into two types, namely reversible and irreversible [15]. Reversible electroporation is widely applied in clinical biotechnology and molecular biology to facilitate the introduction of drugs, antibodies, or plasmids into the cytoplasm in vivo. This process typically occurs within an electric field range of 0.5 to 1.5 kV/cm.

Irreversible electroporation in plant or animal tissue occurs at electric field intensities 1.0–3.0 kV/cm, while microbial inactivation requires higher electric field strengths, ranging from 15–40 kV/cm [16]. In food processing, particularly for solid plant–based matrices, the irreversible electroporation range of 1 to 3 kV/cm disrupts cellular membranes without inducing thermal effects. This process eliminates the cellular turgor component responsible for texture and modifies the tissue's viscoelastic properties [17].

PEF treatment is frequently employed to enhance the release of functional compounds during extraction or drying processes. It has been successfully applied as a pre–treatment to improve the extraction of cellular metabolites from plant tissue. PEF treatment at 0.25–0.75 kV/cm increased carotenoid extract in tomato by 188% [18]. A pretreatment at 5 kV/cm boosted the polyphenol extract by 10% and antioxidant activity by 9% [19]. Additionally, oxalate levels in oca tubers decreased by 50% with PEF pretreatment at 0.3–1.2 kV/cm [11]. This result showed that optimal electric field strength varies by sample and the desired outcome.

A study employing a system dynamics model demonstrated that PEF treatment with an electric field strength of 7 kV/cm and a pulse width of 40 μs effectively induced electroporation in animal cell organelles, extending its effects to the cell nucleus [20]. Calcium oxalate is found in almost all plant tissues, localized within specialized cells known as idioblasts [21]. Therefore, it is expected that applying PEF treatment to walur tubers can facilitate the reduction of the oxalate content.

To further enhance the removal of oxalates, the application of Pulsed Electric Field (PEF) treatment, depending on the applied electric field strength, can be combined with a brief soaking step. This method leverages the increased tissue permeability induced by PEF, thereby facilitating more efficient diffusion of oxalates into the wet medium. Recent research

underscores soaking as an effective technique for diminishing soluble oxalates in plant tissues [9]. These findings support the notion that combining PEF with brief soaking can lead to more rapid and efficient oxalate reduction while preserving tissue integrity.

Furthermore, the efficiency of PEF–assisted processes can be further enhanced through sequential or repeated PEF treatments, which strengthen the electroporation effect. The research indicated that consecutive PEF applications on beef muscle improved membrane disruption and mass transfer without causing excessive thermal damage [22]. This suggests that sequential PEF may further augment the permeability of plant tissue, thereby promoting greater oxalate diffusion during soaking.

Nonetheless, the use of PEF to decrease oxalate levels in walur tubers, along with the optimization of parameters such as electric field strength, soaking duration, and sequential treatment, has not been explored before. The objective of this study was to investigate the effects of varying electric field strengths, soaking time, and sequential PEF treatment to determine optimal conditions for reducing oxalate contents in walur tubers. This study presents the first application of Pulsed Electric Field (PEF) technology to diminish oxalate content in walur tubers that systematically assesses the effects of electric field strength, soaking duration, and sequential PEF treatments. The combination of PEF with brief soaking periods introduces an energy–efficient strategy that enhances oxalate leaching compared to traditional thermal methods, all while preserving tissue integrity.

2 Material and Method

2.1 *Material*

PEF equipment configured in a batch treatment system was used to process walur tubers. The treatment chamber had a depth of 50 mm, with consistent electrode spacing at 3.5 cm, 2 cm, 1 cm, and 0.5 cm. Analytical instruments included a Hantek DS05102P digital oscilloscope with high-voltage probes, slicers, analytical balances, tray dryers, a High–Performance Liquid Chromatography (HPLC) system, polarization microscopes, Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM), conductometers, and various glassware for analysis. The primary materials used in this study were walur tubers obtained from Saradan, Madiun, East Java; oxalic acid standards; membrane filters; H_2SO_4; HCl; and Milli–Q water.

2.2 *Methods*

2.2.1 Effect of electric field strength on oxalate content in walur tuber

Distilled water with a conductivity of 3 nS/cm was added to the PEF chamber until the tuber slices were fully submerged. The tubers were cut into pieces measuring 30 × 5 mm with a thickness of 2 mm. Each treatment was conducted in triplicate as technical replicates. Three separate samples were taken from the same homogenized batch of walur tubers. Air bubbles were removed to ensure the uninterrupted flow of electric current between the electrodes. The total weight of the walur tuber plus water was 17.25 g.

The pulse shape, characterized as a rectangular monopolar wave, was monitored in real time using a Hantek digital oscilloscope. The treatment parameters included an electric current of 0.38 A, a 10 s treatment time with average pulsed width 9.8 µs and average frequency 836 kHz. An electric field strength of 0 (Untreated), 0.6, 1.08, 2.05, and 3.32 kV/cm. Each treatment resulted in energy specific at 0, 2,487 ± 60, 3,747 ± 813, 7,463 ± 260, and 9,204 ± 688 kJ/kg, respectively. After treatment, each piece of walur tuber was removed from the chamber and designated for further analysis.

2.2.2 Effect of soaking time after PEF treatment on oxalate content

After determining the electric field conditions that effectively reduced oxalate levels in the previous stage, this electric field was used to examine the effect of soaking time on oxalate content. Walur tubers cut to the specified dimensions were subjected to PEF treatment using the optimal electric field for 10 s. Following treatment, the samples were immersed in a soaking medium for predetermined durations of 2.5, 5, 10, 15, and 20 minutes. The tuber pieces retrieved from the treatment chamber after each soaking interval were then collected for oxalate content analysis [23].

2.2.3 Effect of Sequential PEF treatment on oxalate content

This study investigated the effects of both soaking time and the number of treatments on oxalate content.

Initially, samples were subjected to PEF treatment under optimal electric field conditions. After each treatment, the samples were removed and re–treated using a fresh medium. The effects of varying the number of treatments—specifically 0, 1, 2, 3, and 4 cycles—were examined. To support these observations, tuber samples collected from the treatment chamber were subsequently analyzed for their oxalate content [23].

2.2.4 Evaluation of oxalate content

Immediately after PEF treatment, each tuber sample was removed from the PEF chamber. Total oxalate extraction was performed [23] by homogenizing 5 g of accurately weighed, chopped samples with 2 M HCl. The mixture was then placed in a water bath at 80 °C for 15 min. After cooling, the solution was transferred to a 100 mL volumetric flask and brought to volume with 2 M HCl. The mixture was centrifuged at 3000 rpm for 15 min, and the resulting supernatant was carefully filtered through a 0.45 µm cellulose acetate membrane. A 5 µL aliquot of the filtrate was then injected into an HPLC system equipped with a UV detector set at 210 nm. Chromatographic separation was carried out using a 7.8 mm Bio–Rad Aminex ion–exclusion column (HPX-87H) under isocratic elution at a flow rate of 0.6 mL/min, with 0.0125 M H_2SO_4 as the mobile phase. The analytical column was maintained at ambient temperature. Quantification of oxalic acid in each sample was performed using external standards.

2.2.5 Structural analysis

Polarized Light Microscope

Before observation, the microscope was calibrated using Köhler illumination to ensure optimal and uniform light distribution. The polarizer and analyzer were positioned in a crossed configuration (at a 90° orientation) to enhance the contrast of birefringent structures. The thin section specimens were examined under both low and high magnifications, using objective lenses ranging from 100× to 1000×. Images were captured with a digital camera attachment for documentation and further analysis.

Scanning Electron Microscope (SEM)

Walur tuber pieces treated with PEF were cut into small portions and dried. Each sample was mounted on a circular aluminum stub and coated with platinum in a vacuum using a sputter coater. The sample morphology was examined using a field emission scanning electron microscope (FE-SEM, ZEISS SUPRA 55VP) equipped with energy–dispersive X–ray spectrometry (EDX). The microscope operated at an accelerating voltage range of 0.2–30 kV, with a beam current of up to 400 nA and a vacuum level between a few pA and 300 nA. In this study, an accelerating voltage of 3 kV was used for imaging, while 15 kV was applied for EDX analysis, except for husk samples, where 10 kV was sufficient.

Transmission Electron Microscope (TEM)

For TEM, ultrathin sections (80–90 nm thick) were cut using a diamond knife on an ultramicrotome (Ultracut N, Reichert) and placed on 300–mesh grids. The sections were stained with osmium tetroxide followed by uranyl acetate to visualize the internal ultrastructure of the tubers. The stained sections were then examined at an accelerating voltage of 100 kV using a TEM Talos microscope.

2.2.6 Statistical analysis

The statistical analysis of oxalate reduction in walur tubers was performed using a one–way ANOVA design with three replications for each treatment. Data obtained from the experiments were analyzed using SPSS software, applying a Completely Randomized Design (CRD) approach with a single–factor ANOVA with a Tukey's post hoc test.

3 Result and Discussion

3.1 *Effect of electric field on oxalate content in walur tuber*

The application of electric field treatment has been increasingly recognized as a promising pretreatment method for plant–derived materials. In the case of walur tuber, exposure to PEF is particularly significant, as it effectively reduces oxalate content. This antinutritional compound restricts the safe consumption and utilization of the tuber. Reducing oxalate levels is essential to ensure the safety of walur tuber for food use.

Table 1: Effect of electric field strength on oxalate content (g/100 g) of walur tubers.

No.	Electric Field (kV/cm)	Oxalate Level (g/100 g)	Oxalate Reduction (%)
1	0.0000	1.1453 ± 0.0833a	0
2	0.6200	0.6138 ± 0.0599b	46
3	1.0800	0.3221 ± 0.0237c	72
4	2.0500	0.2769 ± 0.0154c	76
5	3.3200	0.2319 ± 0.0158c	80

Values represent mean ± standard deviation (n = 3). Different superscript letters within the column indicate significant differences (p-value < 0.05). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple range test.

The PEF treatment applied to walur tubers using electric field strengths of 0, 0.6, 1.08, 2.05, and 3.32 kV/cm showed that increasing the electric field intensity enhanced oxalate reduction in the tubers (Figure 1). Treatment at 3.32 kV/cm achieved the most significant decrease in oxalate content, reaching up to an 80% reduction (Table 1). However, statistical analysis (p-value > 0.05) indicated no significant differences among treatments at 1.08, 2.05, and 3.32 kV/cm.

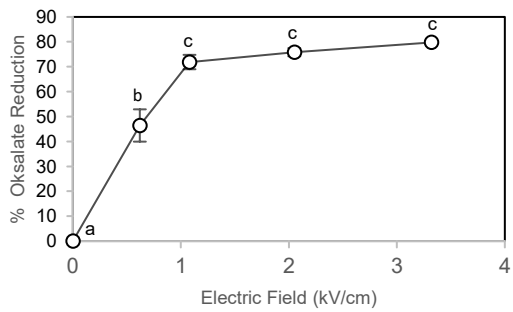

Figure 1: Effect of electric field strength on oxalate reduction. Error bars represent standard deviations of triplicate measurements. Results with different letters are significantly different (p-value<0.05).

This finding suggests that oxalate release had reached a saturation point at lower field intensities, consistent with the principle of irreversible electroporation [24]. Treatment at 1.08 kV/cm resulted in a 72% reduction in residual oxalate, lowering it to 0.3221 from 1.1453 g/100 g (Table 1). This reduction was statistically comparable to that achieved at higher field intensities. Therefore, 1.08 kV/cm was selected for further analysis as the minimum effective dose, as it provides energy efficiency while preserving tissue integrity [25].

This trend is consistent with previous findings as noted by [26], who found that increasing the external polarizing resistance in a microbial fuel cell enhanced the voltage while reducing current, and improved organic matter removal from compost leachate—highlighting the importance of optimizing electrical load in bioelectrochemical systems. This parallel supports the concept that electrical–parameter optimization is critical in systems where electrical input must be balanced with biochemical conversion efficiency.

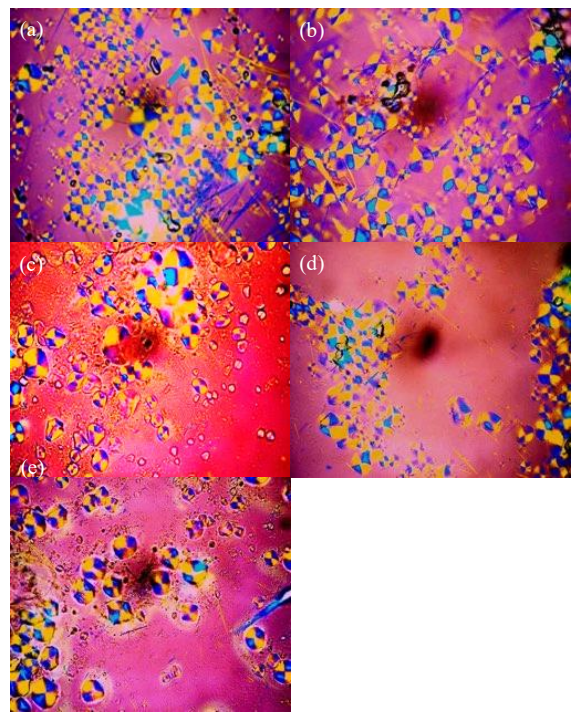

Figure 2: Polarized Light Microscope analysis of thin section walur tuber with 1000x magnification (a) untreated; (b) 0.6 kV/cm; (c) 1.08 kV/cm; (d) 2.05 kV/cm; (e) 3.32 kV/cm.

The optimized electrical condition identified above also corresponds with observable structural changes at the microscopic level. The findings were further supported by visual analysis using a polarized light microscope, as shown in Figure 2(a)–(e). The images revealed distinct differences in the abundance and morphology of calcium oxalate crystals between the control sample (Figure 2(a)) and those subjected to PEF treatment (Figure 2(b)–(e)).

The observations indicate that increasing the applied electric field corresponds to a progressive

decline in the crystals' abundance and size, with the lowest crystal density observed at 3.32 kV/cm. The calcium oxalate crystals in untreated walur tubers (Figure 2(a)) and those treated with PEF at an electric field strength of 0.6 kV/cm (Figure 2(b)) remain relatively dense and elongated. Whereas tubers exposed to 1.08 kV/cm to 3.32 kV/cm (Figure 2(c)–(e)) exhibit visibly fewer and smaller crystals, indicating substantial fragmentation and partial dissolution induced by the PEF. These qualitative findings align with the quantitative oxalate assessments, validating that the disintegration of crystals is related to the chemical reduction seen in the treated samples.

These results are further supported by the SEM analysis (Figure 3), which compares untreated tubers with those treated at 1.08 kV/cm. The SEM images demonstrate that walur tubers exposed to PEF treatment at 1.08 kV/cm contain fewer calcium oxalate crystals, which are smaller in size and show signs of fracturing. The fragmentation of calcium oxalate crystals during PEF treatment can be attributed to several concurrent mechanisms, including electroporation, ionic polarization, dielectrophoretic, and electrohydraulic phenomena such as streamer discharges and microcavitation, which generate localized impulse pressures.

The electroporation induced by PEF facilitates the movement of biomolecules across the cell membrane. External Ca^{2+} ions can enter the cytosol, and intracellular calcium is simultaneously released from the endoplasmic reticulum in pores created from PEF treatment [27]. Electroporation in plant tissues occurs at electric field strengths ranging from 0.8 to 3 kV/cm, with the specific values depending on factors such as pulse duration and cell size [28]. Transmembrane potential reaches around 1 V at these field intensities, which induces pore formation [24]. The solubility and stability of calcium oxalate crystals are influenced by several factors, including pH, Ca^{2+} ion activity, and the ionic strength of the surrounding medium [29]. Therefore, the ion redistribution triggered by electroporation can alter the local ionic composition around the cell or crystal, which promotes dissolution or weakens the crystal surface, thereby making the outer layer more susceptible to cracking.

Furthermore, an applied electric field can induce polarization ions and charges on the crystal surface, causing dielectric stress within the crystal lattice. For particles embedded in a biological matrix, this dielectrical stress translates into internal mechanical strain. When the internal voltage exceeds a critical threshold, the probability of crack initiation within the crystal increases. This phenomenon is analogous to the piezoelectric effect, in which the application of an electric field causes deformation in a non-centrosymmetric crystalline structure [30]. Experimental studies have also reported polarization in biological colloid, such as casein micelles, where electric field application resulted in increased zeta potential, reduced particle size, and an enlarged surface area [31]. Overall, these findings indicate that polarization induced by the electric field can generate sufficient lattice stress to cause microcracks in calcium oxalate crystals within plant tissues, aligning with the morphological evidence observed in this study.

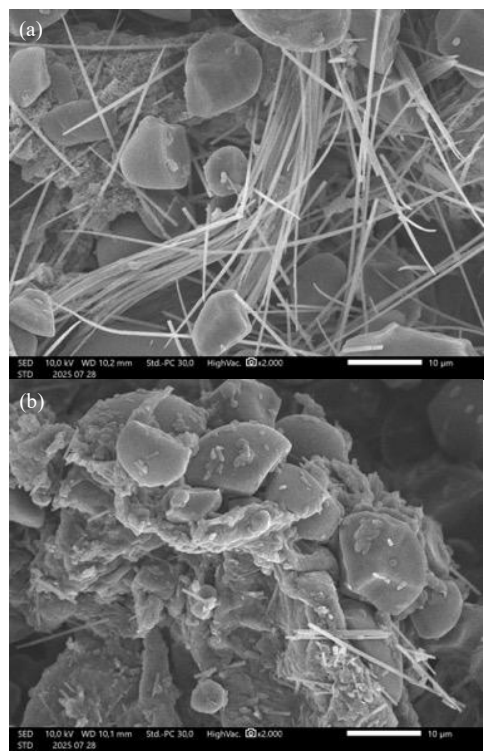

Figure 3: Scanning Electron Microscope analysis of coated walur tuber cell (a) untreated sample; (b) PEF treated sample at an electric field of 1.08 kV/cm.

When applied as a pretreatment for extraction, PEF induces structural damage to cell membranes, facilitating the release of intracellular compounds. Plant cells typically consist of a cell wall surrounding a plasma membrane, which encloses the cytoplasm.

The damage caused by PEF can be either reversible, allowing the membranes to reseal after treatment, or irreversible, leading to cell rupture or dissolution, depending on the specific electrical parameters used. [32]. Figure 3b shows that small fragments are deposited around and on the surface of starch crystals in walur tubers. These fragments are interpreted as cellular debris, resulting from the disruption of cellular structures caused by the PEF treatment.

This observation aligns with the characteristics of irreversible electroporation, in which membrane disruption leads to the accumulation of debris. For instance, SEM imaging of *B. pumilus* spores exposed to PEF at 7.5 kV/cm revealed morphological changes and the presence of cell debris. At the same time, TEM confirmed extensive internal damage to cellular compartments [33]. Similarly, irreversible electroporation (IRE) at high voltages is known to induce cell necrosis, leading to the accumulation of cellular and tissue debris, often accompanied by inflammatory responses [34].

The findings are further supported by TEM analysis (Figure 4), which highlights the differences in cellular structures between untreated walur tubers (Figure 4(a)–(c)) and those treated with PEF at 1.08 kV/cm (Figure 4(d)–(f)). In the control sample (Figure 4(a)), the cell wall appears intact, with well–organized lamellae and no visible damage. Starch granules, calcium oxalate crystals, and various organelles are observed adhering to the surface of the cell membrane.

In contrast, the PEF–treated samples shown in Figure 4(d)–(f) exhibit pronounced cracks in the cell wall, disorganized lamellae, and the formation of dark cavities resembling large pores. Certain areas show breakage with irregular edges, ranging from minor cracks to substantial fractures several tens of micrometers in size, devoid of starch granules, calcium oxalate crystals, or cellular organelles. These observations indicate that PEF–induced damage and pore formation facilitated the release of starch and calcium oxalate crystals from the cells, as discussed previously. Collectively, the findings from Figures 2–4 demonstrate that PEF treatment at an electric field strength of 1.08 kV/cm effectively induces irreversible electroporation in walur tubers.

The changes observed at an ultrastructural level under transmission electron microscopy (TEM) suggest that PEF treatment caused irreversible electroporation in cellular membranes, enhancing mass transfer and the diffusion of molecules. Additionally, these microstructural modifications were also evident in the proximate composition of walur tubers, as demonstrated in ash, moisture, protein, carbohydrate, and fat levels. Table 2 showed that PEF treatment affected the water, ash, and fat content of walur tubers. Water content increased slightly after PEF treatment due to membrane electroporation, which facilitated water uptake during the soaking period.

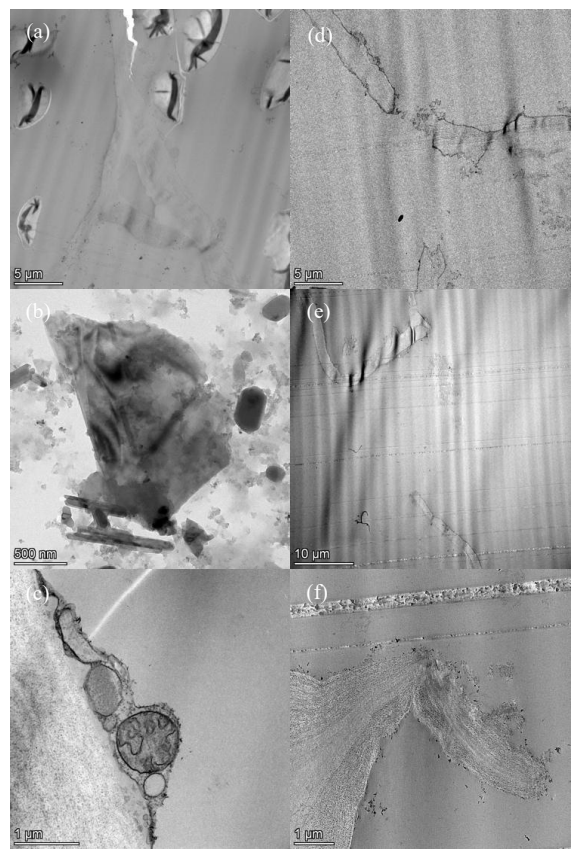

Figure 4: Transmission Electron Microscope (TEM) analysis of ultra–thin section walur tuber cells (a) untreated sample; (b) calcium oxalate crystal at an untreated sample; (c) organelle cell at an untreated sample; (d–f) treated sample at an electric field of 1.08 kV/cm.

Conversely, the ash content decreased due to the leaching of calcium oxalate crystals, which were the dominant mineral fraction in walur tubers, because of damaged cell membranes. Calcium oxalate forms mineral crystals within plant tissues [35]. As a biomineral, it likely contributes to the mineral fraction in proximate analysis. A similar mechanism has been reported in algal biomass, where PEF application

promoted mineral leaching and significantly reduced ash content due to enhanced membrane permeability and electropermeabilization–induced ion migration [36]. These findings collectively suggest that the reduction in ash observed in walur tubers arises from the release of calcium oxalate and other mineral components facilitated by PEF-induced electroporation.

Table 2 showed that fat content is decreased from 0.22% in the control to 0.13% after PEF treatment at 1.08 kV/cm, corresponding to a relative reduction of about 41%. This decline may be attributed to membrane destabilization and lipid migration following electroporation. Similar results reported [37] that PEF modifies the structure and organization of lipid bilayers in fish tissues, facilitating the release of membrane–bound lipids.

Table 2: Effect of electric field strength on oxalate content (g/100 g) of walur tubers.

No	Content	Control (g/100 g)	PEF 1 kV/cm (g/100 g)
1	Water	68.60	76.13
2	Ash	1.34	0.78
3	lipid	0.22	0.13
4	Protein	1.55	1.47
5	Carbohydrate	21.32	21.49

Values represent mean ± standard deviation (n = 3). Different superscript letters within the column indicate significant differences (p-value < 0.05). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple range test.

No significant changes were observed in the protein and carbohydrate content of walur tubers following PEF treatment at 1.08 kV/cm for 10 seconds (Table 2). Previous studies indicate that the effects of PEF on proteins and carbohydrates are highly dependent on the applied electric field and treatment energy. For example, [38] reported that PEF increases protein extraction by up to 11% and carbohydrate extraction by up to 105% from *Agaricus bisporus* at 2.5 kV/cm with a specific energy of 125 kJ/kg. Additionally, PEF treatment at 2.8 kV/cm with 3000 pulses of 20 µs by [39] showed an increase in proteins and reducing sugars from brewer's spent grains. These comparisons suggest that significant changes in protein and carbohydrate levels typically happen at field strengths exceeding 2 kV/cm or with higher specific energy inputs. Consequently, there are no alterations in carbohydrate and protein in this research, corresponding to a mild electroporation, where ionic and low–molecular–weight compounds, such as oxalates, are mobilized without notable modifications to macromolecular nutrients.

Oxalate content in oca tubers (*Oxalis tuberosa*) was reported to decrease by 50% when subjected to an electric field of 1.2 kV/cm [11]. The differences observed compared to the present study may result from several factors influencing electroporation, including electric field intensity, pulse shape, pulse width, treatment duration, pulse–specific energy, number of pulses, and frequency [24]. The present study employed a custom-built PEF generator that delivered monopolar square waves with pulse widths ranging from 400 ns to 19.4 µs and operated at frequencies between 313 and 1980 kHz for a duration of 10 seconds. In contrast, their study used bipolar square waves with a pulse width of 20 µs at a frequency of 50 Hz.

The quantitative assessment of pulse–specific energy indicates that the present treatment delivered a substantially greater cumulative electrical input than the conditions reported by Liu *et al*. [11], despite operating at a comparable field strength (1.08 kV/cm versus 1.2 kV/cm). This difference reflects the higher pulse frequency and longer treatment duration applied in the current protocol, resulting in a markedly higher total energy exposure to the tissue. Previous studies have shown that nanosecond pulsed electric fields (nsPEFs), ranging from 30 to 500 ns, can penetrate intracellular compartments and induce effects beyond the plasma membrane. In contrast, more extended pulse widths, such as 100 µs, primarily affect the plasma membrane [40]. Frequencies in the 1–6 kHz range enable high throughput while maintaining effective permeabilization and minimizing thermal damage [41], and spectra from 1 kHz to 1.9 MHz had increased membrane permeability, reduced the electrical resistance, and altered the impedance of plant cells [12]

In addition to electric field strength, pulse width, and frequency, pulse polarity plays a significant role in electroporation efficiency. Research shows that unipolar (monopolar) square pulses produce more substantial cumulative effects compared to bipolar pulses. This difference is attributed to bipolar cancellation, in which the second phase of a bipolar pulse counteracts the impact of the first phase, reducing overall membrane permeabilization. Consequently, electroporation efficiency decreases notably when using bipolar nanosecond pulses due to this cancellation effect [42].

3.2 *Effect of soaking time after PEF treatment on oxalate content*

In food processing, especially for solid plant-based matrices, an electric field in the range of 1–3 kV/cm can induce irreversible electroporation, causing nonthermal rupture of cell membranes. This process eliminates the cellular turgor component of the texture and alters the viscoelastic properties of the tissue [17]. PEF is commonly used to enhance the release of functional compounds during extraction or drying processes. The electroporation induced by the applied electric field disrupts cell membranes, allowing intracellular compounds to be released.

Based on the results presented previously, PEF treatment at an electric field of 1.08 kV/cm was selected as it represents the minimum effective dose, ensuring both efficiency and impairment of tissue integrity. Using this electric field strength, different soaking times were evaluated to optimize oxalate reduction. The soaking times analyzed in this study were 0, 2.5, 5, 10, 15, and 20 minutes following PEF treatment. From Table 3, it is known that the highest decrease occurred in the PEF treatment of 1.08 kV/cm, followed by soaking for 5 minutes, with a reduction of 84%, leaving oxalate in walur tubers as much as 0.1777 g/100 g

Table 3: Effect of soaking time after PEF treatment on oxalate content (g/100 g) of walur tubers.

No.	Soaking Time (s)	Oxalate Level (g/100 g)	Oxalate Reduction (%)
1	0	0.3221 ± 0.0237^{a}	72
2	2.5	0.3036 ± 0.0096^{ab}	73
3	5	0.1777 ± 0.0136^{d}	84
4	10	0.2354 ± 0.0241^{c}	80
5	15	0.2541 ± 0.0173^{bc}	78
6	20	0.3138 ± 0.0253^{a}	72

Values represent mean ± standard deviation (n = 3). Different superscript letters within the column indicate significant differences (p-value < 0.05). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple range test.

Figure 5 showed that soaking for 2.5 to 5 minutes reduced total oxalate levels, whereas soaking for 10 to 20 minutes increased oxalate content. PEF induces the formation of transient pores in the cell membrane, facilitating ionic movement and the influx of water via osmosis. This process enables small, water-soluble compounds, such as soluble oxalate, to rapidly exit the cells [25].

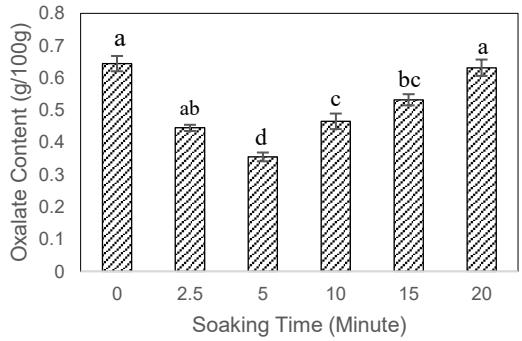

Figure 5: Effect of soaking time after PEF treatment (1.08 kV/cm) on oxalate content. Error bars represent standard deviations of triplicate measurements. Results with different letters are significantly different (p-value < 0.05).

The mechanism explains the early leaching of soluble oxalates, especially when the tissues remain immersed in water after PEF treatment, as soaking helps maintain the concentration gradient and prolongs mass transfer [26]. Following PEF treatment, compounds such as YO–PRO–1, propidium, and calcein can enter the cell, while calcein can also be released from it [25]. In contrast, calcium oxalate (CaOx) crystals are insoluble and contained within specialized vacuoles. Their release requires structural disruption of idioblasts, either through irreversible electroporation or subsequent mechanical disintegration [21].

Soaking can significantly reduce soluble oxalates by promoting their transfer into the soaking liquid. However, soaking primarily affects the soluble fraction and has little impact on insoluble calcium oxalate. Therefore, combining PEF treatment with soaking provides a complementary approach in which PEF disrupts cell membranes and enhances diffusion pathways, while soaking sustains the leaching process and prolongs the release of compounds.

Figure 5 shows that oxalate content increases between 10 and 20 minutes of soaking. Several mechanisms can explain this phenomenon. Following pore formation and initial ion efflux, the cell membranes remain partially electroporated, allowing bidirectional movement of small molecules. In this condition, metabolites such as oxalate ions may diffuse either from the soaking medium into the cells or from intracellular compartments into adjacent tissues, leading to a temporarily higher oxalate concentration detected in the samples [25].

The soaking process modifies the ionic and osmotic environment, as the soaking solution has different osmotic values and ion concentrations compared to intracellular fluids. This can promote the partial dissolution of insoluble calcium oxalate crystals or alter their hydration state, ultimately increasing the amount of oxalate detectable in the analysis [21]. Furthermore, prolonged soaking after PEF treatment softens the tissue matrix, potentially increasing the accessibility of oxalates that were previously bound or sequestered within cell walls or idioblast compartments. This process, in turn, leads to an apparent increase in the measurable total oxalate content [11].

Statistical evaluation demonstrated that the duration of soaking had a significant impact on oxalate levels (p-value < 0.05). The error bars displayed in Figure 5 illustrate the standard deviations derived from triplicate measurements, and varying letters signify statistically significant differences identified through one-way ANOVA followed by Tukey's test. The soaking procedure was conducted under regulated conditions at 28 °C with distilled water (conductivity = 3 µS/cm), ensuring that variations in oxalate levels were exclusively due to the soaking duration.

3.3 *Effect of sequential treatment on oxalate content*

The sequential application of PEF to a biological tissue can produce cumulative effects on membrane permeability. A single PEF treatment typically forms temporary nanopores that allow the release of small, water-soluble metabolites. However, applying treatments in rapid succession before the membrane fully reseals can enlarge existing pores or generate new ones, thereby increasing the extent of leakage. A theoretical framework indicates that continuous exposure can gradually transition cells from RE to IRE [43]. This phenomenon accounts for the observation that multiple treatments can enhance the diffusion of metabolites while simultaneously heightening the risk of structural damage.

PEF treatments were applied at the previously determined electric field strength of 1.08 kV/cm. Each treatment cycle used a fresh soaking medium to allow independent diffusion of the released compounds with 3 µS/cm of medium conductivity. During PEF treatment, the solid sample (1.25 g) was immersed in 16 mL of water while the temperature stayed within a non-thermal range.

The energy absorbed by the sample in this study was 3,747 ± 813 kJ/kg per cycle, which is substantially higher than the levels typically required to induce cell membrane permeabilization in plant tissues. Prior studies suggest that energy levels between 1 and 40 kJ/kg are sufficient to achieve reversible or partially irreversible electroporation, enhancing mass transfer without causing structural failure [44]. For example, [45] reported that reversible permeabilization occurred at approximately 2.5 kJ/kg in broccoli tissues, whereas [46] indicated that energy levels up to 80 kJ/kg in carrots led to irreversible cell damage and disruption of tissue structure, including degradation of phenolic compounds

The energy applied in this study exceeded reported thresholds by one to two orders of magnitude, resulting in irreversible electroporation. TEM micrographs (Figure 4) showed structural damage and lasting disruption of the membrane at an electric field strength of 1.08 kV/cm, suggesting that the treatment resulted in irreversible permeabilization instead of just temporary pore formation. This resulted in a permanent loss of membrane integrity and significant release of oxalate from idioblasts and other compartments. This concludes that the high energy input resulted in complete electropermeabilization. This explained the notable decrease in oxalate content during early treatment cycles. Table 4 shows the decline in oxalate content from the untreated sample, 1.1453 g/100 g, to 0.3221 g/100 g (72% reduction) after the first cycle. After the second cycle, it decreased to 0.2306 g/100 g (80% reduction), confirming that sequenced PEF exposures enhanced ion diffusion and promoted cumulative oxalate release.

Table 4: Effect of sequential PEF treatment on oxalate content (g/100 g) of walur tubers.

No.	PEF Treatment	Oxalate level (g/100 g)	Oxalate reduction (%)
1	0	1.1453 ± 0.0833^{a}	0
2	1	0.3221 ± 0.0237^{bc}	72
3	2	0.2306 ± 0.0260^{d}	80
4	3	0.3622 ± 0.0137^{b}	68
5	4	0.3299 ± 0.0126^{bc}	71

Values represent mean ± standard deviation (n = 3). Different superscript letters within the column indicate significant differences (p-value < 0.05). Statistical analysis was performed using one–way ANOVA followed by Tukey's multiple range test.

After the second cycle, the oxalate levels stopped decreasing linearly. Instead, a minor rise was

noted in the following treatments, suggesting that continuous or repeated exposure might modify the diffusion–precipitation balance within the tissue. This change probably indicates subsequent physical and chemical processes that occur after significant membrane disruption. Figure 6 showed that applying PEF three or four times resulted in a slight increase in oxalate concentration.

The increase in oxalate content observed during the third and fourth PEF treatments can be explained by several factors. First, crystal fragments generated during earlier PEF treatments may redistribute, with some debris remaining within the tissue or adhering to its surface, contributing to the measured oxalate in subsequent analyses. Second, the initial fragmentation produces small particles that do not dissolve immediately; these particles may detach from idioblasts in later treatment cycles, resulting in an apparent increase in oxalate content [21]. Third, changes in the ionic balance of the medium can trigger in situ recrystallization, where calcium ions released from the cell wall rebind to soluble oxalates, forming new crystals that contribute to an increase in the insoluble oxalate fraction [47].

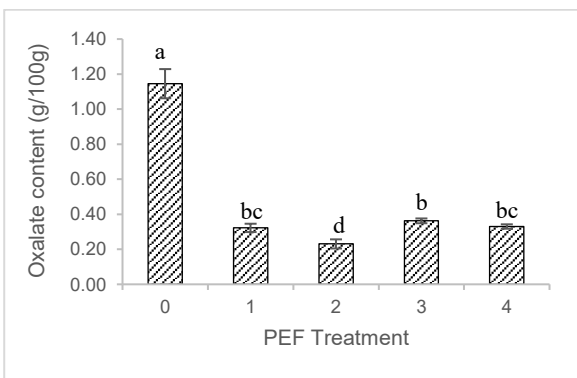

Figure 6: Effect of sequential PEF treatment (1.08 kV/cm) on oxalate content of walur tuber. Error bars represent standard deviations of triplicate measurements. Results with different letters are significantly different (p-value < 0.05).

Considering that a fresh soaking medium was used for each PEF cycle, the observed increase in oxalate concentration cannot be attributed to accumulation from previous solutions. Instead, the increase observed during the third and fourth PEF treatments can be interpreted as a gradual release and redistribution of oxalate forms within the tissue, rather than a contradiction of the initial diffusion mechanism. These findings suggest that the effects of repeated PEF applications are not linear but are governed by the dynamics of cell structure, the ionic conditions of the medium, and the physicochemical properties of oxalate.

A study on *Scutellaria baicalensis* found that applying two PEF treatments to the plant roots was optimal, resulting in higher flavonoid extraction while minimizing growth impairment [13]. However, a third PEF treatment not only promoted the release of specific metabolites but also induced increased physiological stress. Research on meat quality demonstrated that the first treatment cycle effectively disrupted protein structures and improved tenderness. In contrast, the second and third cycles did not consistently provide additional benefits and, in some cases, led to greater water loss [22]. These findings indicate a threshold of cellular tolerance for repeated PEF exposure: once pores are formed during the initial cycle, subsequent treatments can exacerbate cellular damage.

During the initial cycle, the application of an electric field increases transmembrane tension, creating temporary pores in the lipid bilayer. These reversible pores provide the initial pathways for intracellular compounds to diffuse out of the cell matrix [43]. Most of these pores remain relatively small, permitting only limited metabolite release. Following a subsequent PEF treatment, the existing pores typically enlarge and new pores form, enhancing membrane permeability. This process facilitates faster diffusion of metabolites and allows a broader range of compounds to be released.

4 Conclusions

PEF treatment effectively reduced oxalate contents in walur tubers. An electric field strength of 1.08 kV/cm was identified as the minimum effective dose, achieving results comparable to higher intensities while improving energy efficiency. Structural analyses using SEM and TEM confirmed that the treatment induced irreversible electroporation, facilitating oxalate release. When combined with a 5-minute soaking period, oxalate reduction was optimized, yielding the lowest oxalate content among all treatments. The application of two consecutive sessions also reduced oxalate levels, although this decrease was not as significant as that achieved through the combination of PEF and soaking. This finding suggests that although continuous exposure

can enhance the effectiveness of electroporation, the mechanism aided by diffusion during soaking plays a more substantial role in oxalate removal. From a broader perspective, these results highlight the potential of PEF as a non-thermal and energy-efficient method for improving the safety and nutritional quality of high-oxalate root and tuber crops. However, this research was conducted at a laboratory scale, and further studies are required to evaluate scalability, energy cost, and possible structural or textural changes during continuous operation. Future research should focus on optimizing pulse parameters, assessing industrial-scale feasibility, and exploring the applicability of this approach to other tuber varieties.

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Author Contributions

The role of each author is outlined as follows: RA conceived the idea, conducted the experiment, and wrote the initial draft of the manuscript. EHP, PH, FK, and ATS guided the conceptual framework, supervised the experiments, and provided critical revisions of the paper. All authors engaged in discussions about the results and contributed to the final version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors utilized the ChatGPT tool to enhance the language and readability of the manuscript.

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