



Pulsed electric field-assisted fermentation: a novel approach for the kombucha production

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ABSTRACT

Kombucha is a globally popular fermented beverage valued for its health-promoting properties, yet its traditional production is often hindered by slow and inconsistent fermentation kinetics. Non-thermal technologies, such as low-intensity Pulsed Electric Fields (PEF), offer a promising strategy to stimulate microbial activity without compromising product quality. This study investigated the application of low-intensity PEF to stimulate and accelerate kombucha fermentation. Seven PEF treatment conditions were screened to identify optimal parameters for inducing reversible electroporation in the Symbiotic Culture of Bacteria and Yeast (SCOBY). The most promising condition (1.14 kV/cm, 100 pulses, 17.78 kJ/kg) was evaluated over a 14-day period against an untreated control. Results demonstrated that optimised PEF treatment significantly accelerated microbial proliferation, with lactic acid bacteria and yeast counts reaching up to 2.5 log units higher than the control during early fermentation. This enhanced metabolic activity drove accelerated substrate utilization, resulting in a threefold increase in acetic acid and a 2.9-fold increase in ethyl acetate by day 14. Furthermore, PEF treatment positively modulated the volatile organic compound profile by enriching fruity esters and extending the enzymatic biotransformation of tea-derived polyphenols, preserving the beverage's high antioxidant capacity. In conclusion, low-intensity PEF serves as a promising lab-scale, precision tool capable of significantly modulate specific fermentation kinetics (sugar consumption and key metabolites production) while modifying its aromatic profile and preserving its nutritional and functional attributes.

1. Introduction

In recent years, the global food and beverage industry has witnessed a paradigm shift, moving away from ultra-processed staples toward functional, natural, and health-promoting alternatives (Hassoun et al., 2024; Shetty & Singh, 2025). This could also be translated into a real rediscovery of fermented products. Once valued primarily for their ability to extend shelf-life in the absence of refrigeration, fermented products, in particular beverages, are now known for their potential health benefits, particularly regarding gut health, immune system support, and enhanced bioavailability of nutrients (Ibrahim et al., 2023; Siddiqui et al., 2023; Skowron et al., 2022). The global market for fermented beverages is projected to experience robust growth over the next decade, driven by an increasingly health-conscious consumer base that

actively seeks products rich in probiotics, prebiotics, and bioactive compounds (Giri et al., 2023). The functional-fermented beverage sector has evolved beyond a niche market into a mainstream industry, needing scalable industrial solutions that preserve the nutritional integrity of traditional artisanal processes and reduce the processing time while maintaining the same quality standards.

Within this expanding landscape, kombucha has emerged as a standout category (Kim & Adhikari, 2020). A fermented tea beverage produced through the Symbiotic Culture Of Bacteria and Yeast (SCOBY), kombucha is recognized worldwide for its sensory profile and its rich composition of organic acids, polyphenols, and vitamins (Tran et al., 2020b), and for its health-promoting potentialities (Senthilkumar & Muthiah, 2026). However, the rapid industrialization of kombucha presents specific challenges. Traditional fermentation can be slow,

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inconsistent and lacks process reproducibility; indeed, it is quite difficult to control or understand what's driving quality, safety, and consistency at the microbial level (Singh & Kumar, 2025).

In order to address this gap, non-thermal technologies could be the answer. Ultrasound (US), Pulsed Electric Fields (PEF), High-Pressure Processing (HPP), Cold Plasma (CP), and Pulsed Light (PL) are emerging as versatile tools in food fermentation for their ability to control microbial development and enhancing the process without the detrimental heat effects of conventional methods (Souza et al., 2025).

Among these emerging technologies, PEF stands out for its versatility and potential to enhance the fermentation process. PEF involves the application of short, high-voltage pulses (10–80 kV/cm with microsecond to millisecond durations) to a food matrix placed between two electrodes. The applied electric field induces a modification in the microbial cell membrane, increasing the transmembrane potential and causing electroporation (Töpfl, 2006; Yan et al., 2025). Depending on the given electric field, the cell membrane could undergo reversible or irreversible electroporation.

Irreversible electroporation employs high-intensity electric fields (typically defined in the literature as >10 kV/cm, up to 80 kV/cm) to cause permanent, lethal damage to microbial cell membranes, leading to cell death and serving as a key mechanism for non-thermal microbial inactivation in food preservation (Saulis, 2010). Reversible electroporation involves using low-intensity electric fields which, according to the literature, are defined by electric field strengths generally below 5 kV/cm (Zand et al., 2021). Unlike high-intensity treatments, this specific low-intensity range (from 0.5 to 5 kV/cm) is insufficient to cause lethal membrane disruption (Yousfi et al., 2025). Instead, it creates temporary, nanoscale pores in cell membranes, allowing cells to survive and recover their integrity while facilitating beneficial processes like increased nutrient uptake and metabolic stimulation (Zand et al., 2021). Indeed, low-intensity PEF can have a variety of uses in the food sector, as reported by Demir et al., 2023. One interesting application is the enhancement and stimulation of the fermentation process. Indeed, low-intensity PEF treatments are known to induce a mild, transient cellular stress. To repair the electroporated membranes and restore intracellular homeostasis, microorganisms upregulate their central metabolic activity and nutrient uptake (Kanafusa et al., 2021; Miranda-Mejía et al., 2024). As recently highlighted, this physical stimulus triggers metabolic reprogramming that redistributes metabolic fluxes toward improved productivity, often relying on calcium-mediated signalling to provide the energetic support necessary for stress adaptation (Martins-Filho & Silva, 2026). This stress-response mechanism has been directly correlated with enhanced fermentation efficiency, as the increased metabolic activity required for membrane repair promotes increased substrate utilization and the overproduction of both primary and secondary metabolites. For example, PEF has been used to modulate stress-response pathways in lactic acid bacteria, thereby enhancing metabolite production without compromising cell viability. In *Lactiplantibacillus plantarum* during watermelon juice fermentation, PEF applied in the logarithmic growth phase increased L-lactic acid (19%), D-lactic acid (6.8%), and acetic acid (15%) in a voltage-dependent manner, with no negative effects on cell survival (Kanafusa et al., 2021). Similarly, PEF treatment of *Lactobacillus rhamnosus* and *Lactocaseibacillus paracasei* increased lactic acid production by 10% after 24 h and promoted the release of peptides and proteins relevant for postbiotic production (Miranda-Mejía et al., 2024).

To date, this non-thermal technology has been applied mainly as pasteurization beverages (inducing an irreversible electroporation) to few fermented beverages; reports are available about pasteurized beer, fermented beverages and kombucha (Vazquez-Cabral et al., 2016; Walkling-Ribeiro et al., 2011; Zhang et al., 2010). However, to the authors' best knowledge, there are no reports investigating the use of PEF on the kombucha microbial consortium to modulate its performance.

Therefore, this study aimed to investigate the application of low-intensity PEF as a fermentation-stimulation strategy in kombucha

production, evaluating its potential to modulate the fermentation kinetics while preserving or improving microbial activity, metabolite and biomass production and the overall beverage quality. In particular, this work explores the use of reversible electroporation to directly stimulate the SCOBY consortium, assessing its effects on fermentation kinetics, microbial dynamics, and the development of key chemical and volatile markers, positioning the study as a preliminary proof-of-concept for future industrial-scale studies.

2. Materials and methods

2.1. Reagents and standards

The Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), gallic acid ($\text{C}_7\text{H}_6\text{O}_5$), rutin ($\text{C}_{27}\text{H}_{30}\text{O}_{16}$), tannic acid ($\text{C}_{76}\text{H}_{52}\text{O}_{46}$), sodium acetate ($\text{C}_2\text{H}_3\text{O}_2\text{Na}$), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ethanol ($\text{C}_2\text{H}_5\text{OH}$), methanol (CH_3OH), ethyl acetate ($\text{C}_4\text{H}_8\text{O}_2$) and acetic acid (CH_3COOH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydroxide (NaOH), potassium chloride (KCl), sodium nitrate (NaNO_3), and aluminium chloride (AlCl_3) were obtained from Carlo Erba Reagents (Milan, Italy). The DPPH (2,2-diphenyl-1-picrylhydrazyl) was obtained from Glentham Life Sciences (Corsham, UK).

Buffered peptone water (BPW) and De Man–Rogosa–Sharpe (MRS) agar were purchased from Liofilchem Srl (Roseto degli Abruzzi, Italy). Dichloran Rose Bengal Chloramphenicol (DRBC) agar and Plate Count Agar (PCA) were obtained from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Acetobacter Agar was acquired from Microbiol Srl (Uta, Italy). All chemicals and reagents were analytical grade.

2.2. Kombucha preparation

The kombucha preparation was done following the Pompei et al. (2026) procedure. The kombucha base was prepared by infusing 1% (w/v) black tea leaves (Black Lemon, Teahouse Company) in boiling distilled water for 15 min. Following infusion, the leaves were removed using a stainless-steel sieve, and sucrose was added at a concentration of 7.5% (w/v). The sweetened tea was then cooled to 15 °C. To initiate fermentation, 25% (w/v) of SCOBY and 10% (v/v) of active pre-fermented kombucha (starter) were inoculated into the mixture.

The fermentation vessels were secured with paper covers and maintained in a dark environment at a controlled temperature of approximately 20 °C throughout the entire study. All measurements were performed at days 0, 1, 3, 7, 10, and 14 of fermentation, at room temperature (25 ± 2 °C), and in biological triplicate.

2.3. Pulsed electric field treatment

The PEF treatment was performed using a lab-scale PEF unit Mod. EPULSUS®-BM1A-15, which delivers a maximum output current and voltage of ± 15 kV and 200 A, respectively (Energy Pulse System, Lisbon, Portugal). A ratio of 1:5 (SCOBY:kombucha) was placed in a rectangular treatment chamber (4.5 cm length $\times$ 5 cm width $\times$ 2.5 cm height) consisting of two parallel stainless-steel electrodes (3 mm thick). The PEF treatments were applied to the samples at 25 °C after 24 h of SCOBY inoculum for all different samples. Bipolar square pulses were applied at field strength in the range of 1.14 kV/cm to 3.15 kV/cm with 20–200 pulses and specific energy ranging from 1.43 to 62.75 kJ/kg. The frequency of the treatment was maintained constant for all the trials (20 Hz). The total specific energy (W_{spec} , in kJ/kg) delivered to the samples was calculated using the following equation:

$$W_{\text{spec}} = \frac{n \cdot U \cdot I \cdot \tau}{m}$$

where U is the voltage (kV), n is the number of pulses, τ is the pulse

width (s), I is the current (A), and m is the mass of the sample, which is constant for all the treatments.

The pH and conductivity of the treated samples were measured before and after the PEF treatment. No significant differences in pH were observed among the treated samples, and the conductivity values were around 500 $\mu\text{S}/\text{cm}$ across all treatments. Based on the applied specific energies, the temperature increases due to the short duration of the pulse, and the treatment time was monitored and was negligible, ensuring that the observed effects on the microbial consortium were primarily attributable to the electric field-induced electroporation rather than thermal effect.

The characteristics of each treatment are described in Table 1.

2.4. Physicochemical analysis (pH, °Brix, color, SCOBY weight)

The pH was measured using a portable pH meter (Mettler Toledo, Columbus, UK) equipped with a liquid probe. Soluble solids content (°Brix) was determined using a digital refractometer (BM-095 Refractometer, USA). Color parameters were instrumentally evaluated according to the CIEL*a*b* method system using a portable colorimeter (Konica Minolta CR-410, Tokyo, Japan). pH was reported at three time points: initial (t_0), after 48 h ($t_{48\text{h}}$), and at the final sampling time point (t_f); while the Brix° at two different timepoints: initial (T_0) and final timepoints (T_f).

The SCOBY was filtered from the rest of the kombucha and weighed using an analytical weighing balance. The weight measurement was performed in duplicate, and the increase percentage was determined following the formula:

$$\% \text{increase} = \frac{W_f - W_i}{W_i} \times 100$$

where:

- W_f = final SCOBY weight
- W_i = initial SCOBY weight

Based on these parameters (pH, °Brix, color, SCOBY weight), three different PEF treatments were chosen for the further investigation. Finally based on the overall analysis (microbiological, chemical and physicochemical parameters) the most promising PEF treatment was selected for the final comparison with the untreated control sample.

2.5. Microbiological analysis (total mesophilic aerobic bacteria, mesophilic lactic acid and acetic acid bacteria, moulds and yeasts)

Each day of analysis, an aliquot of kombucha from each preparation, the untreated kombucha sample (Norm K.) and the PEF-treated kombucha sample (Pef K.), was sampled to quantify the total mesophilic aerobic bacteria count (TMAC), mesophilic lactic acid bacteria (LAB), moulds, yeasts and acetic acid bacteria (AAB) following the procedure previously described by Pompei et al. (2026).

To characterize the native microflora of the initial SCOBY, 20 g of the sample were aseptically homogenized for 1 min at 230 rpm in 180 mL of

BPW using a sterile Stomacher bag. Serial dilutions (10^{-1} – 10^{-8}) were prepared and plated as previously described (Pompei et al., 2026).

After incubation, plates containing 30–300 colonies were selected for enumeration. Microbial counts were expressed as CFU/mL for kombucha samples and CFU/g for SCOBY. All analyses were performed in triplicate.

2.6. Spectrophotometric analysis (DPPH, TPC, TFC, TTC)

All the spectrophotometric analysis have been carried out following the procedure highlighted in Pompei et al. (2025), and the absorbance has been measured using an Agilent Cary 8454 UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA).

Antioxidant activity was assessed using the DPPH radical scavenging assay; absorbance was measured at 517 nm, and the results were expressed as percentage of total antioxidant capacity.

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method, based on the oxidation of phenolic compounds by the Folin reagent under alkaline conditions (sodium carbonate). Absorbance was recorded at 765 nm, and results were expressed as mg gallic acid equivalents (GAE)/mL.

Total flavonoid content (TFC) was quantified using the aluminium chloride colorimetric method. Absorbance was measured at 415 nm, and results were expressed as mg rutin equivalents (RE)/mL.

Total tannin content (TTC) was evaluated using the Folin–Ciocalteu at 715 nm, with results expressed as mg tannic acid equivalent (TAE)/mL. All analyses were performed in triplicate.

2.7. HS-SPME-GC–MS quantification of acetic acid and ethyl acetate

The quantitative analysis of Acetic Acid and Ethyl Acetate was carried out using headspace solid-phase microextraction (HS-SPME) followed by gas chromatography–mass spectrometry (Agilent 8890 GC coupled to a 5977B MSD; Agilent Technologies, Santa Clara, CA, USA). After an equilibration time of 15 min at 60 °C, a CAR/PDMS-coated fiber (Supelco, Bellefonte, PA, USA) was exposed into the vial headspace for 30 min. The fiber was then manually introduced into the GC inlet for desorption, using a splitless straight liner (Agilent Technologies, Santa Clara, CA, USA). The injector temperature was set at 250 °C, and desorption was performed in splitless mode for 2 min. The fiber was kept in the injector for 15 additional minutes to ensure complete cleaning before the next extraction cycle. Helium was used as carrier gas, with a constant flow of 1.2 mL min⁻¹. Separation was achieved using a DB-WAX capillary column (60 m × 0.25 mm × 0.25 μm ; Agilent Technologies, Santa Clara, CA). The oven temperature program was set as follows: initial temperature 40 °C (held 3 min), ramped at 5 °C min⁻¹ up to 150 °C, then at 20 °C min⁻¹ up to 250 °C and held for 1 min. Ion source and quadrupole temperatures were 230 °C and 150 °C, respectively. Mass spectrometer operated in SIM (single-ion monitoring) using two-time windows: SIM group 1 from 0.00 to 18.99 min: m/z 43 (quantifier), 45 and 61 (qualifiers) and SIM group 2 from 19.00 to 22.00 min: m/z 43 (quantifier), 45 and 60 (qualifiers).

Quantification was performed using a five-points external calibration for each compound (Ethyl Acetate $R^2 = 0.9994$; Acetic Acid $R^2 = 0.9962$). Calibration standards were prepared using distilled water, and 3 mL of each solution was transferred into 20 mL headspace vials for analysis. Since calibration standards were prepared in distilled water rather than in a matrix-matched medium, a potential matrix effect cannot be excluded. However, since both samples share the same complex fermentation matrix, any matrix-induced bias affects both groups equally.

Each sample was analysed in triplicate.

2.8. HS-SPME-GC–MS analysis of volatile compounds (VOCs)

Volatile compounds were characterized following the methodology

Table 1

Experimental conditions used for pulsed electric fields treatments.

Trial	U (kV)	I (A)	n	τ (μs)	E (kV/cm)	Total W_{spec} (kJ/kg)
1	15.76	24	100	83.1	3.15	62.76
2	15.71	20	20	82.9	3.14	10.41
3	6.21	20	100	82.1	1.24	20.36
4	5.89	7.36	20	82.3	1.17	1.43
5	10.21	13.69	50	52.4	2.04	7.32
6	5.71	20	100	82.3	1.14	17.78
7	5.81	20	200	82.3	1.15	32.66

E = Electric field strength (kV/cm).

previously described (Pompei et al., 2026). For solid phase micro-extraction, a DVB-CAR-PDMS fiber (Supelco, Bellefonte, PA, USA) was chosen over the CAR-PDMS one to enhance the detection of a wider range of compounds. Mass spectrometer operated in SCAN mode with a mass range of 29–500 *m/z*. Compounds identification was performed using the NIST 20 library (National Institute of Standards and Technology, Gaithersburg, MD, USA). Each sample was analysed in triplicate. Volatile compounds were determined in semi-quantitative mode, and their relative abundances were expressed as a percentage of the total peak area of all identified compounds.

2.9. Scanning electron microscope (SEM)

The cellulosic pellicle formed was collected at the end of the fermentation, cut into thin slices and washed with double-distilled water. The washed cellulose fragments were centrifuged at 4500 rpm for 2 min (IEC CL10 Centrifuge, Thermo Fisher Scientific, Waltham, USA) and the supernatant was discarded. The sample were then freeze-dried and then before the analysis, were prepared adding a conductive thin film of chromium with QUORUM Q150T instrument. Once each sample had been treated, it was analysed with ZEISS SIGMA 300 FESEM (Field Emission SEM) microscope supplied with a Gemini column.

2.10. Statistical analysis

Statistical analysis was carried out on experimental data using XLSTAT statistical software (Addinsoft 1995–2024, VERSION 2021.4.1, France). The analysis of variance (ANOVA) was performed on all the analytical replicates on both samples (Pef K. and Norm K.) across the different fermentation times, with Tukey's post-hoc correction. While the *t*-test was conducted on the analytical replicates within the same day to compare the two groups (Pef K. and Norm K.). The significance level used was *p* ≤ 0.05.

3. Results and discussion

3.1. Identification of the best treatment conditions

The pretials were designed to evaluate the effect of different pulsed electric field (PEF) treatments through modulation of electric field strength and pulse numbers on kombucha fermentation performance, with the aim of identifying the most promising treatment conditions for further comparison with the untreated sample. Seven runs were assessed alongside a non-treated control sample, covering a wide range of field strengths (1.14–3.15 kV/cm) and pulse numbers (20–200), with corresponding specific energy inputs (1.43–62.76 kJ/kg), as detailed in Table 1.

Fermentation outcomes for all seven trials were evaluated through measurements of pH evolution, sugar content (°Brix), and SCOBY biomass gain. The selection of the trials was based on the highest SCOBY biomass formation and consistent acidification. Following this initial assessment, the three most promising samples were selected for more

detailed characterization; the choice for the best trial was based on the metabolite production and the increase in microbial growth.

As shown in Table 2, all trials exhibited a consistent decrease in pH across the fermentation period, from initial values around 3.46–3.56 (*t*₀) to final values in the range of 2.88–2.97 (*t*_f), reflecting the expected acidification driven by the SCOBY consortium. The control sample, however, showed a notably higher final pH of 3.11 ± 0.01, indicating a substantially lower degree of acidification in the absence of PEF stimulation. This suggests that even low-intensity PEF application exerted a measurable stimulatory effect on the metabolic activity of the microbial community, consistent with the reversible electroporation mechanism described for lactic acid bacteria in previous studies (Kanafusa et al., 2021; Miranda-Mejia et al., 2024).

In terms of sugar consumption (°Brix), all trials began at 7.9°Brix and reached comparable final values between 6.2 and 6.5°Brix, indicating broadly similar substrate utilization across the tested conditions, with no clear differentiation attributable to field intensity alone.

The weight increase of the SCOBY biofilm varied considerably between trials, ranging from 130 to 180%. The control achieved only an increase of 77%, confirming the essential stimulatory role of PEF in increasing cellulosic biofilm formation. Notably, Trial 1, which employed the highest field intensity (3.152 kV/cm) and the highest total specific energy (62.76 kJ/kg), did not yield proportionally superior biomass formation (156 ± 6%), possibly suggesting that beyond a certain energy threshold, additional PEF intensity does not translate into further enhancement of SCOBY biofilm growth and may instead cause irreversible membrane damage rather than beneficial reversible electroporation (Demir et al., 2023; Saulis, 2010; Vaessen et al., 2019).

Based on the obtained results, Trials 1, 5, and 6 were selected for further analysis, including total mesophilic aerobic bacterial counts and quantification of the principal fermentation biomarkers, ethyl acetate and acetic acid (Pompei et al., 2026).

TMAC (Total Mesophilic Aerobic Bacteria) were recorded at 2, 5, and 14 days for the selected trials (trials 1 = 3.15 kV/cm, 5 = 2.04 kV/cm and 6 = 1.14 kV/cm) and reported in Table 3. All PEF-treated trials demonstrated a substantially higher microbial population than the control at all time-points. By day 14, TMAC declined across all samples, a pattern consistent with progressive acidification and nutrient depletion during late-stage fermentation (Liao et al., 2024). Trial 6, corresponding to a moderate field intensity, maintained the highest final microbial count (3.28 log₁₀ CFU/mL), suggesting that this intermediate-

Table 3
TMAC (total mesophilic aerobic bacteria count) of the different trials.

Sample	TMAC (log ₁₀ CFU/mL)		
	2 day	5 day	14 day
Control	3.48	6.86	1.45
1	5.70	6.97	3.41
5	5.30	6.91	3.38
6	4.00	6.91	3.28

Table 2
pH, °Brix and SCOBY weight of the different trials.

Sample	pH			Brix°		% w increase
	<i>t</i> ₀	<i>t</i> _{48h}	<i>t</i> _f	<i>T</i> ₀	<i>T</i> _f	
Control	3.52 ± 0.05	3.35 ± 0.03	3.11 ± 0.01	7.9	6.4	77 ± 4%
1	3.46 ± 0.02	3.33 ± 0.03	2.88 ± 0.04	7.9	6.4	156 ± 6%
2	3.53 ± 0.03	3.36 ± 0.02	2.89 ± 0.05	7.9	6.4	183 ± 5%
3	3.53 ± 0.06	3.38 ± 0.05	2.97 ± 0.03	7.9	6.4	166 ± 3%
4	3.56 ± 0.04	3.37 ± 0.06	2.97 ± 0.01	7.9	6.4	136 ± 9%
5	3.52 ± 0.4	3.36 ± 0.01	2.91 ± 0.02	7.9	6.2	158 ± 3%
6	3.53 ± 0.02	3.35 ± 0.02	2.92 ± 0.04	7.9	6.4	163 ± 2%
7	3.52 ± 0.05	3.37 ± 0.01	2.96 ± 0.03	7.9	6.5	179 ± 4%

Results are expressed as mean ± standard deviation of three independent replicates.

energy treatment offered a sustained stimulatory effect without inducing excessive microbial stress.

The quantification of fermentation markers presented in Fig. 1 further reinforces the differentiation between treatment conditions. Ethyl acetate concentrations increased progressively over the 14-day fermentation period across all monitored trials. Trial 6 exhibited markedly higher ethyl acetate accumulation by day 14, reaching approximately 67 mg EA/kg, compared to the control (around 13 mg EA/kg). This elevated ester production in Trial 6 may reflect enhanced yeast metabolic activity resulting from optimised membrane permeabilization.

For acetic acid, Trial 6 again displayed a similar pattern, reaching concentrations of approximately 12,000 mg AA/kg at days 3 and 14, considerably higher than Trials 1, 5 and the control, which converged to lower values (3000–5000 mg AA/kg) by the end of fermentation. The pronounced acetic acid accumulation in Trial 6 is consistent with a stimulated *Acetobacter* activity and aligns with the findings of (Kanafusa et al., 2021), who reported voltage-dependent increases in acetic acid production following PEF treatment of lactic acid bacteria.

Scanning Electron Microscopy (SEM) analysis presented in Fig. 2, revealed that PEF treatments performed at electric field strengths between 1.15 and 3.14 kV/cm caused progressive alterations in the microstructure of the kombucha consortium. In particular, SEM micrographs qualitatively demonstrated a marked release and dispersion of lactic acid bacteria and yeasts from the SCOBY matrix with increasing electric field intensity. In contrast, Control image revealed cells that retained a smooth, intact surface morphology with no visible disruption. This phenomenon could be attributed to the electroporation-induced weakening of cellular and extracellular structures, which enhanced microbial availability in the fermentation broth. Therefore, the direct mechanistic link between this physical dispersion and the observed improvements in fermentation kinetics, while highly plausible, remains speculative.

The increased presence of metabolically active microorganisms in the liquid phase could potentially contribute to improved fermentation kinetics, as evidenced by the enhanced performance observed during kombucha fermentation. Therefore, the application of the field strengths applied in Trial 6 (1.15 kV/cm) within the studied range appears to

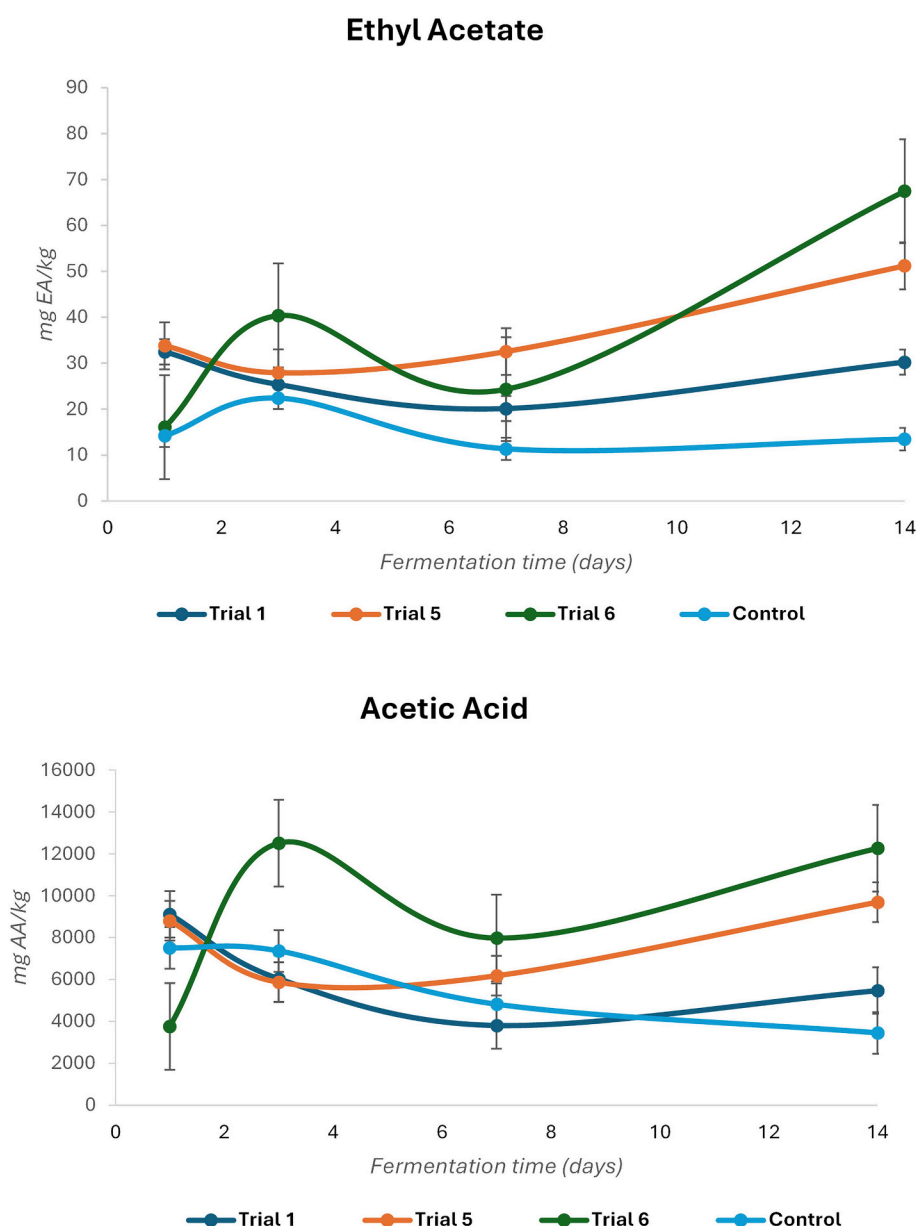


Fig. 1. Quantification of ethyl acetate and acetic acid in the different pretrials (Trial 1, 5, 6) and control sample.

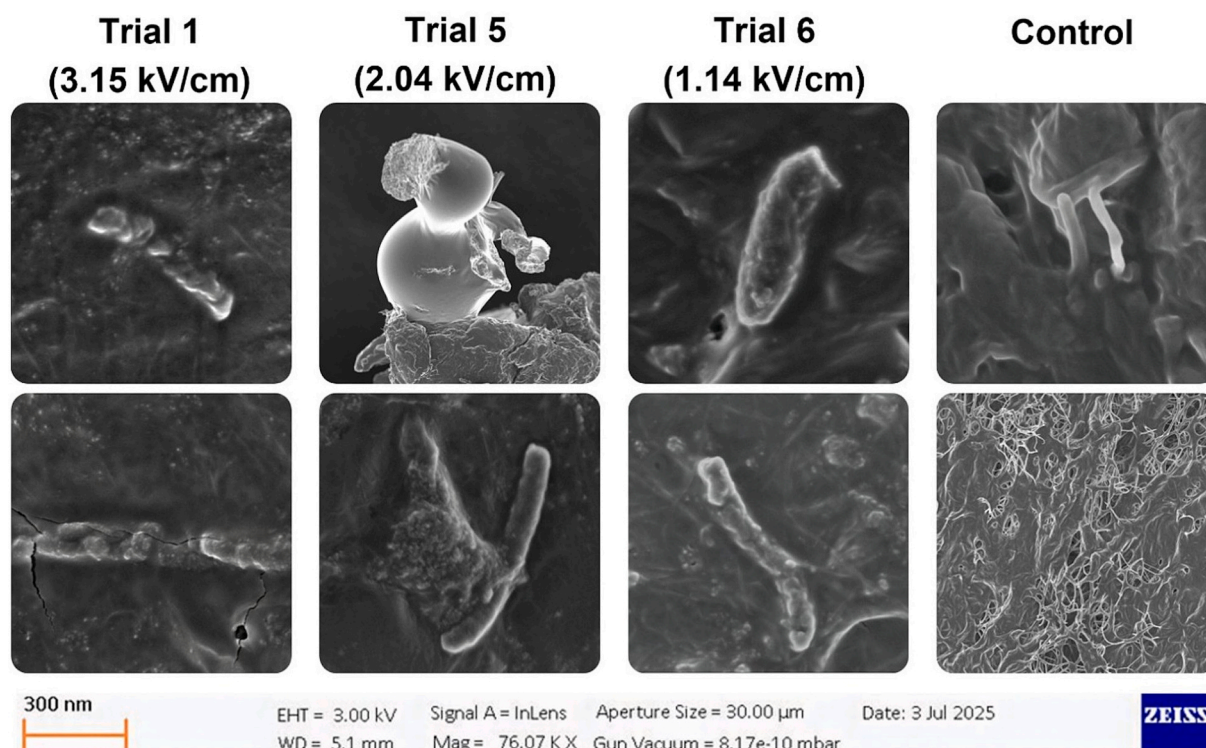


Fig. 2. Scanning electron microscope images showing PEF-treated SCOBY and untreated (control) SCOBY.

favour microbial accessibility and activity, which may support a more efficient fermentation process.

Overall, the fermentation kinetics, total microbial counts, key fermentation markers and SEM observations indicate that Trial 6 (electric field strength of 1.14 kV/cm over 100 pulses at total specific energy of 17.78 kJ/kg) represents the most promising treatment condition among those evaluated, combining consistent acidification, high biomass yield, sustained microbial growth, and the most pronounced accumulation of both acetic acid and ethyl acetate used as biomarkers of the fermentation process. This condition was therefore selected for further investigation in optimised fermentation trials.

3.2. Fermentation kinetics and bioactive compounds profile

Fermentation kinetics were monitored through pH and °Brix evolution (Figs. 3 and 4), while the bioactive compounds profile was assessed

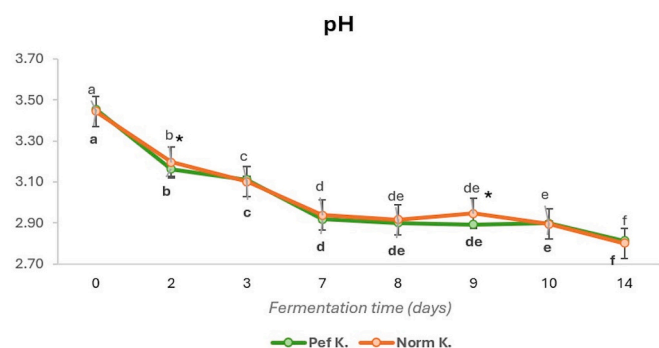


Fig. 3. Changes in pH values of Norm K. and Pef K. (Trial 6) during the fermentation period (Day 0, Day 1, Day 3, Day 7, Day 10, and Day 14). Different letters indicate statistically significant differences during the fermentation process for each type of kombucha. The asterisk (*) means statistical difference ($p \leq 0.05$) between the two formulations at each fermentation day.

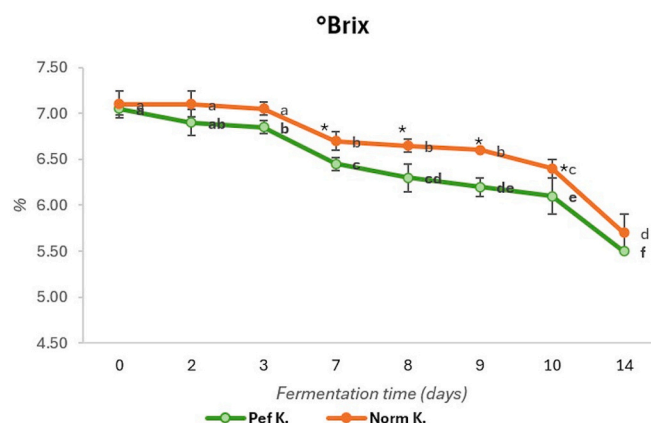


Fig. 4. Soluble solids content (°Brix) of Norm K. and Pef K. (Trial 6) during the fermentation period (Day 1, Day 3, Day 7, Day 10, and Day 14). Different letters indicate statistically significant differences during the fermentation process for each type of kombucha. The asterisk (*) means statistical difference ($p \leq 0.05$) between the two formulations at each fermentation day.

through DPPH radical scavenging activity, total polyphenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC) (Fig. 5).

As shown in Fig. 3, both samples exhibited a progressive and consistent decrease in pH throughout the fermentation period, starting from comparable initial values of approximately 3.46 (Pef K.) and 3.44 (Norm K.) at Day 0. The pH drop was rapid during the first three days, with both samples reaching approximately 3.18–3.20 and continuing to decline until Day 7, where pH values stabilised at 2.88–2.96; at the end of fermentation (Day 14), the pH decreased only slightly to about 2.80. Within each sample, statistically significant differences were observed between fermentation time points, as indicated by the letter groupings, confirming that the fermentation progressed meaningfully over time.

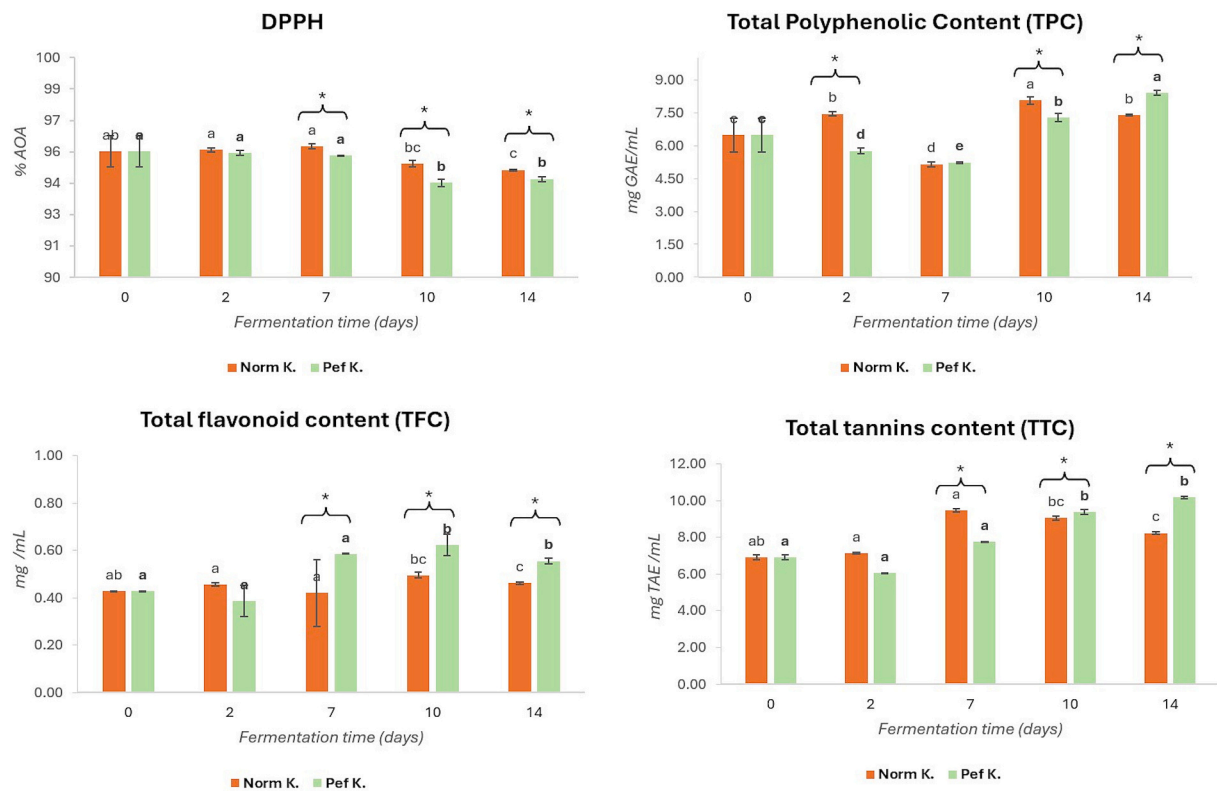


Fig. 5. DPPH radical scavenging activity, total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC) of Norm K. and Pef K. (Trial 6) during fermentation period.

Different letters indicate statistically significant differences during the fermentation process for each type of kombucha.

The asterisk (*) means statistical difference ($p \leq 0.05$) between the two formulations at each fermentation day.

Notably, no statistically significant differences between the two samples were detected at most time points, except for Day 2 and Day 9, where marginal but statistically significant differences were observed. These results indicate that the PEF treatment did not substantially accelerate the overall pH drop during kombucha fermentation. Thus, while pH and acetic acid concentration are related, they are not equivalent descriptors of fermentation; indeed, they exhibit a nonlinear relationship (Chong et al., 2024; Tran et al., 2020a). The observed pH evolution can be attributed to the buffering capacity of the kombucha matrix, particularly in the low pH range (2.8–3.1), where the system approaches an acidification plateau, and pH becomes relatively insensitive to further acid accumulation (Breidt & Skinner, 2022). The lack of a significant pH difference between Pef K. and Norm K. does not indicate similar metabolic activity but instead highlights the limited sensitivity of pH as the main measure of fermentation.

Related to the °Brix trend (Fig. 4), both samples started at similar initial values of around 7.1 and showed a steady, statistically significant decline throughout fermentation. From Day 7 onward, however, a clear difference appeared, with Pef K. consistently displaying lower °Brix values than Norm K. at each subsequent time point. This accelerated sugar consumption in the PEF-treated sample is consistent with a stimulation of microbial metabolic activity induced by reversible electroporation, which has been shown to enhance membrane permeability and nutrient uptake in fermentative microorganisms (Demir et al., 2023).

Related to the bioactive compounds profile (Fig. 5), the DPPH radical scavenging activity remained high throughout fermentation in both samples, consistently exceeding 94% antioxidant activity, reflecting the inherently strong antioxidant capacity of the tea polyphenol matrix (Jakubczyk et al., 2020, 2025). However, statistically significant differences between the two samples were observed at Days 7, 10, and 14, with Norm K. showing slightly but consistently higher DPPH activity

than Pef K. at these time-points. Despite this, the overall antioxidant capacity of Pef K. remained within a comparable range throughout fermentation, suggesting that the PEF treatment did not adversely compromise the antioxidant activity of the beverage.

Total phenolic content (TPC) increased in both formulations from Day 0 to Day 14, with a slight drop on Day 7. This trend mainly depends on early microbial biotransformation and release of phenolic compounds from the tea matrix (Liang et al., 2024). Results were in line with the literature, where a gradual increase in TPC content has been identified in the kombucha beverage (Kaewkod et al., 2019)(Teixeira Oliveira et al., 2023). Significant differences between Pef K. and Norm K. were observed at Days 2, 10, and 14, with Norm K. showing slightly higher values early on, while Pef K. exhibited a greater phenolic content only at Day 14. Suggesting that the PEF-treated sample may have extended the biotransformation of tea phenolic compounds into the late fermentation phase, ultimately yielding a slightly higher phenolic accumulation in the treated sample by the end of the fermentation period.

A broadly similar trend was observed for both total flavonoid content (TFC) and total tannin content (TTC). Both of the samples started at comparable TTC and TFC values at Day 0, Pef K. showed markedly higher levels from Day 7 onward till the end of fermentation. Thus, statistically significant differences were detected at multiple time points (Days 2, 7, 10, and 14 for flavonoids and Days 2, 7, 14 for tannins), with Pef K. consistently showing higher levels from Day 7 onward.

PEF treatment likely enhanced the release and transformation of tannins and flavonoids by increasing microbial membrane permeability, thereby stimulating SCOBY metabolic and enzymatic activity (Fauster et al., 2020). This improved nutrient uptake, metabolite exchange, and enzyme secretion, promoting the enzymatic biotransformation of tea-derived polyphenols. During fermentation, the combined activity of

yeast and acetic acid bacteria facilitates the degradation, conversion, and structural modification of these compounds. Concurrently, progressive acidification promotes hydrolysis, oxidation, and rearrangements, contributing to the sustained accumulation and transformation of tannins and flavonoids during the mid-to-late stages of fermentation (Chandran et al., 2026).

3.3. Microbial characterization of the kombucha sample

The microbial dynamics of the two kombuchas, namely Norm K. and Pef K. (Trial 6) were monitored over a 14-day fermentation period by tracking four key microbial groups: total mesophilic aerobic counts (TMAC), mesophilic lactic acid bacteria (LAB), acetic acid bacteria (AAB), moulds and total yeasts (TY) (Fig. 6). The initial microbial reference for both samples was provided by the SCOBY itself, which exhibited a high concentration of all four monitored groups, with counts in the range of 6.0–6.5 $\log_{10}$ CFU/mL for TMAC, LAB, and TY. In contrast, AAB counts were notably lower ($\sim 1.80 \log_{10}$ CFU/mL). This is consistent with studies showing that SCOBY communities are dominated by yeasts and bacteria, although their microbial composition remains complex and varies depending on the inoculum source, environmental conditions, and geographical origin (Harrison & Curtin, 2021; Wang et al., 2025).

As shown in Fig. 6, the two samples displayed markedly different TMAC from the very onset of fermentation. While Norm K. started from a near-zero count at Day 0 and increased gradually to approximately 2.6 $\log_{10}$ CFU/mL by Day 2 and 3.6 $\log_{10}$ CFU/mL by Day 7, Pef K. exhibited a substantially more rapid and pronounced growth, reaching approximately 5.9 $\log_{10}$ CFU/mL by Day 2 and peaking at approximately 6.3 $\log_{10}$ CFU/mL at Day 7. This 2.5 $\log_{10}$ unit difference at Day 2 was statistically significant ($p \leq 0.05$) and persisted throughout the whole fermentation. Following the peak at Day 7, TMAC counts declined in

both formulations through Days 10 and 14, consistent with the progressive accumulation of acetic acids and ethyl acetate and depletion of fermentable substrates during late-stage fermentation (Villarreal-Soto et al., 2019). The accelerated microbial growth in Pef K. during the early fermentation phase is consistent with the stimulatory effect of reversible electroporation on microbial membrane permeability, which has been shown to enhance nutrient uptake and promote metabolic activity in fermentative microorganisms (Demir et al., 2023; Kanafusa et al., 2021).

Mesophilic LAB growth closely mirrored the TMAC pattern; indeed, Pef K. showed faster growth, reaching 4.37 $\log_{10}$ CFU/mL by Day 2 compared with 2.57 $\log_{10}$ CFU/mL in Norm K., and peaked the highest amount at Day 7 (5.97 $\log_{10}$ CFU/mL in Pef K. and 3.41 $\log_{10}$ CFU/mL in Norm K.). Throughout fermentation, the PEF-treated sample consistently maintained LAB counts approximately 2 $\log_{10}$ units higher than untreated ones. The high mesophilic LAB counts sustained in Pef K. through the whole fermentation are of relevance from a functional food perspective, as LAB populations in kombucha have been associated with the production of beneficial metabolites including lactic acid, exopolysaccharides, and bioactive peptides (Siddiqui et al., 2023; Skowron et al., 2022).

The PEF-mediated stimulation of mesophilic LAB proliferation may thus represent an additional functional benefit of the treatment beyond merely accelerating fermentation kinetics. Notably, the stimulation of LAB proliferation and metabolic activity by low-intensity PEF aligns with previous findings by Kanafusa et al. (2021), who demonstrated that reversible electroporation significantly enhanced the growth and organic acid production of *Lactiplantibacillus plantarum* during watermelon juice fermentation. This consistency confirms PEF's role in modulating LAB across substrates and extends its possible positive effects to the complex SCOBY ecosystem.

Total yeast (TY) counts followed a broadly similar trend to that of

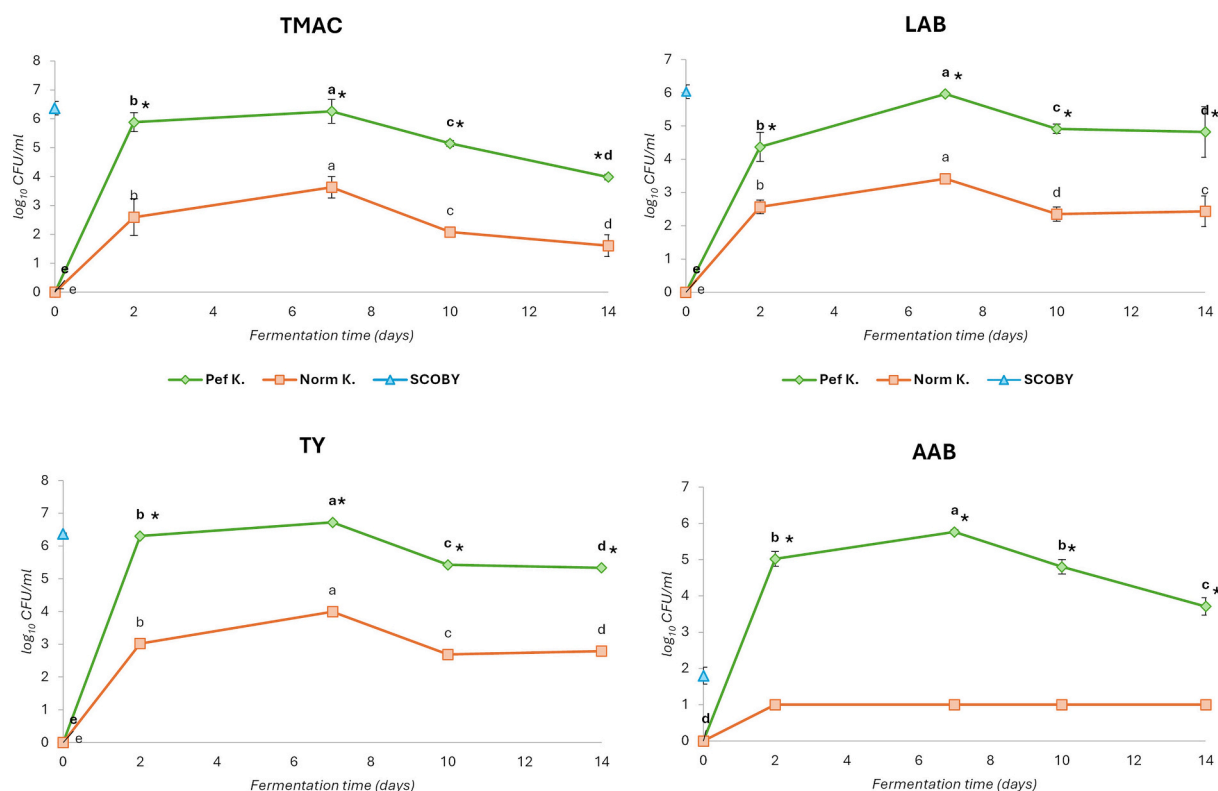


Fig. 6. Changes in microbial population of total mesophilic aerobic counts (TMAC), acetic acid bacteria (AAB), mesophilic lactic acid bacteria (LAB), and total yeasts (TY) in Norm K. and Pef K. (Trial 6) during the fermentation period.

Different letters indicate statistically significant differences during the fermentation process for each type of kombucha.

The asterisk (*) means statistical difference ($p \leq 0.05$) between the two formulations at each fermentation time.

TMAC and mesophilic LAB, with Pef K. again demonstrating substantially higher growth rates throughout fermentation. Pef K. reached approximately $6.1 \log_{10}$ CFU/mL by Day 2 and maintained this peak through Day 7 at approximately $6.4 \log_{10}$ CFU/mL. Norm K., by contrast, showed a more modest proliferation, reaching approximately $3.0 \log_{10}$ CFU/mL by Day 2 and approximately $4.0 \log_{10}$ CFU/mL at Day 7. Statistically significant differences between the samples were confirmed at Days 2, 7, 10, and 14. Following the Day 7 peak, TY counts declined in both samples through Days 10 and 14, with Pef K. maintaining counts approximately $2 \log_{10}$ units above Norm K. at each time point. The rapid and abundant yeast proliferation in Pef K. is consistent with the enhanced sugar consumption observed in the °Brix data, as yeasts are the primary drivers of sucrose hydrolysis and ethanol production, the first step in the sequential metabolic interplay that ultimately fuels AAB organic acid production (Tran et al., 2020a; Villarreal-Soto et al., 2019). This result is in line with the literature, in which PEF treatment enhanced the growth of *Saccharomyces cerevisiae*, resulting in 30% decrease of the fermentation time compared with the control samples (Mattar et al., 2015).

AAB dynamics revealed the greatest differences between the two samples. Thus, Norm K. maintained near-one $\log_{10}$ throughout the entire fermentation time with values consistently at or below the detection limit, likely reflecting the low initial concentration of AAB also in the starter SCOBY. In contrast, Pef K. showed a rapid and substantial increase in AAB counts from Day 0, reaching approximately $5.0 \log_{10}$ CFU/mL by Day 2 and peaking at approximately $5.5 \log_{10}$ CFU/mL at Day 7, before declining to approximately $4.5 \log_{10}$ CFU/mL at Day 10 and approximately $3.8 \log_{10}$ CFU/mL at Day 14. This pronounced stimulation of AAB growth in Pef K. is of key mechanistic importance, as genera such as *Komagataeibacter*, *Acetobacter*, and *Gluconobacter* are main producers of acetic acid and gluconic acid in kombucha and are responsible for SCOBY cellulosic pellicle formation (Marsh et al., 2014; Villarreal-Soto et al., 2019).

The AAB counts directly explain the increase in pellicle formation and high acetic acid concentration in the treated samples, furthermore it provides a coherent mechanistic link between PEF stimulation, AAB metabolic activation, and the enhanced organic acid production of Pef K. Furthermore, in literature there were only studies documenting the inactivation of acetic acid bacteria but not its possible enhancement, this highlights the novelty of this observation and identifies an important area for future research to clarify the mechanisms and potential applications of this phenomenon (Perminaitė et al., 2023; Teng et al., 2025).

The concurrent stimulation of both yeast and AAB populations in Pef K. suggests that PEF treatment enhanced the overall fermentative capacity of the SCOBY consortium in a synergistic manner, accelerating the entire metabolic cascade from sugar utilization through to organic acid accumulation.

3.4. Quantification of key fermentation biomarkers (ethyl acetate and acetic acid)

The volatile profile analysis revealed significant differences in the concentration and composition of key metabolites between the PEF-treated kombucha (Pef K.) and the control (Norm K.) after 14 days of fermentation.

As illustrated in Fig. 7, PEF treatment resulted in a substantial enhancement of both acetic acid and ethyl acetate production. At Day 0, both samples started from comparable levels (around 1860 mg/kg), reflecting the acetic acid content of the starter culture.

From Day 2 onward, statistically significant differences emerged between the two samples. Pef K. demonstrated accelerated acetic acid production and accumulation, reaching approximately 2400 mg/kg at Day 2 compared with 1200 mg/kg in Norm K. This difference became more pronounced over time. Thus, in the late fermentation stage (Day 10), Pef K. contained approximately 6000 mg/kg acetic acid, representing a 73% increase compared with Norm K. (~3500 mg/kg). By Day

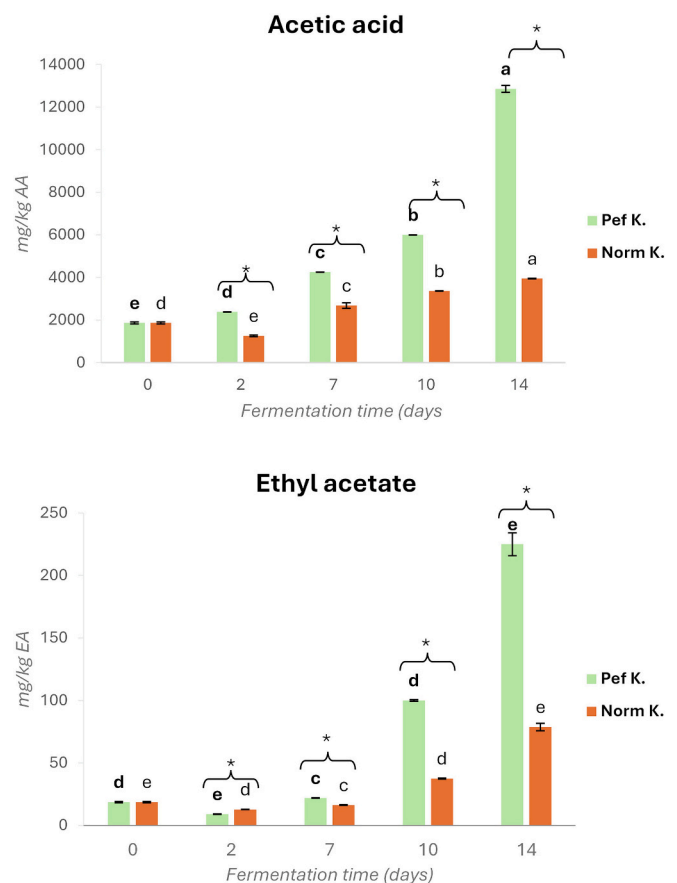


Fig. 7. Quantification of ethyl acetate and acetic acid in Norm K. and Pef K. (Trial 6).

Different letters indicate statistically significant differences during the fermentation process for each type of kombucha.

The asterisk (*) means statistical difference ($p \leq 0.05$) between the two formulations at each fermentation day.

14, Pef K. reached a remarkable concentration of around 12,800 mg/kg, which is more than three times higher than the value observed in Norm K.

Acetic acid is produced by acetic acid bacteria (primarily *Komagataeibacter* and *Acetobacter* spp.) through the activity of two membrane-bound enzymes, alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH), via membrane-bound PQQ-dependent ADH and ALDH oxidize ethanol to acetic acid in an aerobic respiratory process (Yakushi & Matsushita, 2010). This pathway is central to kombucha acidification and is directly linked to the sequential metabolic activity of yeasts (ethanol producers) and AAB (ethanol oxidizers) within the SCOBY consortium (Tran et al., 2020a; Villarreal-Soto et al., 2019). This substantial enhancement in acetic acid production directly correlates with the stimulated AAB proliferation observed in Section 3.3 and confirms the effectiveness of reversible electroporation in enhancing acetobacter metabolic activity.

Ethyl acetate exhibited a similar trend to acetic acid; indeed, both samples started from similar initial concentrations at Day 0 (~19 mg/kg), but their amount diverged significantly during fermentation.

During the early fermentation phase (Days 0–7), ethyl acetate accumulation was comparable in both samples, with values remaining below 25 mg/kg. However, from Day 10 onward, Pef K. exhibited exponential ester production, reaching approximately 100 mg/kg, compared to only 38 mg/kg in Norm K.

By Day 14, the amount of ethyl acetate in Pef K. was about 225 mg/kg ethyl acetate, representing a 2.9-fold increase compared with Norm K. (78.69 mg/kg). This could represent a possible direct effect of PEF

treatment on the beverage and suggest enhanced esterification activity, likely resulting from the synergistic stimulation of both yeast (ethanol production) and AAB (acetic acid production) populations, which provide the substrates for ethyl acetate synthesis (Zhang et al., 2020).

In kombucha, ethyl acetate is primarily biosynthesized by yeast alcohol acetyltransferase (AAT) enzymes, which catalyse the condensation of ethanol (derived from yeast-mediated sugar fermentation) with acetyl-CoA or acetic acid produced by acetic acid bacteria. This symbiotic metabolic interplay, enhanced by the progressively acidic fermentation environment, directly links SCOBY microbial dynamics to the accumulation of this key ester and the development of the beverage's characteristic fruity aroma (Jayabalan et al., 2014; Tran et al., 2020a; Villarreal-Soto et al., 2019).

The semiquantitative volatile organic compound (VOCs) profiling further defined the metabolic differences between PEF-treated and normal kombucha fermentation (Fig. 8). A total of 33 compounds were

identified across the different fermentation stages, including organic acids, esters, alcohols, ketones, aldehydes, and terpenes.

The organic acids profile reflected the enhanced metabolic activity induced by PEF treatment. Acetic acid dominated the volatile acid fraction, increasing from 21% at Day 0 to 28% in Pef K., compared with 24% in Norm K., consistent with the quantitative data discussed above.

While medium-chain fatty acids exhibited a different behaviour, hexanoic acid decreased substantially from 18% at Day 0 to 4.8% in Pef K. and 2.0% in Norm K. Indeed, it is an important precursor for ester biosynthesis; its depletion indicates active metabolic conversion, consistent with a yeast–yeast interaction driven by the exchange and consumption of fatty acids and peptide (Tong et al., 2026; Tran et al., 2022).

Octanoic acid, isobutyric acid, isovaleric acid and nonanoic acids showed similar and comparable trend between the different samples.

The ester profile demonstrated the most pronounced treatment-

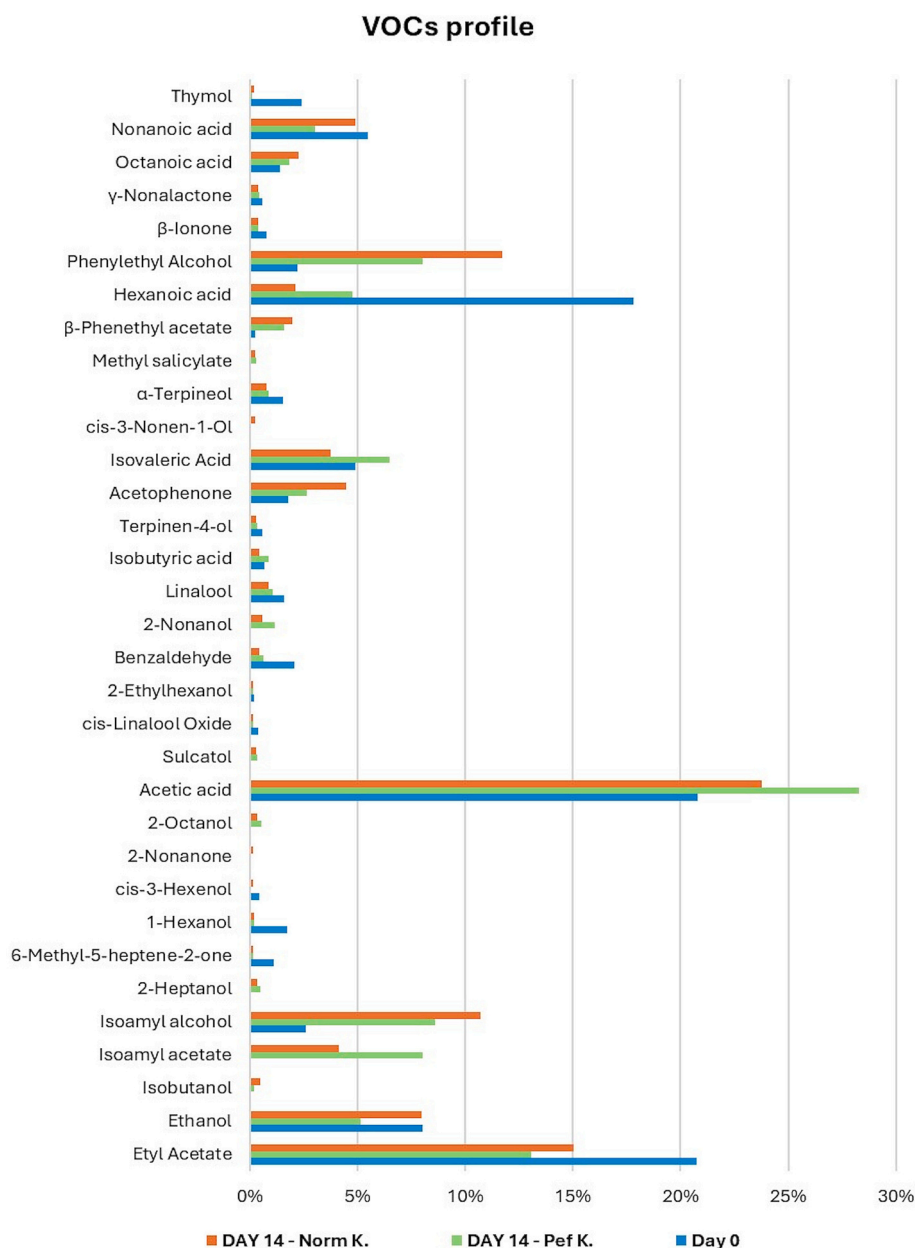


Fig. 8. Concentrations of the volatile organic compounds (VOCs) in Norm K. and Pef K. (Trial 6) at fermentation Day 14 compared with Day 0. VOCs concentrations are expressed in percentage compared to the total compounds identified.

The detection of the peak itself had an accuracy greater than 70% of the compounds match according to NIST 2020 Mass Spectral Library.

specific differences, with PEF treatment generally enhancing ester biosynthesis. Ethyl acetate, the predominant ester, decreased from 21% at Day 0 to 13.1% in Pef K. and 15% in Norm K., this difference indicates that while ethyl acetate biosynthesis was enhanced, the concurrent accumulation of other volatiles (e.g., alcohols, esters, terpenes) altered the compositional balance of the overall aroma profile.

Isoamyl acetate, absent at Day 0, exhibited a remarkable two-fold enhancement in Pef K. (8.0%) compared with Norm K. (4.0%). These compounds, together with isovaleryl isoamyl acetate, serve as the dominant fruit aroma contributors, imparting intense sweet banana and pineapple notes (Martínez-Lüscher & Kurtural, 2023). Their synthesis relies on alcohol acetyltransferase (AATase) encoded by the ATF1 gene in yeast, which catalyses the esterification reaction between acetic acid and phenethyl alcohol (acetic acid + phenethyl alcohol → ethyl phenylacetate), thus the increase of ester compounds is strictly related to the increase in both yeast and acetic acid bacteria by PEF treatment (Liao et al., 2025; Qiao et al., 2026).

The alcohol profile revealed a treatment-specific pattern reflecting the balance between yeast fermentation activity and subsequent oxidation/esterification. Ethanol decreased from 8% at Day 0 to 5.1% in Pef K., compared with 8% in Norm K., consistent with enhanced AAB-mediated oxidation to acetic acid in the PEF-treated sample.

Isoamyl alcohol increased from 3% at Day 0 to 8.6% in Pef K. and 11% in Norm K., indicating that conventional fermentation favoured greater alcohol accumulation. Phenylethyl alcohol, derived from phenylalanine, followed a similar pattern, rising from 2% to 8.0% in Pef K. and 12% in Norm K. Phenethyl alcohol and isoamyl alcohol, likely by the *Saccharomyces* genus, leads to ester formation, such as ethyl acetate, during kombucha fermentation (Suffys et al., 2023). Thus, the lower higher-alcohol content in Pef K., coupled with enhanced ester production, further confirmed that the PEF treatment increases yeast activity.

Several minor alcohols uniquely appeared or accumulated in the fermented samples at 14 days. 2-Heptanol, 2-octanol, 2-nonanol and sulcatol were detected in slightly higher percentages in the PEF sample, indicating a progressive accumulation during the fermentation period in both samples.

The terpene profile, primarily derived from tea substrate biotransformation, showed a similar trend across the different samples (Chandran et al., 2026). Linalool, a monoterpene alcohol contributing floral notes, decreased from 2% at Day 0 to 1.1% in Pef K. and 1% in Norm K., suggesting similar biotransformation rates (Dartora et al., 2023).

The same trend is followed by the other terpenes, indeed, cis-linalool oxide, terpinen-4-ol, β -ionone and α -terpineol decreased during the fermentation without being affected by the treatment applied, with similar amounts in both samples after 14 days of fermentation.

Collectively, these findings demonstrate that PEF treatment could reshape the kombucha volatile aroma profile by accelerating AAB-driven acidification, redirecting yeast metabolic pathways toward ester biosynthesis, and enriching key aroma compounds. However, the increase in acetic acid concentration in Pef K. raises the possibility of potential negative sensory effects, such as excessive acidity. Therefore, while the treatment successfully enriches fruity esters, the ultimate impact on the beverage's sensory acceptability and overall flavor requires careful sensorial validation.

4. Conclusion

This study successfully demonstrates, for the first time, the application of low-intensity PEF as a non-thermal strategy to stimulate and modulate specific kombucha fermentation kinetics (sugar consumption, fermentation metabolite production and microbial growth). Through systematic screening, an optimal treatment condition (1.14 kV/cm, 100 pulses, 17.78 kJ/kg) was identified to induce reversible electroporation within the SCOBY consortium, significantly enhancing microbial growth. This targeted physical intervention accelerated substrate

utilization, promoted robust stimulation of lactic and acetic acid bacteria and yeasts, and substantially increased the production of key fermentation markers, notably a threefold rise in acetic acid and a 2.9-fold increase in ethyl acetate by Day 14. Concurrently, PEF treatment modulated the volatile organic compound profile by enriching fruity esters while reducing alcohol accumulation and extended the enzymatic biotransformation of tea-derived polyphenols, thereby preserving and enhancing the beverage's bioactive potential.

From a technological standpoint, these findings position PEF as promising precision tool capable of significantly modifying kombucha fermentation kinetics in a laboratory set-up. By modulating microbial dynamics and tailoring metabolic outputs within a controlled batch environment, PEF-assisted fermentation offers a promising approach to modulate the fermentation kinetics while preserving the nutritional and functional attributes and modulating the aromatic profile of the final beverage.

The current full-mixture batch treatment was deliberately chosen for its operational simplicity; however, to further optimize industrial feasibility, future research should evaluate whether pre-treating only the liquid starter can achieve comparable results.

Future investigations will prioritize comprehensive sensory analysis to quantitatively assess the consumer acceptance and perception of the different kombucha samples. Additionally, subsequent research will focus on pilot-scale validation of these batch processing scenarios as well as interpreting the kinetic dynamics and underlying mechanisms of interspecies interactions within the SCOBY microbiome following PEF-induced reversible electroporation. Understanding how reversible electroporation alters microbial competition, syntrophic cross-feeding, and post-lysis metabolite exchange will provide critical insights into community resilience and process control under varying electric field intensities.

CRedit authorship contribution statement

Francesca Pompei: Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Mattia Acito:** Writing – review & editing, Formal analysis, Data curation. **Silvia Tappi:** Writing – review & editing. **Junior Bernardo Molina Hernandez:** Writing – review & editing, Validation, Formal analysis, Data curation. **Sauro Vittori:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Cinzia Mannozi:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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