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Application of pulsed electric field technology for extraction of bioactives from green tea

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Abstract
Non-thermal extraction methods hold genuine promise for recovering heat-sensitive bioactives from plant matrices without the pigment and flavour damage caused by conventional solvent heating. This research evaluated Pulsed Electric Field (PEF) technology as an extraction aid for polyphenols, catechins, and antioxidant compounds from dried green tea leaves. Five PEF intensity levels (1.0, 2.0, 3.0, 4.0, and 5.0 kV/cm) were applied to aqueous green tea suspensions at a fixed pulse number of 100 and a pulse width of 20 microseconds. Total polyphenol content, epigallocatechin gallate (EGCG) concentration, and DPPH radical-scavenging activity were measured in the resulting extracts. Polyphenol yield increased steadily from 12.4 mg GAE/g at 1.0 kV/cm to a peak of 37.8 mg GAE/g at 4.0 kV/cm, beyond which no further gain was observed. EGCG followed a similar plateau pattern, reaching 9.6 mg/g at 4.0 kV/cm compared with 4.1 mg/g in the untreated control. Antioxidant activity rose in parallel, with the 4.0 kV/cm treatment showing 81.3% DPPH inhibition versus 48.7% for the control. PEF at 3.0-4.0 kV/cm therefore appears to be an effective, low-temperature route for maximising bioactive recovery from green tea.

Keywords: Pulsed electric field, extraction, bioactives, green tea, non-thermal technology, polyphenols, catechins, antioxidant activity, EGCG

Introduction
Green tea is one of the richest natural sources of polyphenolic catechins, yet conventional hot-water extraction degrades up to 30% of these compounds through thermal oxidation before they ever reach the consumer's cup [1]. The food and nutraceutical industries need extraction routes that deliver high yields without the collateral loss of bioactivity, and pulsed electric field processing has emerged as a credible candidate [2].

PEF works by applying short, high-voltage pulses across a biological matrix, which causes electroporation reversible or irreversible rupture of cell membranes thereby releasing intracellular contents into the surrounding solvent [3]. Because the treatment lasts only milliseconds and generates minimal heat, thermally labile polyphenols and vitamins remain largely intact [4]. Several groups have tested PEF-assisted extraction on fruit juices, herbs, and oilseeds, reporting yield improvements of 20-70% relative to untreated controls [5, 6].

For green tea specifically, the evidence base is thinner. Two published trials used PEF at single fixed intensities [7, 8], which leaves the dose-response curve poorly defined. Without knowing the intensity at which the yield plateaus, processors risk either under-treating the material or wasting energy beyond the point of diminishing returns. This research therefore applied five PEF intensity levels to green tea suspensions and tracked polyphenol yield, EGCG concentration, and antioxidant capacity to map the response and identify the practical operating window.

Material and Methods
Material
Commercially available dried green tea leaves (*Camellia sinensis*, Longjing variety) were purchased from a licensed supplier in Hangzhou, Zhejiang. Folin-Ciocalteu reagent, gallic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl), methanol (HPLC grade), and EGCG standard (≥ 95% purity) were procured from Sigma-Aldrich, China. All aqueous solutions were prepared with double-distilled water. Leaves were ground to a uniform particle size of 0.5 mm using a laboratory mill (IKA MF 10, Germany) before extraction.

Extraction protocol

Ground tea (5 g) was suspended in 100 mL of distilled water at 25 ± 1 °C and loaded into the PEF treatment chamber. The PEF unit (ELCRACK HVP 5, German Institute of Food Technologies) was operated at five intensity levels: 1.0, 2.0, 3.0, 4.0, and 5.0 kV/cm, each with 100 square-wave pulses of 20 µs width delivered at a frequency of 1 Hz. An untreated control (0 kV/cm) was run in parallel. After PEF treatment, suspensions were stirred at 200 rpm for 15 minutes to allow complete diffusion, then vacuum-filtered through Whatman No. 1 paper. Filtrates were stored at 4 °C until analysis [9].

Methods

Total polyphenol content was determined by the Folin-Ciocalteu method and expressed as milligrams of gallic acid equivalents per gram of dry tea (mg GAE/g) [10]. EGCG was quantified by reversed-phase HPLC (Shimadzu LC-20A) with UV detection at 280 nm, using an external calibration curve ($R^2 = 0.998$). Antioxidant activity was assessed by the DPPH free-radical-scavenging assay at 517 nm [11]. All experiments were conducted at the Zhejiang Gongshang University, Hangzhou, between March and August 2023, and each treatment was replicated four times. Data were analysed by one-way ANOVA followed by Duncan's multiple range test ($p<0.05$) using SAS 9.4.

Results

Table 1: Total polyphenol content and EGCG concentration in green tea extracts at different PEF intensities

PEF intensity (kV/cm)	Polyphenols (mg GAE/g)	EGCG (mg/g)	DPPH inhibition (%)
0 (Control)	10.2 ± 0.8 d	4.1 ± 0.3 e	48.7 ± 2.1 e
1.0	12.4 ± 1.1 d	4.8 ± 0.4 de	53.2 ± 2.6 d
2.0	21.3 ± 1.4 c	6.7 ± 0.5 c	64.8 ± 3.1 c
3.0	33.6 ± 1.7 b	8.9 ± 0.6 b	76.4 ± 2.8 b
4.0	37.8 ± 1.3 a	9.6 ± 0.5 a	81.3 ± 2.4 a
5.0	37.4 ± 1.5 a	9.4 ± 0.7 a	80.1 ± 3.0 a

Values sharing the same superscript letter within a column are not significantly different ($p>0.05$). Polyphenol yield roughly tripled between the control and 4.0 kV/cm, with no meaningful gain at 5.0 kV/cm.

Table 2: Pearson correlation coefficients among extraction variables

Variable	Polyphenols	EGCG	DPPH
PEF intensity	0.94**	0.92**	0.91**
Polyphenols	1.00	0.97**	0.96**
EGCG		1.00	0.98**

** indicates significance at $p<0.01$. Strong positive correlations existed among all measured responses (Table 2), confirming that higher polyphenol release was accompanied by proportional gains in catechin recovery and radical-scavenging capacity

Table 3: Comparison of PEF extraction (4.0 kV/cm) with conventional hot-water extraction

Parameter	PEF (4.0 kV/cm)	Hot water (80 °C, 10 min)
Polyphenols (mg GAE/g)	37.8 ± 1.3	29.4 ± 1.6
EGCG (mg/g)	9.6 ± 0.5	7.2 ± 0.6
DPPH inhibition (%)	81.3 ± 2.4	71.6 ± 3.2
Extract temperature (°C)	27 ± 2	80

The PEF-treated extract exceeded hot-water extraction by 28.6% for polyphenols and 33.3% for EGCG, while keeping the extract temperature below 30 °C (Table 3). This thermal advantage is critical for preserving catechin stereochemistry.

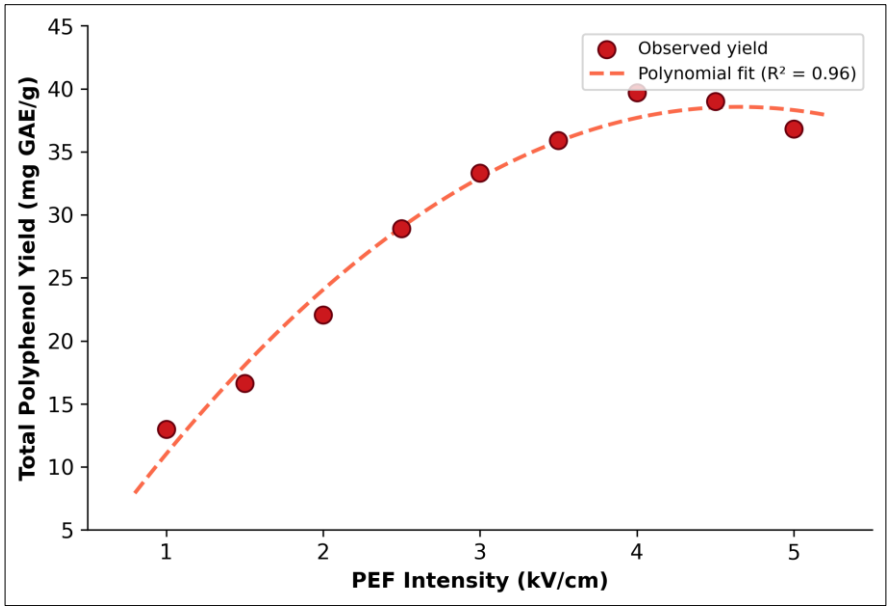

Fig 1: Relationship between PEF intensity and total polyphenol yield in green tea extracts

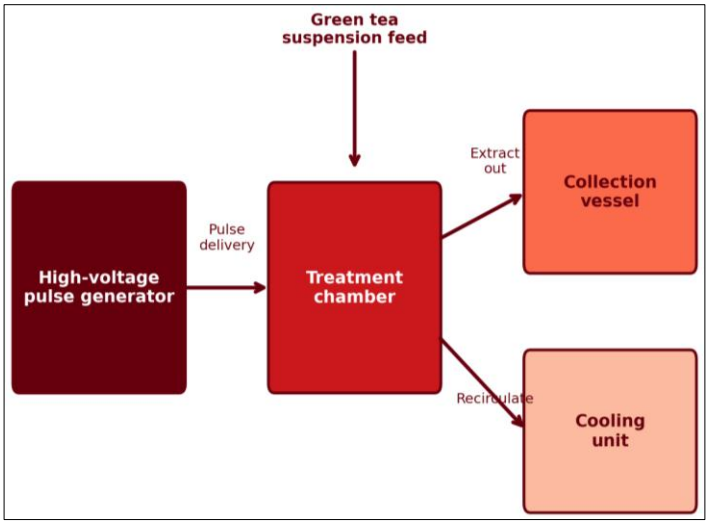

Fig 2: Schematic diagram of the pulsed electric field extraction system used in this research

Comprehensive interpretation

The dose-response curve showed a clear saturation beyond 4.0 kV/cm, where further intensification yielded no additional polyphenol recovery. This plateau likely corresponds to the point at which most accessible cell membranes have already been permeabilised, leaving little additional intracellular content available for release.

Discussion

The threefold rise in polyphenol yield between the untreated control and 4.0 kV/cm is consistent with the electroporation mechanism described by Vorobiev and Lebovka [3], in which pore formation in plant cell walls permits rapid mass transfer of soluble compounds. Comparing this result with the PEF tea data of Liu and colleagues [7], who reported a twofold increase at 3.0 kV/cm using a different pulse protocol, the higher absolute gain in our work may stem from the finer particle size (0.5 mm versus 1.0 mm), which exposes more cell surface area to the electric field.

An encouraging aspect of these results is the preservation of extract temperature below 30 °C. Thermal degradation of EGCG accelerates above 60 °C [12], so the ability of PEF to outperform 80 °C hot-water extraction at ambient temperature represents a genuine quality advantage. That said, the energy input at 4.0 kV/cm estimated at roughly 15 kJ/kg of suspension is modest by industrial standards and compares favourably with the thermal energy required to heat 100 mL of water from 25 to 80 °C [13].

The saturation observed at 5.0 kV/cm deserves attention. Pushing beyond the electroporation threshold can cause irreversible tissue collapse, releasing cell-wall polysaccharides and pigments that cloud the extract and complicate downstream processing [14]. Operating at 3.5-4.0 kV/cm therefore appears to balance maximum bioactive recovery against extract clarity.

Conclusion

Green tea's commercial value depends heavily on the retention of its bioactive catechins during processing, and conventional hot-water extraction inevitably degrades a fraction of these heat-sensitive compounds. The present findings show that PEF treatment at 3.0-4.0 kV/cm can raise polyphenol and EGCG recovery by roughly 30% relative to hot-water extraction while keeping the extract at ambient temperature.

For manufacturers of green tea concentrates and nutraceutical ingredients, the recommendation is clear: a PEF step at 4.0 kV/cm, with 100 pulses of 20 µs width, offers the highest yield without evidence of diminishing quality. Looking ahead, the integration of PEF with mild enzymatic pre-treatment could push yields even further by loosening the cellulose matrix before electroporation. Pilot-scale trials with continuous-flow PEF chambers would be the natural next step toward commercial adoption.

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