Application of Pulsed Electric Field for Squid Salting: A Study on Technical Efficiency and Economic Feasibility

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ABSTRACT

The production of salted squid in Indonesia through soaking and boiling often is a long process under conditions that support halophilic bacteria, such as *Vibrio parahaemolyticus*. Boiling reduces the effective salt content and damages proteins, thereby negatively impacting the texture of the product. This study evaluates the application of Pulsed Electric Field (PEF) to improve the quality of salted squid. PEF treatment at 7 kV/cm for 30 s resulted in the highest salting rate (9.81%) with a 47.5% reduction in bacteria, accompanied by a decrease in sarcoplasmic and myofibrillar proteins of 17.56% and 11.33%, respectively. Increasing the electric field to 10.5 kV/cm resulted in a salt content of 8.62%, comparable to

the control samples (8.61%). Still, it increased bacterial reduction efficiency to 97.6%, with relatively low reductions in sarcoplasmic protein (18.44%) and myofibrils (12.67%). Conversely, boiling treatment produced the lowest salt content (6.81%) with 97.9% bacterial reduction, but caused significant protein loss, marked by a decrease in sarcoplasmic protein (46.22%) and myofibril protein (39.11%). Microstructural observations of the mantle tissue confirmed the occurrence of electroporation, which increased tissue permeability and accelerated salt mass transfer. From an economic perspective, the operational costs of PEF are lower (± Rp 10,630/kg) compared to boiling (± Rp 15,250/kg), salt solution loss is less significant, and it allows for product diversification and an increase in the sales value index by up to 1.7 times. Overall, PEF improves product quality, microbiological safety, cost efficiency, and potential added value for the industry.

Keywords-pulsed electric field; salted squid; salting; protein; boiling

I. INTRODUCTION

Salted squid is an important processed fishery product in Indonesia, with quality primarily determined by moisture content, salt content, and texture. This product generally contains salt >6% (w/w) and is mainly produced from *Uroteuthis duvaucelii* [1]. As consumer awareness of food quality and safety increases, demand for salted squid with a softer texture, even salt distribution, and consistent quality continues to rise, requiring innovation in more controlled salting processes. Conventional salting methods involving soaking and boiling still dominate, but often result in inconsistent physical quality. Prolonged soaking causes uneven salt penetration, strengthens protein interactions, reduces texture quality, and potentially supports the survival of halophilic bacteria [2]. Conversely, boiling as a thermal process accelerates processing but triggers denaturation of sarcoplasmic and myofibrillar proteins, which increases hardness and reduces tissue elasticity, making the product less suitable for consumer preferences [3]. The sarcoplasmic and myofibrillar protein fractions control the texture of salted squid, so utilizing a processing strategy that can increase salt penetration without damaging protein integrity is important. Pulsed Electric Field (PEF) technology enhances tissue permeability and accelerates salt mass transfer without significant thermal effects [4], while also potentially improving microbiological safety [5]. This study evaluates the application of PEF as a method to produce salted squid with improved texture and quality. The proposed method has potential applicability for Small and Medium Industries (SMIs).

II. RESEARCH METHOD

A. Research Design

This study used a completely randomized experimental design consisting of four treatment groups: (1) control (soaking only), (2) boiling after soaking, (3) PEF treatment at 7 kV/cm after soaking, and (4) PEF treatment at 10.5 kV/cm after soaking. All treatments were evaluated for physicochemical, microbiological, structural, and techno-economic parameters.

B. Production and Processing of Salted Squid

Fresh squid was cleaned and soaked in a 15% salt solution (w/w) for approximately 30 min. After the soaking process, the samples were divided into three treatment groups, namely control (no further treatment), boiling, and PEF. In the boiling treatment, the salted squid was boiled at 85°C for ±2 min after soaking, then drained at room temperature. The soaking step was applied uniformly to all groups to standardize the initial salt concentration and ensure comparable osmotic conditions prior to further treatment. In the PEF treatment, after soaking, the salted squid was treated with an electric field of 7 kV/cm and 10.5 kV/cm for 30 s, and then drained according to [6, 7].

C. Determining Salt and Moisture Content

The salt content of salted squid was analyzed using the Mohr method [8]. A 10 g sample of salted squid mantle was extracted using sterile distilled water, then titrated with silver nitrate ($AgNO_3$) solution as titrant and potassium chromate (K_2CrO_4) as indicator. The salt content was expressed as a percentage of wet weight (% w/w). The moisture content of salted squid was determined by the oven-drying method at 105°C using a 100 g salted squid sample [9].

D. Calculation of Number of V. parahaemolyticus Bacterial Colonies

The analysis of *V. parahaemolyticus* colony counts was performed in accordance with the SNI 2719.3:2011 standard [10]. A total of 10 g of salted squid samples were homogenized in 90 mL of 0.9% NaCl physiological solution, then serial dilutions were performed. The appropriate dilution was inoculated on selective Thiosulfate Citrate Bile Sucrose Agar (TCBSA; Himedia, India) medium and incubated according to standard procedures. The colony count was expressed as CFU/g and converted to $\log_{10}$ CFU/g.

E. Measurement of Sarcoplasmic and Myofibril Protein Content

Measurement of sarcoplasmic and myofibrillar proteins was based on [11] with slight modifications. For sarcoplasm measurement, 10 g of salted squid was ground and then homogenized in 10 mL of 20 mM Phospate Buffer Saline (PBS; Oxoid, UK) (pH 7.5) and distilled water in a 1:1 ratio, while for myofibril measurement, only PBS was used as the solvent. The dissolved samples were then treated with a homogenizer at a speed of 4500 rpm for 30 s. The homogenization process was carried out twice with an interval of 1 min. The sample solution was centrifuged at a speed of 8,000 rpm at a temperature of 4°C for 10 min. The resulting supernatant was the sarcoplasmic protein fraction, which was then tested using the Bradford method.

F. Texture Profile Analysis

Squid mantle samples were cut into 2 × 2 × 2 (length × width × height) cm cubes using a TA-XT Express material analyzer. Salted squid meat was analyzed using a physical analysis tool. The measurement conditions were as follows:

SMS P/36 probe, pre-test speed of 2 mm/sec, test speed of 1 mm/sec, and post-test speed of 2 mm/sec. The ambient temperature was 20–25°C, and the measurements were repeated three times [12].

G. Molecular Weight Profile of Salted Squid Protein

The molecular weight profile of sarcoplasmic and myofibrillar proteins was analyzed based on references from [13] with several modifications. The electrophoresis profile of each treatment of sarcoplasmic and myofibrillar proteins was previously performed by Polyacrylamide Gel Electrophoresis (PAGE) using Sodium Dodecyl Sulfate (SDS) separation conditions in a discontinuous gel (4% upper gel and 10% lower gel) according to the method described in [14]. Protein samples were incubated for 1 hr at room temperature and heated at 95°C for 5 min. An aliquot of 25 µL containing 12.5 µg of protein was loaded into each lane. The gel was electrophoresed at 95 V using a Mini-PROTEAN Tetra Cell system, then stained with Coomassie Brilliant Blue R-250 solution (0.125% w/v) containing methanol (40% v/v) and acetic acid (7% v/v). The gel was stained with methanol (50% v/v) and acetic acid (10% v/v). Gel images were collected using a GS-800 densitometer (Bio-Rad laboratory chemicals, model GS-800, Hercules, CA).

H. Scanning Electron Microscopy Observation

Scanning Electron Microscopy (SEM, Thermo Scientific Prisma E, USA) was performed based with modifications [15]. Squid samples (1 × 1 cm) were taken from each treatment. The sections were placed in a Quorum E3100 drying cabinet (Quorum Technologies) at 45°C for 12 hr. Next, the samples were coated with 90% Au and 10% Pd using a Cressington Sputter Coater 108 Auto (Cressington Scientific Instruments). Histological observations followed established procedures [16], and were made using an FEI Quanta FEG 250 (FEI Company, Eindhoven, NL) at 500× magnification, WD, 10.9 mm, and 3 kV. All samples were fixed in 10% formalin. The samples were embedded in epoxy resin in a HistoEmbedder, and thick sections (5 µm thick) were prepared using a microtome. The sections were stained with hematoxylin and eosin and observed under an optical microscope.

I. Techno-Economic Feasibility Analysis

Technical-economic feasibility analysis was conducted using the capital expenditure (CAPEX) and operating expenditure (OPEX) approaches to compare thermal (boiling) and non-thermal PEF salting methods. CAPEX was calculated based on the investment requirements for main and supporting equipment for each method, assuming a technical life of 5–10 years. OPEX was calculated from water and salt solution requirements and electricity consumption during the process and treatment time using the equation:

$$\text{Energy (kWh)} = \text{electricity (kW)} \times \text{time (hr)} \quad (1)$$

J. Statistical Analysis

All data were analyzed using a parametric statistical approach through One-Way Analysis of Variance (ANOVA). The results are presented as mean values ± standard deviation from three independent replicates (n = 3). The relationship between changes in protein profile and texture characteristics of salted squid was analyzed using Pearson's correlation test, focusing on sarcoplasmic proteins related to cohesiveness parameters and myofibrillar proteins related to flexibility parameters. Each relationship was analyzed at a significance level of $p < 0.05$ using IBM SPSS Statistics software version 22.

III. RESULTS AND DISCUSSION

A. Salt Content and Water Content

The salt content of salted squid with various treatments is shown in Table I. An electric field of 7 kV/cm produced the highest salt content (9.81%). The salt content decreased at 10.5 kV/cm (8.62%). This phenomenon shows that the intensity of the electric field significantly affects salt penetration ($p<0.05$). The application of PEF 7 kV/cm significantly increased the salt content of salted squid through increased membrane permeability due to electroporation, which facilitated the diffusion of salt ions into the tissue [17]. Conversely, increasing the intensity to 10.5 kV/cm reduces salt content, disrupts tissue integrity, and reduces effective ion retention, in accordance with the membrane permeability theory [18].

TABLE I. SALT AND MOISTURE CONTENT OF SALTED SQUID (%)

Treatment	Total salt and moisture content of salted squid (% w/w)	
	Salt content	Water content
Salted squid control*	8.61±0.4[b]	80.5±0.4[a]
Salted squid PEF 7 kV/cm	9.81±0.45[a]	79.2±1.4[a]
Salted squid PEF 10.5 kV/cm	8.62±0.01[b]	78.6±2.4[a]
Boiling salted squid	6.81±0.01[d]	64.6±12.7[b]

*Salted squid soaked in 15% salt water for 30 minutes

Different superscript letters within the same column indicate significant differences ($p < 0.05$).

Boiling treatment resulted in the lowest salt content (6.81%) due to salt dissolution and protein denaturation, which weakened the binding capacity of the tissue [18]. Table I shows a decrease in water content of 1.15% (PEF 7 kV/cm) and 2.36% (PEF 10.5 kV/cm), while boiling causes a much greater decrease (19.75%). These findings confirm that increasing the intensity of the electric field increases the degree of electroporation and accelerates the mass transfer of water and salt ions ($p<0.05$), while protein denaturation due to heating is the main factor in the decrease in water content, as reflected in the changes in tissue microstructure (Figure 2).

B. The Effect of PEF and Boiling on Colonies of V. parahemolyticus in Salted Squid

The effects of PEF and boiling on *V. parahaemolyticus* bacterial colonies are shown in Table II.

TABLE II. PEF TREATMENT AND BOILING ON *V. PARAHAEMOLYTICUS* IN SALTED SQUID

Treatment	Number of colonies (CFU/g)
Salted squid control*	2.14±0,03[a]
Salted squid PEF 7 kV/cm	1.86±0,01[b]
Salted squid PEF 10.5 kV/cm	0.51±0,07[c]
Boiling salted squid	0.47±0,15d

*Salted squid soaked in 15% salt water for 30 min.

Different letters in the column indicate significantly different values ($p<0.05$).

The results of PEF treatment at electric fields of 7 kV/cm and 10.5 kV/cm are consistent with reports on lactic acid

bacteria. At an intensity of 7.5 kV/cm, a reduction of 0.3–0.6 log CFU/mL was reported, while at around 10 kV/cm, the reduction increased to 1.0–1.5 log CFU/mL [19]. Meanwhile, the boiling treatment is also in line with the findings in [20], which show the effectiveness of heating in reducing the number of bacteria. The salted squid produced in this study is a raw product that will undergo cooking during consumption, where increased temperature plays a crucial role in bacterial inactivation. The remaining *V. parahaemolyticus* population after PEF is highly likely to die during cooking at 68–74°C [21]. Both PEF treatments reduced *V. parahaemolyticus* below 2.0 log CFU/g, which is generally considered a low-risk level for raw seafood prior to cooking according to international food safety guidelines [21].

C. Effects of PEF and Boiling on the Protein Profile

The protein content of the sarcoplasm and myofibrils of control salted squid is ± 4.5 mg/g. The similarity in the amount of sarcoplasm and myofibril protein content is influenced by the solvent used, which is PBS 1:1 and distilled water. The sarcoplasm fraction is more soluble in water or dilute salt than myofibrils with a salt concentration of 0.2-0.5 M [22]. The protein content of salted squid is shown in Table III.

TABLE III. SARCOPLASMIC AND MYOFIBRILLAR PROTEIN CONTENT

Treatment	Total sarcoplasmic and myofibrillar protein (mg/g)	
	Sarcoplasmic	Myofibrillar
Salted squid control*	4.5±0.05[a]	4.5±0.04[a]
Salted Squid PEF 7 kV/cm	3.71±0.04[b]	3.99±0.02[b]
Salted Squid PEF 10.5 kV/cm	3.67±0.03[c]	3.93±0.05[c]
Boiling salted squid	2.42±0.003[d]	2.74±0.001[d]

*Salted squid soaked in 15% salt water for 30 min.

Different letters in the column indicate significantly different values ($p<0.05$).

Treatment with PEF at 7 kV/cm and 10.5 kV/cm reduced sarcoplasmic and myofibrillar proteins. This reduction was associated with increased cell membrane permeability, thereby accelerating protein release, as supported by microstructural observations showing pore opening and tissue reorganization (Figure 2). In contrast, boiling treatment caused a much greater decrease in sarcoplasmic and myofibrillar proteins due to changes in protein structure. Statistically, the effect of boiling was more significant than that of PEF ($p<0.05$). Compared to boiling treatment, PEF at 7 and 10.5 kV/cm was more effective in maintaining protein content close to the control, as confirmed by SDS-PAGE analysis (Figure 1).

D. Molecular Weight Profile of Sarcoplasmic and Myofibrillar Proteins

Qualitative variations in the protein fraction of salted squid mantle using SDS-PAGE, PEF treatment, and boiling are shown in Figure 1. Samples in the sarcoplasmic protein molecular weight test, both control and PEF treatment (7 kV/cm and 10.5 kV/cm), were detected at molecular weights of 67.29 kDa, 53.41 kDa, 37.78 kDa, 31.77 kDa, 25.22 kDa, and 17 kDa. Proteins with very high molecular weights (above 100 kDa) are generally not sarcoplasmic proteins. These proteins are most likely myofibrillar proteins, such as myosin (~200 kDa), or stromal proteins that have huge molecular weights [23]. Proteins with molecular weights of 17 kDa and 25.22 kDa are likely parvalbumin, which functions in muscle relaxation. Bands at 31.77 kDa and 37.78 kDa may be related to glycolytic isoforms and enzymes. Molecular weights of 53.41 kDa and 67.29 kDa may indicate other metabolic proteins or structural enzyme variants. Bands at 67.29 kDa and 84.76 kDa may be associated with large enzyme oligomers or regulatory proteins. Meanwhile, bands with molecular weights of 100.78 kDa and 134.39 kDa may be large structural enzymes or protein complexes that regulate muscle energy and structure [23].

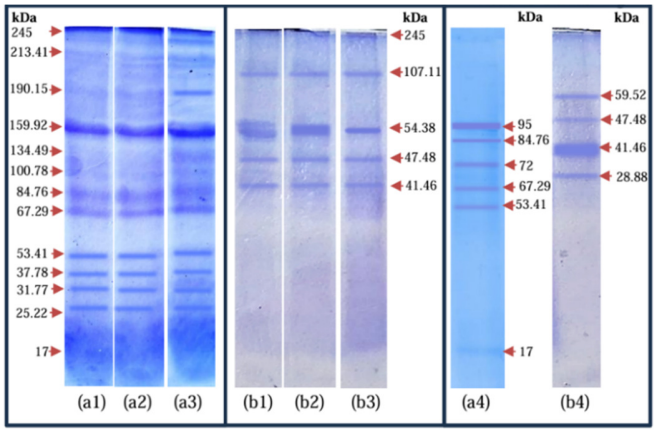

Fig. 1. Molecular weight of sarcoplasmic and myofibrillar proteins in salted squid. Notes: code (a) sarcoplasmic protein test and code (b) myofibril protein test. Numbers (1) Control sample, (2) 7 kV/cm sample, (3) 10.5 kV/cm sample, and (4) boiled sample.

The molecular weight of myofibrillar protein in the control sample and PEF (7 kV/cm and 10.5 kV/cm) was detected at molecular weights of 245 kDa, 107.11 kDa, 54.38 kDa, 47.48 kDa, and 41.46 kDa. These results are consistent with the findings in [24], which states that the four mantle muscles of squid consist of four main myofibrillar proteins, namely heavy chains of myosin with a molecular weight of approximately 200 kDa, paramyosin of approximately 100 kDa, actin of approximately 43 kDa, and tropomyosin of approximately 35 kDa. The control sample and PEF have the same molecular weight, indicating that PEF 7 kV/cm and 10.5 kV/cm do not affect molecular weight. Meanwhile, the boiling treatment causes differences in sarcoplasm and myofibrils. In sarcoplasmic proteins, the molecular weights that remained were 17 kDa, 53.41 kDa, and 67.29 kDa, and new bands appeared at 72 kDa, 84.76 kDa, and 95 kDa, which were suspected to be serum albumin. Myofibrillar proteins had a weight of 29 kDa and were suspected to be tropomyosin and troponin. The remaining molecular weights, 41.46 kDa and 47.48 kDa, are considered to be actin, and 59.52 kDa is considered to be myosin, which is soluble in salt [24].

E. Effect of PEF and Boiling on Texture in Salted Squid

The changes in the texture of salted squid after boiling and PEF treatment are shown in Table IV. The 7 kV/cm PEF treatment reduced cohesiveness by 7.69% and springiness by 1.82%, while the 10.5 kV/cm PEF treatment caused a reduction of 10.77% and 3.64%, respectively. The decrease in these texture parameters is thought to be related to the phenomenon of electroporation, which increases the permeability of salted

squid tissue, thereby weakening the integrity of the tissue structure [25].

TABLE IV. COHESIVENESS AND SPRINGINESS VALUES

Treatment	Cohesiveness	Springiness (%)	Hardness (g)
Salted squid control*	0.65±0.01[b]	55±0.01[b]	41.2 ± 3.1[c]
Salted squid PEF 7 kV/cm	0.6±0.08[bc]	54±0.06[b]	42.0 ± 2.7[c]
Salted squid PEF 10.5 kV/cm	0.58±0.01[c]	53±0.01[b]	48.6 ± 3.4[b]
Boiling salted squid	0.86±0.1[a]	85±0.13[a]	62.50±2.4[a]

*Salted squid soaked in 15% salt water for 30 min.

Different letters in the columns indicate that the values are significantly different ($p<0.05$).

The boiling treatment significantly increased cohesiveness by 32.31% and springiness by 54.55%, indicating the formation of a more springy texture. This phenomenon is associated with the denaturation of muscle proteins due to heating, which triggers the shrinkage of myofibrillar and sarcoplasmic proteins and the folding of the squid mantle layer [26]. In contrast, PEF treatment showed better ability to maintain the physical properties of salted squid. Correlation analysis revealed that a decrease in sarcoplasmic protein was strongly negatively correlated with cohesiveness ($r \approx -0.805$), while a decrease in myofibrillar protein showed a very strong negative correlation with flexibility ($r \approx -0.92$), confirming the dominant role of protein degradation in determining texture changes. These findings confirm that variations in sarcoplasmic and myofibrillar protein levels significantly affect the physical quality of salted squid, which was further analyzed with a mantle-layer permeability approach as the main structural factor controlling the texture response to process treatment.

F. Observed Permeability of the Mantle Layer

Observation of the salted squid mantle shows the epidermis in direct contact with the salting environment, as shown in Figure 2.

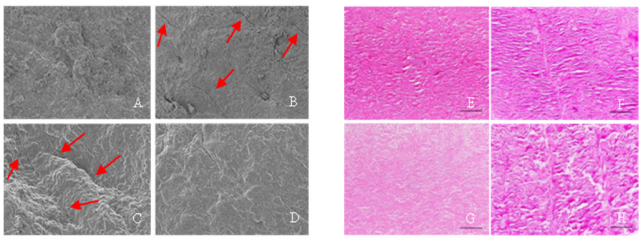

Fig. 2. Observation of squid mantle permeability. Notes: A to D are SEM observations, and E to H are histological observations. The arrows in the SEM indicate open pores, while the faded histological color indicates a reduction in squid mantle tissue fibers. Scale bars represent 20 µm for SEM images and 50 µm for histological images. SEM images were acquired at 1,000× magnification, while histological images were captured at 400× magnification

Figure 2 (A–E) represents the structure of untreated salted squid tissue. The application of PEF 7 kV/cm (B, F) shows an increase in tissue membrane permeability. A more extensive increase in permeability was observed in the 10.5 kV/cm PEF treatment (C, G), confirming the occurrence of open pores in the membrane of salted squid tissue and correlating this with increased salt ion diffusion. In contrast, boiling treatment (D, H) caused tissue structure damage characterized by tissue folding and tightening. These changes were associated with protein denaturation and reorganization, which were consistent with changes in the protein molecular weight profile in SDS-PAGE analysis (Figure 1), and implied a decrease in salt and water content. Overall, these results confirm that the electroporation mechanism consistently influences protein characteristics, salt content, water content, and texture.

G. Techno-Economic Feasibility

When conducting an energy cost analysis to compare conventional boiling methods and PEF, the rates used refer to those set by the Indonesian government. Electricity and gas tariffs were calculated based on official Indonesian regulations [27, 28]. The CAPEX and OPEX analysis is shown in Table V.

TABLE V. COMPARISON OF CAPEX, OPEX, AND SALES POTENTIAL OF PEF AND BOILING PRODUCTS (100 kg OF RAW MATERIALS)

Component	Boiling (thermal)	PEF (non-thermal)
CAPEX*	± IDR 150,000	± IDR 300,000
Electricity	–	0.1 kWh/kg × IDR 1,300 = IDR 130/kg
Gas	0.5 kg LPG/kg × IDR 16,500 = IDR 8,250/kg	–
Water and salt	IDR 5,000/kg	IDR 5,000/kg
Salt loss	High (±20%) = IDR 2,000/kg	Low (±5%) = IDR 500/kg
Total OPEX/kg	± IDR 15,250	± IDR 10,630
Product type	1 (undercooked)	3 (raw, undercooked, dry)
Yield & sales volume	80% or 80 kg/day	Raw 35 kg, undercooked 30 kg, dry 20 kg to 85 kg/day
Sales value index **	1.0× (baseline)	1.5–2.0× (averages 1.7×)

*CAPEX assuming an economic life of 5–10 years and amortization of main and supporting machinery (boiler vs. PEF generator).

**Sales value index = (weighted average selling price of treatment products) / (selling price of baseline products)

Using the same raw materials, the boiling method produces one type of product with a sales volume of around 80 kg/day due to process shrinkage and market limitations. In contrast, PEF allows for product diversification, increasing the total sales volume to ±85 kg/day, driven by market segmentation flexibility (raw, under-cooked, and dried products) and reduced shrinkage due to salting efficiency. In addition, PEF OPEX is lower due to the elimination of Liquefied Petroleum Gas (LPG) and reduced salt loss. Although PEF's CAPEX is higher, the combination of greater sales volume, lower OPEX, and an average sales value index of 1.7× shows that PEF provides a net economic advantage over the boiling method. Thus, PEF is more techno-economically feasible for the industrialization of salted squid oriented towards added value and product diversification.

IV. CONCLUSION

PEF treatment at 7 kV/cm is the optimal condition for salting squid, as it increases the salt content (9.81%) via electroporation without damaging tissue structure. Unlike boiling, which causes protein denaturation, significant water loss, and an overly burdensome texture, PEF can maintain sarcoplasmic and myofibrillar proteins and a texture close to that of the control. SEM, histology, and SDS-PAGE analyses confirm that PEF does not alter the main protein profile and

effectively reduces the presence of *Vibrio parahaemolyticus* to meet food safety standards. The novelty of this research lies in integrating accelerated salt diffusion, protein structure preservation, and techno-economic evaluation, demonstrating PEF as a viable and sustainable non-thermal alternative for salted squid processing.

DECLARATION OF COMPETING INTERESTS

The authors declare that this study was conducted without any financial ties or personal interests that could influence the reported results. The authors also declare that there are no conflicts of interest of any kind that could affect the objectivity, integrity, and interpretation of the data in this study.

DATA AVAILABILITY

The data used and/or analyzed in this study are available from the corresponding author upon reasonable request. The data have not been publicly released due to institutional policy restrictions, but remain accessible for the purpose of verifying and replicating the study results.

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ARTIFICIAL INTELLIGENCE STATEMENT

This study was conducted without the use of artificial intelligence (AI) at any stage of the research process, including design, experimental implementation, data processing, and analysis.

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