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# EFFECT OF PULSED ELECTRIC FIELD ON THE NUMBER AND CELL MEMBRANE OF *Vibrio parahaemolyticus* IN SALTED SQUID

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**Abstract**

Squid are susceptible to bacterial contamination by *Vibrio parahaemolyticus*, with a prevalence of 80%. Squid preservation is generally achieved by drying after brine salting, which does not always completely stop the growth of *V. parahaemolyticus*. To reduce bacterial numbers, a boiling process is usually conducted before drying. This study aimed to determine the optimal electric field and duration for inactivating *V. parahaemolyticus* and evaluating the effectiveness of pulsed electric fields (PEF) technology on salted squid. PEF technology spec-ifications with a current strength of 2 amperes using electric fields (3.5, 7, and 10.5 kV/cm) and time durations (10, 20, and 30 s). Bacterial reduction by electric fields was observed using colony counts, followed by counting of dead cells using a flow cytometer. Bacterial damage was observed using a scanning electron microscope. The results showed that PEF with the highest intensity (10.5 kV/cm for 30 s) reduced *V. parahaemolyticus* by 66.12% at high contamination levels (approximately $10^6$ CFU/g) and 97.63% at low contamination levels (approximately $10^2$ CFU/g) in salted squid. These results were comparable to those obtained after boiling treatment (2 min, 85°C). Damage to the bacterial cell membrane increased due to the increasing electric field, as observed by increasing in red fluorescing cells by flow cytometry and cell damage by SEM. PEF is a promising alternative technology for producing salted squid.

Keywords: bacterial reduction, cell damage, contamination, flow cytometer, SEM

# Pengaruh Medan Listrik Berdenyut terhadap Jumlah Total dan Membran Sel *Vibrio parahaemolyticus* pada Cumi Asin

**Abstrak**

Cumi-cumi rentan terhadap kontaminasi bakteri *Vibrio parahaemolyticus* dengan prevalensi 80%. Pengawetan cumi-cumi umumnya dilakukan dengan cara pengeringan setelah penggaraman air garam, dengan pertumbuhan *V. parahaemolyticus* tidak selalu terhenti. Pengurangan jumlah bakteri, biasanya dilakukan proses perebusan sebelum pengeringan. Penelitian ini bertujuan menentukan medan listrik dan lama waktu terbaik untuk menonaktifkan *V. parahaemolyticus* serta mengevaluasi efektivitas teknologi *pulsed electric fields* (PEF) pada cumi asin. Spesifikasi teknologi PEF yang digunakan, yaitu kuat arus 2 ampere dengan medan listrik (3,5; 7; dan 10,5 kV/cm) dan lama waktu (10, 20, dan 30 detik). Pengurangan bakteri oleh medan listrik diamati dengan penghitungan koloni, dilanjutkan dengan penghitungan sel yang mati menggunakan *flow cytometer*, sedangkan kerusakan bakteri diamati dengan pemindaian mikroskop

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elektron. Hasil penelitian menunjukkan bahwa PEF dengan intensitas tertinggi (10,5 kV/cm selama 30 detik) dapat mengurangi *V. parahaemolyticus* sebesar 66,12% pada tingkat kontaminasi yang tinggi (sekitar $10^6$ CFU/g) dan 97,63% pada tingkat kontaminasi yang rendah (sekitar $10^2$ CFU/g) pada cumi-cumi asin. Hasil ini sebanding dengan perlakuan perebusan (2 menit, 85°C). Kerusakan pada membran sel bakteri meningkat karena meningkatnya medan listrik, yang diamati dengan meningkatnya sel berpendar merah dengan *flow cytometry* dan kerusakan sel dengan SEM. *Pulsed electric fields* adalah teknologi alternatif yang menjanjikan untuk produksi cumi asin.

Kata kunci: *flow cytometer*, kerusakan sel, kontaminasi, pengurangan bakteri, SEM

## INTRODUCTION

*Vibrio parahaemolyticus* is a mesophilic and halophilic Gram-negative bacterium that is the leading cause of seafood-related outbreaks among various other pathogenic bacteria, mainly resulting from the consumption of poorly handled raw or undercooked seafood (Hackbusch *et al*., 2020; Malcolm *et al*., 2018). *V. parahaemolyticus* is a bacterium that causes seafood-associated diarrheal disease worldwide (Huang *et al*., 2024). After consuming food contaminated with *V. parahaemolyticus*, the presenting symptoms are diarrhea for two to ten days with watery symptoms, abdominal cramps, nausea, vomiting, and headache (DePaola *et al*., 2003; Austin, 2010).

Research suggests that the risk of infection is mainly due to the abundance of these bacteria at harvest, processing conditions, and cross-contamination (Brumfield *et al*., 2023). Most studies have shown that the presence of *V. parahaemolyticus* in seafood is influenced by sea surface temperature, particularly in the temperate regions (Harrison *et al*., 2022; Brumfield *et al*., 2023). Salinity is another frequently reported factor affecting the abundance of *V. parahaemolyticus* in seafood (Kalburge *et al*., 2014; Pazhani *et al*., 2021). *V. parahaemolyticus* is a halophilic bacterium that can spoil seafood and turn it pink (Leisner & Gram, 2014) and can increase the total volatile base nitrogen (TVBN) and thiobarbituric acid reactive substances (TBARS) (Sungsri-In *et al*., 2011; Secci & Parisi, 2016).

Seafood products susceptible to contamination by *V. parahaemolyticus* include salted squid. The production of salted squid usually involves soaking fresh squid in a saline solution, followed by drying to reduce the moisture content and bacterial growth. Alvianti *et al*. (2023) observed that salted squid in traditional Indonesian markets stored at room temperature for 2 weeks had halophilic bacterial contamination of $3.1 \times 10^4$ CFU/g, which increased over time. Vu *et al*. (2022) observed the growth of halophilic bacteria *V. parahaemolyticus* on squid in Vietnamese traditional markets, with 28.57% having bacterial counts reaching 4 log MPN/g. The standard for *V. parahaemolyticus* in squid products is 3 MPN/g (BPOM, 2019).

*Vibrio parahaemolyticus* can infect humans, usually at a colony count of $10^5$-$10^7$ CFU/g (FAO, 2011; Thongjun *et al*., 2013). The prevalence of *V. parahaemolyticus* in squid is 80% (Tan *et al*., 2020). Controlling bacteria, especially *V. parahaemolyticus* in salted squid, uses only hygienic procedures and sun-drying. However, this method is less effective in reducing the number of bacteria. Therefore, some manufacturers use boiling, which affects the product's nutrition due to heat-induced protein denaturation (Torres-Arreola *et al*., 2018; Tsai *et al*., 2021). In addition to the heating process, bacteria are controlled in squid products in some countries using formalin to maintain the appearance of squid and extend its shelf life (Jinadasa *et al*., 2022). However, formalin is carcinogenic and damages DNA (Noda *et al*., 2011), leading to the rejection of Indonesian fishery products containing formalin residues.

Roy et al. (2021) investigated non-thermal technology for reducing *V. parahaemolyticus* in seafood using UV-C and sodium hypochlorite (NaOCl) treatments individually and in combination. The results showed that the combination of UV-C 60 mW × s/cm² with 300 ppm NaOCl resulted in a maximum reduction of 3.78 log CFU/cm² on the surface of shrimp and 3.32 log CFU/cm² on the surface of crab. Zhu *et al*. (2023) used a combination of ultrasonic field (UF) and blue light (BL) to eliminate *V. parahaemolyticus* in fresh salmon, achieving

an elimination rate of 98.81%. Another study using high-pressure processing (HPP) at 250 MPa successfully reduced *V. parahaemolyticus* by 6.2 log CFU/g (Phuvasate & Su, 2015). However, its application in the production of salted squid is less relevant, such as the use of NaOCl chemicals and UV-C light, which is likely to damage the surface of salted squid. Simultaneously, ultrasonic devices require a large area, and the use of HPP is likely to damage the texture of salted squid products.

One method to process seafood without causing direct physical damage is to use pulsed electric fields (PEF), a non-thermal technology that uses electric fields to improve preservation and quality of seafood. PEF processing has little to no adverse effects on food nutrition (Tomasevic *et al*., 2023). This technology uses intense, high-frequency pulsed electric field forces that can modulate the activity of biological membranes. The dielectric breakdown mechanism has been used to explain the bactericidal effects of PEF-treated food (Lytras *et al*., 2024).

The great benefits of applying PEF technology for bacterial reduction have considerable challenges in terms of safety risks, namely high voltage (Machado *et al*., 2010) and the risk of reverse electric current (Holguin *et al*., 2020). This is because PEF typically operates with electric currents ranging from to 5-25 A (Raso *et al*., 2022). PEF research, mainly using 2-ampere current strength, has never been conducted, especially those that study the optimal electric field for the microbial reduction of *V. parahaemolyticus* in fishery products with high salt content, such as salted squid.

This study explored the use of PEF with a current strength of 2 A instead of the usual 10 A and used electric fields from low, medium, and high to reduce the colony and cell number of *V. parahaemolyticus* in salted squid. This study aimed to determine the optimal electric field and duration for inactivating *V. parahaemolyticus* and evaluate the effectiveness of PEF technology on salted squid. Optimal PEF treatment can improve the quality of salted squid products with low bacterial contamination and maintain the nutritional quality.

## MATERIALS AND METHODS

### Treatment on Salted Squid

Salted squid was prepared by soaking fresh squid (*Loligo duvaucelii*) from the Tegal City port, which was 10 cm long. The squid was soaked in a 15% w/v salt solution for ±30 min. The salt used was Refina brand obtained from a local supermarket. The samples were divided into two groups: salted squid with and without artificial contamination with *V. parahaemolyticus*. For the sample without artificial contamination, after 30 min of soaking, the sample was immediately drained in a sterile container. Non-contaminated salted squid samples produced bacterial colonies of approximately $10^2$ CFU/g.

Artificial contamination was conducted by adding 0.01 mL of Tryptic Soy Broth (TSB; Oxoid, United Kingdom) containing *V. parahaemolyticus* ATCC 17802 to salted squid after 30 min of immersion. *V. parahaemolyticus* ATCC 17802 strain was obtained from Thermo Fisher Scientific (USA). Artificially contaminated samples were incubated at 37°C for 24 h in media containing 15% w/v salt solution. After incubation, the artificial contaminant samples were drained into sterile containers. Contaminated salted squid samples produced bacterial colonies of approximately $10^6$ CFU/g. Contaminant and non-contaminant samples without PEF treatment or boiling were used as controls.

Two treatments, boiling and PEF, were applied to salted squid samples. In the boiling treatment, the salted squid was boiled for ± 2 min at 85°C and then drained. In the PEF treatment, electric fields of 3.5, 7, and 10.5 kV/cm for 10, 20, and 30 s were applied using a frequency of 684 Hz and 400 µs and a current strength of 2 A. The selection of the electric field and current strength was based on previous research (Darmawan *et al*., 2024). The electrode shape used was a parallel plate configuration with a tube shape for a uniform electric field distribution (Raso *et al*., 2022). The treatment chamber used a beaker glass containing 15% w/v salt solution with two electrodes between the salted squid samples. The process used a batch method to handle the static volume of the solid food (Raso *et al*., 2022).

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## Viable Count of *V. parahaemolyticus*

Enumeration of *V. parahaemolyticus* in salted squid was performed based on the SNI 2719.3:2011 standard (Badan Standardidasi Nasional [BSN], 2011) by homogenizing 10 g of salted squid in 90 mL of 0.9% NaCl physiological solution, followed by serial decimal dilutions. Appropriate dilutions were plated on Thiosulfate Citrate Bile Sucrose Agar (TCBSA; Himedia, India) for selective enumeration. The number of colonies corresponds to viable microorganisms, expressed as colony-forming units per gram (CFU/g) or their decimal logarithm (log10 CFU/g).

## Membrane Bacterial Cell Damage Assay by *Flow Cytometer*

The effect of PEF on cell membrane integrity was observed using flow cytometry (BD Attune plus, BD Biosciences, USA) as previously described by Falcioni *et al*. (2008) with minor modifications. *V. parahaemolyticus* ATCC 17802 was incubated in TSB media for 24 h at 37°C. Density was measured at a wavelength of 600 nm ($OD_{600}$). To evaluate the effect of medium and high electric field strength on bacterial membrane damage, the culture in TSB medium was treated with pulsed electric field (PEF) at PEF at 7 kV/cm for 20 s and 30 s (representing medium electric field strength), and at 10.5 kV/cm for 30 s (representing high electric field strength). This treatment selection aimed to observe the phenomenon of electroporation, particularly the extent of membrane disruption in bacterial cells, under different field intensities. After treatment, the TSB medium was centrifuged at 9,000 rpm for 15 min at 4°C and washed once with sterile phosphate-buffered saline (PBS; Oxoid, United Kingdom) before flow cytometry analysis.

Propidium iodide (PI; Sysmex, Japan), a membrane-impermeable fluorescent dye, was used to differentiate between dead and live cells. PI selectively penetrates cells with compromised membranes (i.e., dead cells), binds to nucleic acids, and fluoresces upon excitation. Before flow cytometry, PI staining was optimized by observing the fluorescence intensity using a fluorescence microscope. Samples showing optimal fluorescence at a concentration of 0.5 µL/0.5 mL were selected and analyzed using an Attune flow cytometer with a sample volume of 0.5 mL. The flow cytometer was set to detect forward scatter (FS) and side scatter (SS) for single-cell identification. Fluorescence detection was performed using a maximum excitation wavelength of 605 nm and an appropriate bandpass filter.

## Scanning Electron Microscope Observation

Observation of bacterial membrane damage using scanning electron microscopy (SEM; Thermo Scientific Prisma E, USA) is an advanced assay of bacterial reduction after using flow cytometry to prove the presence of electroporation phenomenon. Bacterial observation using SEM was performed as described by Chen *et al*. (2009) with minor modifications to the original protocol. Bacterial suspensions in TSB medium were treated with PEF7 kV/cm for 20 s and 30 s, and at 10.5 kV/cm for 30 s, while untreated suspensions served as controls. The bacterial suspensions were washed with 0.1 M phosphate buffer and fixed in the same buffer containing 2.5% glutaraldehyde and 4% paraformaldehyde. The samples were then fixed in 1% osmium tetroxide, dehydrated, and embedded in epoxy resin (Spurr, 1969) at a magnification of 10,000, a WD of 10.9 mm, and 10 kV.

## Total Protein Determination

The protein content was measured to observe the effects of the various treatments. The PEF treatments were selected based on the medium electric field (7 kV/cm, 30 s) and high electric field (10.5 kV/cm, 30 s), which showed the highest level of bacterial membrane damage among the electric field groups. Salted squid protein determination was carried out by cutting 25 g of mantle squid, followed by deconstruction, distillation, and titration according to the Kjeldahl method (AOAC, 2019).

## Statistical Analysis

Statistical analysis of bacterial colony counts for both artificial and non-artificial

treatments, bacterial cell reduction, and protein content was performed using a completely randomized design with one treatment without grouping. The experimental treatments consisted of various levels of each parameter. For bacterial colony counting, the treatment factors consisted of 10 levels: control (no treatment), boiling, and PEF treatment at 3.5, 7, and 10.5 kV/cm for 10, 20, and 30 s. For bacterial cell reduction, the treatment factors consisted of four levels: control, PEF 7 kV/cm for 20 s, PEF 7 kV/cm for 30 s, and PEF 10.5 kV/cm for 30 s. Protein content analysis was conducted using four levels of treatment: control, boiling, PEF at 7 kV/cm for 30 s, and PEF at 10.5 kV/cm (30 s). All treatments were performed in triplicate (n = 3). Statistical analysis for each dataset was performed using one-way analysis of variance (ANOVA) at a 95% confidence interval ($\alpha = 0.05$) to determine the significance of differences among treatment groups.

## RESULTS AND DISCUSSION

### Bacterial Reduction in Salted Squid

The effects of various treatments on *V. parahaemolyticus* in salted squid are shown in Table 1. The average number of *V. parahaemolyticus* in the artificially contaminated control sample was $2.28\times10^6$ CFU/g. This result indicates that salted squid supports the growth of *V. parahaemolyticus* (Vu *et al*., 2022). PEF treatment at an intensity of 10.5 kV/cm for 30 s reduced the number of bacteria to $7.86\times10^5$ CFU/g, equivalent to a reduction of 0.5 log CFU/g or 66.12%. Furthermore, using 10.5 kV/cm for 30 s for naturally contaminated samples, a 1.62 log CFU/g reduction was found, from $1.4 \times 10^2$ CFU/g in the control sample to $3.3 \times 10^0$ CFU/g in the treatment sample, or equivalent to 97.63% reduction. The results showed that the higher the applied electric field, the greater the bacterial reduction ($p<0.05$), as also indicated by Lee *et al*. (2015). This result also showed that an electric field strength of 10.5 kV/cm with a current strength of 2 A could reduce bacteria, as reported in a previous study (Toepfl, 2006), who used an electric field (~ 10-20 kV/cm) with a current strength of 10 A.

Higher currents, such as 10 A, are usually used and are much more effective for microbial inactivation by forming pores in the bacterial membrane (Raso *et al*., 2022). However, high current strengths can cause increased thermal effects, which can potentially damage food quality. The results of this recent study confirmed that a current strength of 2 A effectively reduced the microflora bacteria. Hence, the potentially damaged food quality can be minimized.

Furthermore, the effect of boiling was evaluated because it is the most common approach for removing *V. parahaemolyticus* from seafood (Ndraha *et al*., 2022). The boiling treatment resulted in bacterial reduction equivalent to 0.51 log CFU/g or 69.31% when the salted squid was contaminated at high levels. Meanwhile, at natural contamination levels, the boiling treatment resulted in a bacterial reduction of 1.68 log CFU/g, equivalent to a 97.86% reduction. The results of the boiling treatment were comparable to those of the highest electric field treatment (10.5 kV/cm for 30 s). These results show that PEF is one of the most promising technologies for inactivating microorganisms and achieving microbial inactivation equivalent to thermal treatment (Barba *et al*., 2015).

In this study, high contamination levels were achieved by artificial contamination using an overnight culture of *V. parahaemolyticus* that reached $10^8$-$10^9$ CFU/g (Chimalapati *et al*., 2020), with a final contamination level of approximately$10^6$ CFU/g. The exponential phase has a higher resistance to electric fields and heat, whereas bacteria in the lag phase are more susceptible to electric fields and heat (Lytras *et al*., 2024; Wang *et al*., 2015). The presence of salt also contributed to bacterial reduction (Yoon *et al*., 2025). Furthermore, although PEF treatments of salted squid with high contamination levels were not as effective as those of salted squid with low levels of contamination, this technology can still be considered promising because it does not involve heat, thus maintaining the nutritional quality of salted squid (Niu *et al*., 2020; Bhat *et al*., 2021).

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Table 1 Effects of various treatments on *V. parahaemolyticus* in salted squid
Tabel 1 Pengaruh berbagai perlakuan terhadap *V. parahaemolyticus* pada cumi asin

| Sample and treatment | Total *V. parahaemolyticus* in salted squid (Log CFU/g) | | | |
|---|---|---|---|---|
| | Artificially contaminated | Reduction (%) | Natural | Reduction (%) |
| Salted fresh squid* | 6.36±0.001[a] | - | 2.15±0.01[a] | - |
| Boiling 2 min at 85°C | 5.85±0.001[i] | 69.31 | 0.46±0.15[i] | 97.86 |
| PEF 3.5 kV/cm (10 s) | 6.31±0.01[b] | 10.87 | 2.13±0.02[b] | 4.03 |
| PEF 3.5 kV/cm (20 s) | 6.29±0.001[b] | 14.89 | 2.11±0.01[b] | 8.30 |
| PEF 3.5 kV/cm (30 s) | 6.24±0.001[c] | 24.14 | 2.1±0.01[c] | 11.42 |
| PEF 7 kV/cm (10 s) | 6.22±0.001[d] | 27.56 | 2.08±0.003[c] | 14.47 |
| PEF 7 kV/cm (20 s) | 6.21±0.003[d] | 29.21 | 1.91±0.003[d] | 42.03 |
| PEF 7 kV/cm (30 s) | 6.17±0.001[e] | 35.43 | 1.87±0.01[e] | 47.26 |
| PEF 10.5 kV/cm (10 s) | 6.07±0.001[f] | 48.71 | 1.48±0.01[f] | 78.87 |
| PEF 10.5 kV/cm (20 s) | 5.97±0.001[g] | 59.26 | 1.30±0.01[g] | 86.00 |
| PEF 10.5 kV/cm (30 s) | 5.89±0.002[h] | 66.12 | 0.52±0.07[h] | 97.63 |

*Soaked in 15% salt solution for ±30 min.
Data is presented as mean ± standard deviation of three independent experiments.
Different superscript letters (a-i) under the same column indicate significant differences ($p$<0.05).

The advantages of PEF in bacterial reduction also have weaknesses, namely, in low and medium electric fields, which only inactivate bacteria under reversible conditions (Demir *et al*., 2023). *V. parahaemolyticus* can still re-grow on raw or undercooked food (Wang *et al*., 2018) with 3-5 log growth rates in 3-5 hours at 37°C (FAO, 2020). Additional processing, such as heating above 50°C or storage at 4°C, is necessary for further bacterial control, especially for foods with high bacterial contamination potential (Zarei *et al*., 2014).

## Bacterial Cell Membrane Damage Observed using Flow Cytometer

The inactivation mechanism of the electric field was further observed by examining the number of *V. parahaemolyticus* ATCC 17802 cells after direct electric field treatment. As shown in Figure 1, the forward scatter and side scatter values detected using flow cytometry shifted from approximately $3.3 \times 10^4$ events/mL dead cells in the control sample (A) to $8.2 \times 10^4$ events/mL dead cells in the sample treated with 10.5 kV/cm for 30 s (D).

The number of red fluorescent cells in flow cytometer measurements indicated cell membrane damage (Pillet *et al*., 2016) because propidium iodide is a membrane-impermeable fluorescent DNA dye. The large number of dead cells or cell damage before electric field treatment was most likely caused by stress or differences in the cell response to sample preparation (Shapiro, 2008; Spidlen *et al*., 2021). Increased cell death or damage is likely caused by the membrane cell stress response to electric field treatment (Shapiro, 2008).

The number of events detected by the flow cytometer is presented in Table 2. The percentage indicates the average number of dead cells in the R1 (gate) region in FSC and SSC flow cytometry (He *et al*., 2018). In various treatments, the longer and higher the electric field, the higher the percentage value of R1, indicating that the number of dead cells

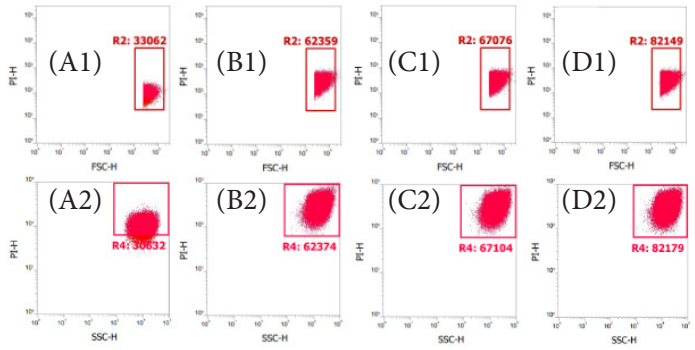


Figure 1 Representative forward scatter (A1–D1) and side scatter (A2–D2) plots of (A) untreated control cells, (B) cells exposed to 7 kV/cm for 20 seconds, (C) cells exposed to 7 kV/cm for 30 seconds, and (D) cells exposed to 10.5 kV/cm for 30 seconds. Forward and side scatter were measured to assess changes in cell size and granularity, respectively.

Gambar 1 Plot sebaran ke depan (A1-D1) dan sebaran samping (A2-D2) dari (A) sel kontrol yang tidak diberi perlakuan, (B) sel yang terpapar 7 kV/cm selama 20 detik, (C) sel yang terpapar 7 kV/cm selama 30 detik, dan (D) sel yang terpapar 10,5 kV/cm selama 30 detik. Sebaran ke depan dan ke samping diukur untuk menilai perubahan dalam ukuran dan granularitas sel.

Table 2 Effect of PEF on reduction of bacterial cells *V. parahaemolyticus* 17802
Tabel 2 Pengaruh PEF terhadap reduksi sel bakteri V. parahaemolyticus 17802

| Sample and treatment | Total cells (log cell/ 0.5 mL) | | Average reduction (%) |
|---|---|---|---|
| | Number of detected cells | Number of dead cells | |
| Control | 5.04±0.03 | 4.59±0.01 | 35.58±1.83[a] |
| Sample 7 kV/cm (20 s) | 5.08±0.04 | 4.82±0.01 | 55.59±4.8[b] |
| Sample 7 kV/cm (30 s) | 5.05±0.06 | 4.85±0.04 | 59.58±1.3[b] |
| Sample 10.5 kV/cm (30 s) | 5.03±0.09 | 4.84±0.07 | 67.73±3.3[c] |

Data is presented as mean ± standard deviation of three independent experiments.
Different superscript letters (a-c) indicate significant differences ($p<0.05$)

is increasing because R1 is an area that shows propidium iodide staining (Wang *et al.*, 2023). High % reduction values in propidium iodide measurements often indicate that cells were exposed to environmental stress or external agents that cause membrane damage to some bacteria (Shapiro, 2008; Power *et al.*, 2021).

In this study, PEF demonstrated great potential for controlling microorganisms in seafood, such as *V. parahaemolyticus* 17802. The high percentage reduction in PEF treatment indicated that this method was much more effective than that of the untreated control. In another study, increasing the voltage and duration significantly reduced *V. parahaemolyticus* in the treated products without causing adverse effects (Alkanan *et al.*, 2024). These data show that the reduction of bacterial cells should still be possible if there is an increase in the electric field or treatment duration.

## Bacterial Cell Damage Observed using Scanning Electron Microscopy

The effect of electric field treatment on the cell morphology of *V. parahaemolyticus* 17802 is shown in Figure 2. Intact *V.*

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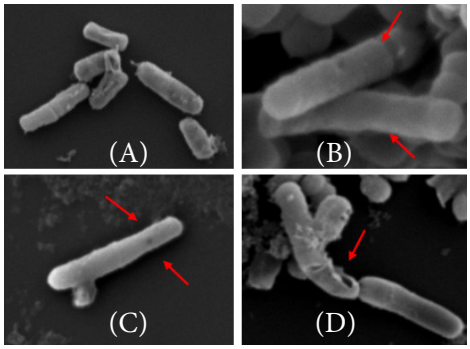


Figure 2 Observation of bacterial membrane shape damage from PEF treatment of *V. parahaemolyticus* ATCC 17802: (A) untreated sample, (B) 7 kV/cm electric field treatment (20 s), (C) 7 kV/cm electric field treatment (30 s), (D) 10.5 kV/cm electric field treatment (30 s).

Gambar 2 Pengamatan kerusakan bentuk membran bakteri dari perlakuan PEF terhadap *V. parahaemolyticus* ATCC 17802: (A) sampel yang tidak diberi perlakuan, (B) perlakuan medan listrik 7 kV/cm (20 detik), (C) perlakuan medan listrik 7 kV/cm (30 detik), (D) perlakuan medan listrik 10,5 kV/cm (30 detik).

*parahaemolyticus* 17802 cells were observed in samples without PEF treatment (Figure 2A). This shape was consistent with a previous study, which stated that *V. parahaemolyticus* 17802 is a rod-shaped Gram-negative bacterium with a small rod shape of approximately 1.5-3.0 µm and width 0.5-0.8 µm and has a polar flagella structure that allows it to move quickly in liquid media (Su & Liu, 2007).

In Figure 2B, the appearance of *V. parahaemolyticus* 17802 cell shape started to change in its integrity with a rough surface shape due to electric field treatment of 7 kV/cm (20 s). These results are consistent with those of previous studies that observed damage to bacterial cells with surface roughness and membrane damage in the form of pore formation, although some were still in sublethal injury conditions and had the potential to return to their initial form (Liu *et al.*, 2016; Li *et al.*, 2021; Croptova *et al.*, 2021). Furthermore, Figure 2C shows that much more damage than the previous process was observed on the cell shape of *V. parahaemolyticus* 17802, with visible holes in the cell shape due to the electric field treatment at 7 kV/cm (30 s). This damaged cell shape was thought to be due to membrane damage worsening through the electroporation process, which increases permeability and causes irreversible damage (Kotnik *et al.*, 2019).

Pillet *et al.* (2016) described the effect of electric fields on bacterial plasma membrane permeability, which irreversibly causes nucleic acid leakage. This phenomenon also shows physical changes in the cell wall, as observed in the SEM images. Figure 2D shows the perforated appearance of *V. parahaemolyticus* 17802 cells due to the 10.5 kV/cm, 30 s electric field treatment. This damage was thought to result in the loss of membrane integrity and cell lysis due to irreversible electroporation (Mahnič-Kalamiza & Miklavčič, 2022).

The inactivation effectiveness of PEF depends on the bacterial species (Niu *et al.*, 2020), and in general, the larger the cell, the more susceptible it is to electric fields (Heinz *et al.*, 2014). In this study, the results showed that the effectiveness of the highest electric field of 10.5 kV/cm for 30 s in damaging the bacterial membrane was higher than that of the lower electric field, with the appearance of a perforated cell shape.

## Effect of PEF on Protein Content of Squid Mantle

The PEF treatment at 7 kV/cm for 30 s did not affect the protein content in the squid mantle ($p<0.05$) (Figure 3). However, at 10.5 kV/cm for 30 s, a decrease in protein content of 14.91% from the control sample, 70.37% w/w to 59.88% w/w occurred. This decrease was also observed after boiling. This decrease

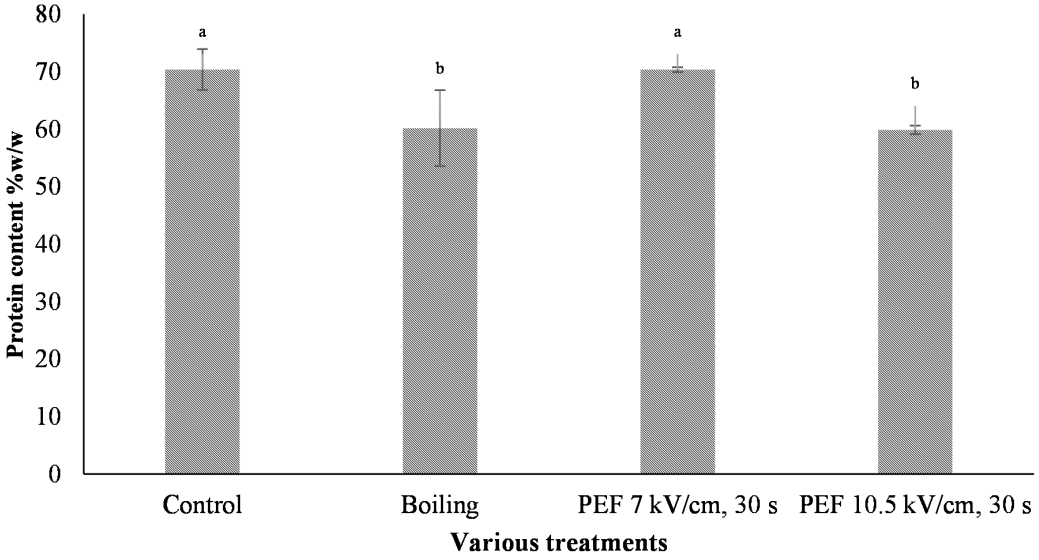


Figure 3 Protein content of salted squid of various treatments; different superscript letters (a-b) indicate significant differences ($p$<0.05)
Gambar 3 Kandungan protein cumi asin dengan berbagai perlakuan; huruf superskrip yang berbeda (a-b) menunjukkan perbedaan yang signifikan ($p$<0,05)

agrees with a previous study (Chen *et al*., 2023) that observed a decrease in the ζ-potential of PEF-treated long bean protein at 10 kV/

This is most likely due to electrostatic interactions that cause several changes in the functional and structural properties of proteins (Han *et al*., 2016; Liu *et al*., 2019), increased proteolysis (Bekhit *et al*., 2014; Bhat *et al*., 2019; Suwandy *et al*., 2015), and secondary structural changes that increase non-covalent cross-linking between protein molecules. This resulted in the leaching of the protein into the media. The results of the effect of electric fields on proteins are consistent with those of previous studies that reported changes in the aggregate structure of proteins when forces using electric fields of 5-20 V/cm resulted in the loss of *α-helix* and *β-turn* content (Chen *et al*., 2022).

Based on the results of this study, PEF treatment at 7 kV/cm for 30 s using a current strength of 2 A can be considered an alternative process for salted squid, since it did not affect the protein content while the bacterial reduction apparently occurred. The PEF treatment at 10.5 kV/cm for 30 s can also be considered an alternative process for heat-processed salted squid, since the effects on protein content and bacterial reduction were comparable with those of boiling treatment.

This finding aligns with previous studies showing that PEF can effectively inactivate microorganisms through cell membrane permeabilization (electroporation) without damaging protein compounds and product sensory quality (Toepfl *et al*., 2006; Barba *et al*., 2015). In addition, PEF can be an alternative to seafood processing, as it ensures microbiological safety and maintains nutritional content (Gómez *et al*., 2019).

The results of this study show that the PEF designed is applicable to the process of making salted squid. However, the research conducted still has a laboratory-scale design. Then it needs testing in a pilot plant scale with a capacity of pilot plant scale with a capacity of 10–20 kg/batch process.

## CONCLUSION

This study found that PEF with a current of 2 A and an electric field of 10.5 kV/cm for 30 s effectively reduced *V. parahaemolyticus* in salted squid, with a reduction rate of 97.63% in non-artificially contaminated samples and

66.12% in artificially contaminated samples. PEF treatment at 7 kV/cm for 30 s did not affect protein quality, whereas 10.5 kV/cm caused a 14.91% reduction, which is negligible compared to its significant effectiveness in reducing *V. parahaemolyticus* and improving the microbiological safety of salted squid products.

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