

Comparative Study of Medium and Low Intensity Pulsed Electric Field and Its Effect on Protein and Fat of Mackerel Tuna and Shrimps

Arry Darmawan¹, Qirom², Arum Restu Widyasti³

¹ Department of Food Science and Technology, IPB University, Bogor, Indonesia

² Department of Electronics, Politeknik Harapan Bersama, Tegal, Indonesia

³ Department of Chemistry, Chulalongkorn University, Thailand

ARTICLE INFO

Article history:

Received January 10, 2024

Revised February 20, 2024

Published May 15, 2024

Keywords:

PEF;
Bacteria;
Low-intensity;
Medium-intensity;
Protein;
Fat

ABSTRACT

Pulsed electric field (PEF) is a well-known non-thermal technology used to inactivate bacteria and improve the physico-chemical properties of food products, including food products from the sea. However, the application of this technology in the seafood industry has a major challenge in terms of safety risk, namely electrical backlash. Therefore, several researchers have begun to study the reduction of PEF current intensity (ampere) to obtain the benefits of food treatment without the risk of electric backlash. A comparison of the performance of both medium and low-intensity PEF devices can be seen from conductivity, reliability, and back electricity. In addition to the device's performance, the effects obtained on seafood from medium and low-intensity PEF treatment on bacterial inactivation and chemical properties of protein and fat need attention. The results showed that low- and high-intensity PEF showed good electrical conductivity in media with various salt concentrations, namely 5%, 15%, and 20% (w/v). The reliability of the components tested were transistors, transformers, and ignition coils. In the return electricity test, PEF with low intensity has low return electricity compared to PEF with medium intensity. The testing process was carried out with the test pen spaced at a certain point from the power source. The effect of medium-intensity PEF on protein and fat was insignificant between the control and treatment samples. Comparison of the effect of medium and low-intensity PEF was highly significant at $P < 0.05$. The effect of low-intensity PEF on protein and fat is much better because it does not reduce the value of protein and fat in fish and shrimp too much. Medium-intensity PEF can be effectively treated quickly, but it takes work to apply in the home industry. Low-intensity PEF needs to be treated longer than medium-intensity PEF but tends to be safer from the risk of electrical backlash.

Corresponding Author:

Corresponding Author

Arry Darmawan, Department of Food Science and Technology, IPB University, Bogor, Indonesia

Email: profarry@apps.ipb.ac.id

1. INTRODUCTION

Pulsed electric field (PEF) is emerging as a non-thermal technique that is recognized for its efficacy in bacterial inactivation [1] and enhancement of food's physical properties [2]. In its application, PEF has two categories at high voltage: medium (10 ampere) and low (2 Ampere) intensity. PEF treatment has been shown to improve meat tenderness in several ways. This includes reducing muscle dimensions and increasing the distance between muscle cells [3]. In addition, PEF increases muscle fiber fragments [4]. The process also involves the formation of pores in the myofibrils, which leads to the release of calcium ions from the sarcoplasm and the enzyme μ -calpain, as well as an increase in the proteolysis process [5]. PEF treatment also affects the temperature at which collagen, a major component of connective tissue, denatures, resulting in increased solubility of connective tissue during the ripening process [6].

PEF treatment of fishery products has little effect on the content of lipid oxidation, free amino acids (FAA), and saturated fatty acids (SFA) in abalone shell samples compared to samples without PEF treatment [2]. The effect of PEF and OMF-based supercooling technology on salmon preservation can extend the storage time of salmon while maintaining its freshness [7]. In addition to fish, PEF research has also been conducted on PEF-treated Pacific white shrimp soaked in Chamuang leaf extract (CLE), showing lower melanosis scores compared to control samples [8].

PEF is the most commonly used technology in microbial inactivation and has been shown to have little impact on physico-chemical and nutritional properties [9]. The advantages of PEF also have drawbacks, namely that it is difficult to industrialize and risky to operate because the electric field voltage reaches several kV [10]. Therefore, it is necessary to have a study that examines PEF with low intensity (2 Ampere) as a comparison of PEF, which is usually used with medium intensity. This study discusses the manufacture of medium-intensity and low-intensity PEF, the effects on bacterial inactivation, and the characteristics of protein and fat as an indicator of the quality of fishery products with samples of tuna and shrimp. Both tools were measured for electrical conductivity, reliability, and electrical return, as well as the effect on protein and fat and inactivation of pathogenic bacteria from fishery products.

2. Related Works

Several researchers have researched PEF, which has the advantage of this technique to maintain the final product's physical, organoleptic, and functional characteristics and minimize changes in flavor, vitamins, and other nutrients [11]. PEF research has also been applied to fishery products, such as research [3] that evaluated the effect of PEF on the microstructure of salmon and lumpfish eggs. The research by modifying the current intensity in PEF has been conducted in the following studies.

Tabel 1. Penelitian PEF yang terkait

Sample	Treatment	Reference
Sea Bass	PEF with 5 and 10% w/w NaCl applied current intensities were set at 10 and 20 A treated with 300 and 600 V cm ⁻¹ , respectively.	[12]
Fish sashimi	Various waveforms with a capacity of 80 μF, an electric field of 15 kV, and a brine conductivity of 5 mS/cm. The peak values of voltage, current, and power reached 15 kV, six kA, and 100 MW, respectively, and the time duration (10% of the peak) was about 380 μs.	[13]
Cow milk (liquid)	Bacterial reduction of >5 log CFU/mL can be achieved at 50-55°C, 0.3 A-0.6 A, and with an initial 5 min intensity of 5 V/cm - 9 V/cm.	[14]
Squid	PEF (6 kV cm ⁻¹ , 300 pulses, 5 Ampere) for 5 minutes with ethanolic custard apple leaf extract	[15]
Sea Bass	PEF 24 kV/cm 100 A for 72 ms, with a frequency of 1 Hz, followed by hydrolysis with porcine pancreatic lipase (PPL) at 42.36 U/g..	[16]

3. METHODS

The design of this research method is first to make a medium-intensity and low-intensity pulsed electric field tool by trial and error method, then test the performance of the voltage released and the resulting electric waveform. After the input and output of the tool are appropriate, the electric field conductivity test is carried out using distilled water, 5%, 15% and 20% salt solution to see the difference in conductivity values that will be submitted in the calculation of the electric field value. The next step is to test the reliability with the life test experiment method, which will take 120 minutes to test the transistor components, ignition coil, and electrodes. Electricity back is tested by setting the highest volt of 50 kV and tested affixed to the high voltage casing.

3.1. Conductivity Testing PEF

The conductivity test was carried out by testing the electrical voltage on the anode in distilled water and salt solution with a concentration of 5%, 15% and 20%; data collection was carried out for 30 seconds using a multimeter with 100 volts-1000 volts. Statistical analysis in this conductivity test uses 2-way ANOVA by looking at the difference between each concentration of salt content with aquadest.

3.2. Reverse electrical testing PEF

The reverse electricity test was carried out by applying a full voltage of 50 kV to the high voltages; then, a pen test was carried out on the electrode and ignition coil at a distance of 30 cm. All static and reverse electricity test data were analyzed with a T-test comparison between PEF medium intensity and low intensity.

3.3 Reliability testing PEF

Reliability testing using an assessment life test experiment with the stress level used is a voltage of 25 kV for 120 minutes continuously with the parameter of temperature increase. Components of concern are transistors, ignition coils and electrodes. The data obtained from this reliability test will be a T-test comparison between PEF medium and low intensity.

3.4 Pathogenic Bacteria Testing

Identification and enumeration of *Vibrio parahaemolyticus* in tuna and shrimp were based on SNI standards [17]. The test procedure weighed 50 g of samples that had been mashed and then diluted 1:10 by homogenizing 50 g of samples using 2% Phosphate Buffer Saline (Himedia) solution for 2 minutes. Then, dissolve 1: 5 by weighing 20 g of homogenate 1: 2 into 80 ml of diluent to obtain the sample homogenate with a dilution of 1: 10 or solution 101. Dilution series 102 by dissolving 1 ml of solution 101 into 9 ml of 3% PBS diluent. Each dilution was shaken at least 25 times. Transfer 1 ml of each dilution into three dilution tubes containing 10 ml of Alkaline Peptone Water (APW) (Himedia) using a sterile pipette. Incubate the tubes at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 16 - 18 hours. Take one full ose from each positive (cloudy) tube at each dilution to a depth of 1 cm from the liquid surface and scratch it into TCBS. Incubate at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 hours.

3.5 Protein and fat testing of PEF effects

Protein and fat testing was carried out with the proximate test of Kjeldahl and soxlet methods; the effect of protein testing was carried out using tuna and shrimp samples treated with 7.14 kV / cm between electrodes for 30 seconds and then testing the content. The test results were then subjected to a T-test comparison between PEF medium and low intensity.

4. RESULTS AND DISCUSSION

The design begins with making high voltages of high voltage pulsed electric fields, in the first stage of compiling and designing how the components used can work properly, such as transformers, frequency generators and converting from DC electricity into pulses.

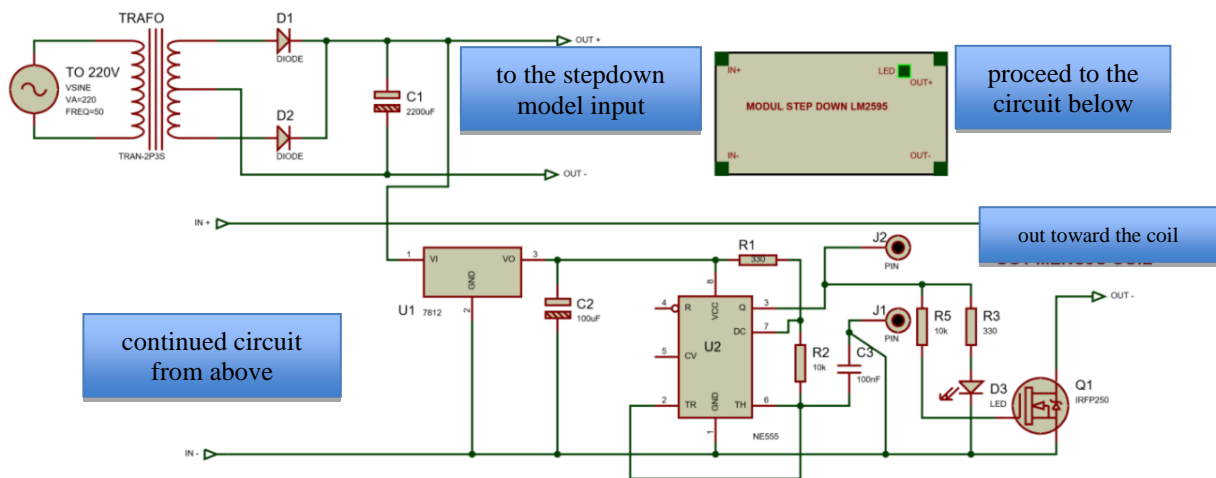
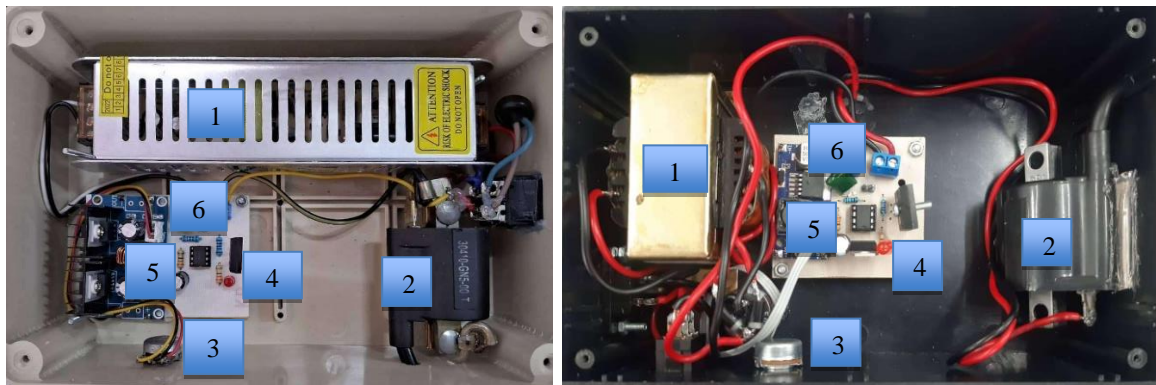


Figure 1. Series of highvoltages PEF

After the high-voltage circuit is designed, the next step is to implement the design drawing into a real form. The design is done by selecting the appropriate components and meeting the criteria for a good electronic circuit. The high voltages designed in **Figure 2** are high voltages with a current of 2 amperes (right) and 10 amperes (left).



(a)

(b)

Figure 2. High voltage pulsed electric field design implementation High voltage pulsed electric field design implementation (a) 10 amperes (b) 2 amperes. Notes: 1. Transformator step down, 3. Potensiometer 5. Dioda Bridge convert AC to DC, 4. Modul LM 2596 DC (DC step down), 6. IC NE555 (raised frequency), 2. Ignition coil.

After implementing the image on the PEF high voltages tool circuit in real life, test the resulting output voltage with an oscilloscope. The resulting output voltage form is pulsed, as shown in **Figure 3**.

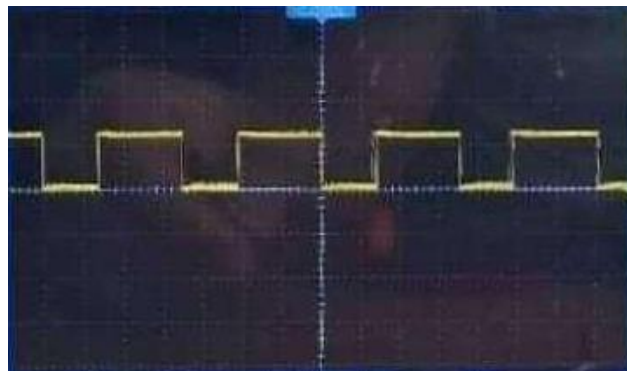
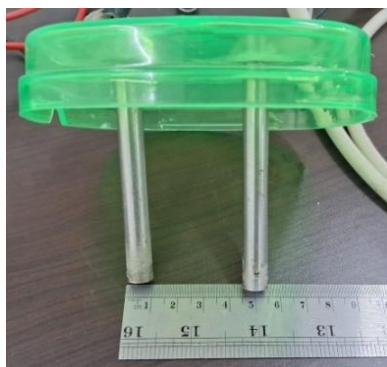


Figure 3. The output voltage is pulsed.

Rectangular pulses with fast-rising and falling edges were chosen because this pulse type is the most efficient process and produces a low-temperature rise [18]. After ensuring that the voltage output is in the form of a square pulse, the ampere was increased from 2 amps to 10 amps to compare the performance of PEF. Both high voltages have tube-shaped electrode outputs with a distance of 3.5 cm.



(a)

(b)

Figure 4. Form electrode PEF (a) distance electrode (b) testing anoda

The design and output of PEF testing results at two amps and ten amps obtained the difference data detailed in Table 2..

Table 2. Comparison of HV PEF 2 ampere and 10 ampere

Specification	Output Low intensity	Output Medium intensity
Voltages	1kV up to 25kV	1kV up to 25kV
Electrode	Parallel tube	Parallel tube
Material electrode	Stainless steel	Stainless steel
Strong electric current/ Intensity	2 Ampere	10 Ampere
Handling	Rectangular	Rectangular
Form dc electrical	square pulse	square pulse
Wave	684 Hz	684 Hz
Wave frequency	400 μ s	400 μ s

The next stage, after obtaining all the data between low-intensity and medium-intensity PEF, is conducting a reverse electricity test to ensure the safe use of PEF. Before testing, personal protective equipment [19] is used, especially insulating gloves, to protect from electrical induction, according to [20], and PEF-induced electricity testing is carried out by putting type [21] to detect areas that are induced by electricity. After that, testing is carried out in certain areas with the potential to have back electricity. In testing the back electricity of PEF low intensity, almost every part is safe from back electricity. However, at PEF 10 ampere, there is back electricity in parts such as electrodes, ignition coil and high voltage casing. The amount of back electricity and the distance used in the measurement is shown in **Figure 5**.

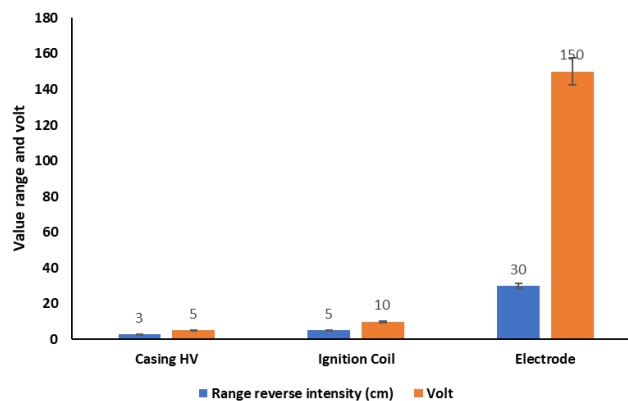


Figure 5. Distance and magnitude of return electricity of each part of PEF medium intensity

Reverse current is when the load forces current back to the power supply source. Pulse reverse current (PRC) is another type of pulse waveform in which the anodic pulse follows the cathodic pulse [22]. After ensuring safety, PEF's durability will be tested using the life test experiment method on several components such as transistors, ignition coils, and electrodes. The testing process lasted up to 120 minutes; the results are shown in **Figure 6**.

In the low-intensity and high-intensity PEF generation trials, there were differences in the temperature rise of some components, so reliability testing was carried out. Reliability testing aims to measure the life of the experimental unit developed. One of the reliability assessment methods is Accelerated Life Testing (ALT), which estimates time to failure by accelerating working conditions to predict changes in material properties over time [23]. ALT identifies the level of stress on the system and increases the stress to observe possible damage, predicting damage under normal use with a shorter test time [24][25].

Stress factors affecting the service life of electronic products include voltage, temperature, and humidity. In this study, two interrelated factors, voltage and temperature, are explored where an increase in voltage corresponds to an increase in temperature. Thermal management is very important in electronic products because it directly affects the device's performance, especially the mobility of power carriers, which decreases at high temperatures [26]. The time selection of 120 minutes is based on the component voltage beyond the normal usage limit.

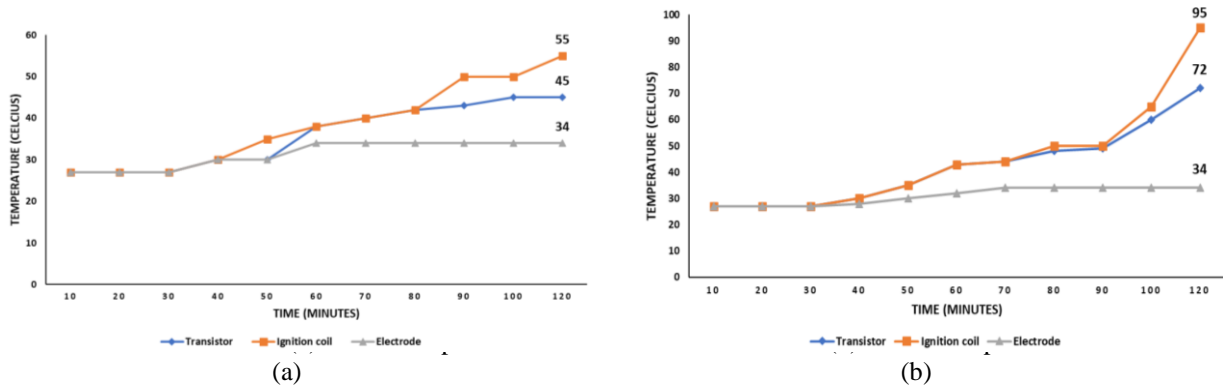


Figure 6. Temperature increase of PEF 2 ampere (a) and PEF 10 ampere (b)

In the reliability testing process, it can be seen that PEF low intensity has less temperature increase than PEF medium intensity with a significant value of $P < 0.05$ due to different electric currents. The largest electrical load occurs on the ignition coil because this component receives pulsed electricity, which flows to the electrodes. This load causes the ignition coil to be the hottest among other components. After knowing the heat, the PEF medium intensity is used for additional fans and PEF in a cool room.

Electrical conductivity testing at various salt levels

After reliability and repair, conductivity tests of various salt concentrations were carried out. Both low and medium-intensity PEFs have almost no difference in conductivity values. The results of the conductivity measurement are shown in Figure 7.

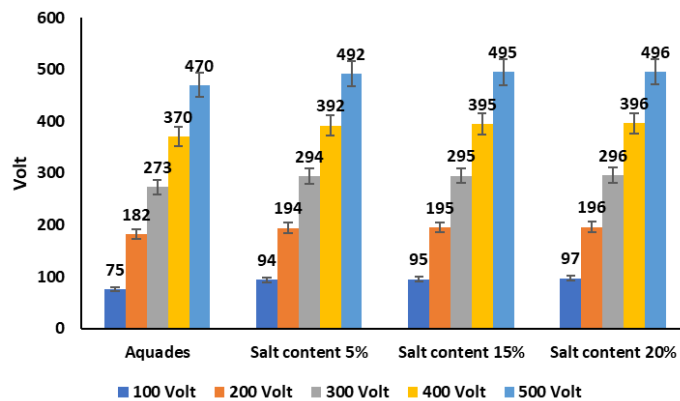


Figure 7. Electrical conductivity values of various salt levels

Electrical conductivity is an evaluation of the ability of a solution to conduct an electric current through the ions contained in it. Ions play a key role in the ability of a solution to conduct electric current, and this conductivity measurement is often used routinely in various industrial and environmental applications to assess the amount of ions in a solution [27]. Assessment of the electrical conductivity of an electrolyte solution involves measuring the resistance of the solution between two electrodes, which can be flat or cylindrical and separated by a fixed distance [28]. The difference in conductivity between distilled water and various salt contents showed significant values at each difference in salt concentration ($P < 0.05$).

Evaluation of PEF on Inactivation of pathogenic bacteria

In the testing process, the sample bacteria is *Vibrio parahaemolyticus* bacteria. *V. parahaemolyticus* is a Gram-negative halophilic bacterium commonly found in seafood [29]. Consumption of raw or undercooked seafood contaminated with *V. parahaemolyticus* can cause severe gastroenteritis, septicemia, and even death in humans [30]. The halophilic bacterium *Vibrio parahaemolyticus* is also a psychrophilic bacterium [31] that can damage foodstuffs under low-temperature conditions and is a foodborne pathogen that causes 39-51% of infections compared to other *Vibrio* types [29]. Raw or undercooked seafood is the main cause of vibrio infections [32].

The working principle of the pulsed electric field against bacteria is the electroporation phenomenon that occurs when the device is turned on. Electroporation is a method that uses electric shock to enlarge the cell membrane's pores to increase the membrane's permeability [33]. The efficiency of PEF in compressing biological cells mainly depends on the part of the applied pulse (time) at which the electric field strength exceeds a certain critical value [34].

Reversible electroporation is when the created pores will close again after the electric field is removed [35]. At the same time, irreversible electroporation is when the pores formed by the electric field can not return to the beginning [36]. The value of bacterial reduction from PEF low intensity and medium intensity can be seen in Figure 8 and Figure 9.

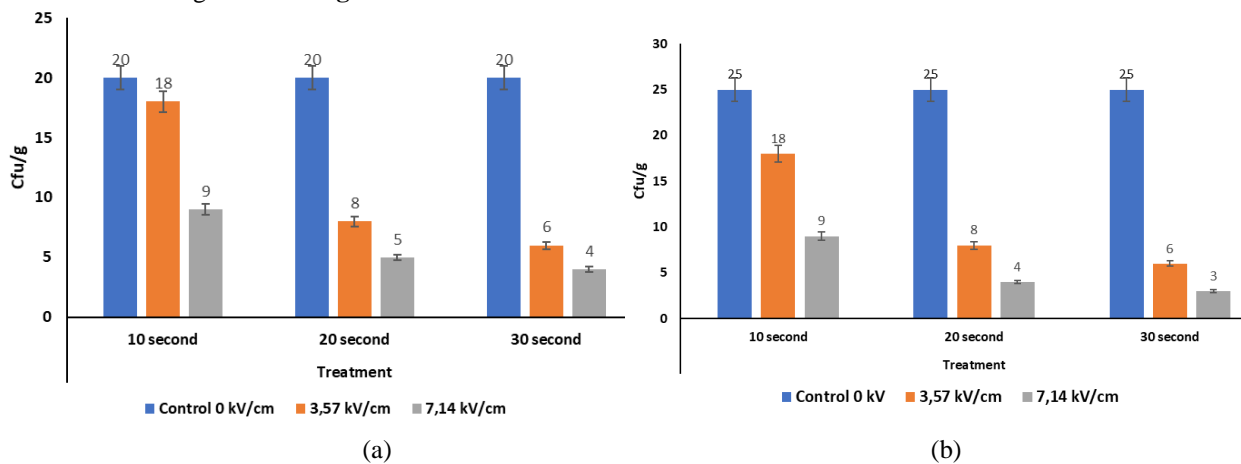


Figure 8. PEF 2 Ampere inactivation of tuna sample (a) and shrimp sample (b).

In Figure 8 a and b, the initial bacterial colonies of 20 cfu/g for tuna and 25 cfu/g for sea shrimp were then treated with 3.57 kV/cm and 7.17 kV/cm with low intensity (2 ampere); the resulting reduction value is not much different from the treatment of 10 seconds to 30 seconds, none of the reduction values exceed 1 log of bacteria, but only able to reduce the number of colonies in each log. The same is true with Figure 9, where using medium-intensity PEF can only reduce some colonies without reaching 1 log of bacteria even though it uses 7.14kV/cm with a 30-second treatment. This is by some previous studies that used treatments generally 15kV/cm [9].

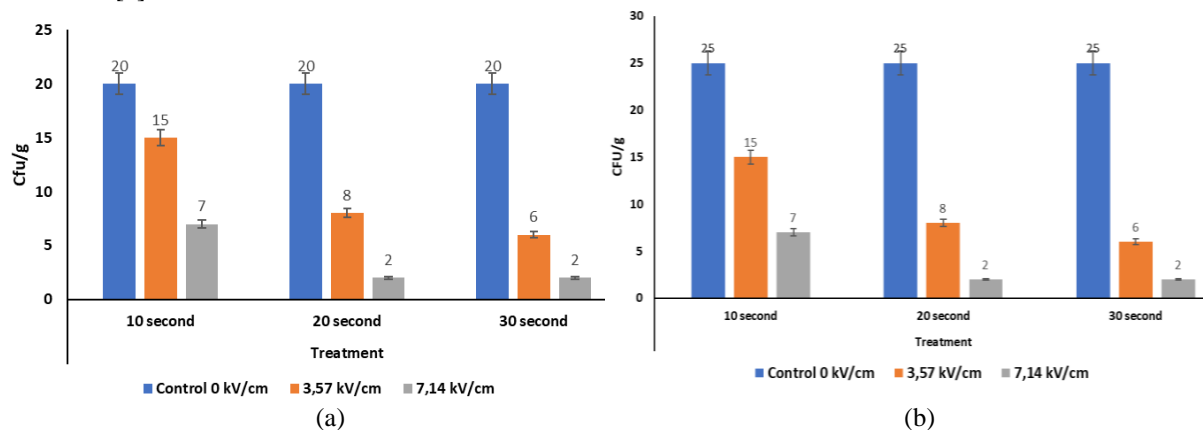


Figure 9. PEF 10 Ampere inactivation of tuna sample (a) and shrimp sample (b)

Both treatments using shrimp and tuna samples had no significant difference in bacterial reduction. The number of initial bacterial colonies obtained results from the growth of TCBS media with sample preparation, which was first washed with clean water. The initial colony of shrimp is higher than that of tuna because shrimp are animals that are susceptible to contamination with *V. parahaemolyticus*. After all, their main food is zooplankton, which is likely to be contaminated with *V. parahaemolyticus* [37].

Testing the effects of PEF on protein and fat

After looking at the process of bacterial reduction in low-intensity and medium-intensity PEF treatments, look at the effects on protein and fat. To assess the quality of good fishery products, it is necessary to see the quality of protein and fat content. The value of protein and fat is in Figure 10. PEF treatment on control and low-intensity samples did not experience too many significant changes, in contrast to medium-intensity control samples. This is because the current strength used in low intensity has not been able to cause too much electroporation in muscle fibers and initial proteolysis like PEF medium intensity.

PEF differs from traditional tenderizing methods such as aging, electrical stimulation, and mechanical intervention and can be applied to various muscles by adjusting its parameters [38]. The effect of PEF on proteins causes the deflation that results from physical disruption of the muscle structure [39]. PEF induces electroporation of cell membranes to promote the movement of intracellular components further, leading to protein hydrolysis and damaging the ultrastructure of meat to increase meat tenderness [40]. The conductivity of the sample plays an important role in the effectiveness of PEF treatment [41] since the degradation of myofibrillar proteins largely determines meat tenderness. This biochemical process takes time to work [42].

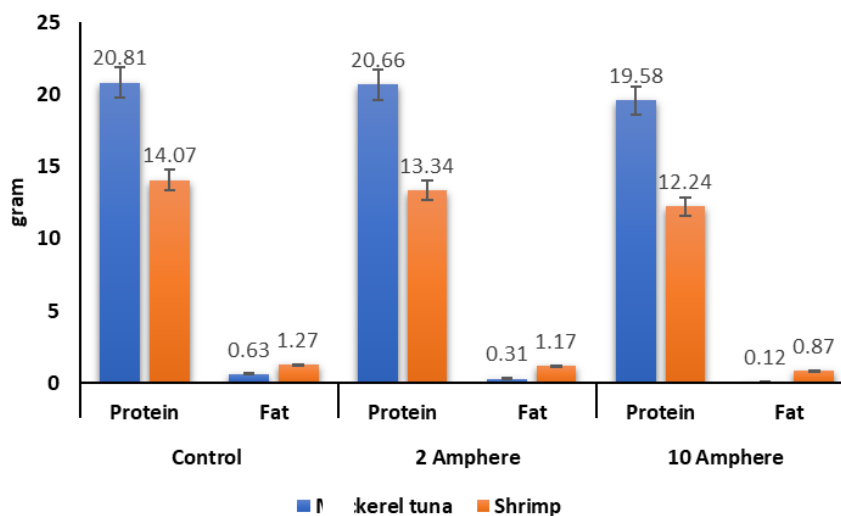


Figure 10. Decrease in protein and fat due to PEF treatment

The increased lipid reduction may be due to PEF-mediated electroporation and hydrodynamic and deformation-relaxation phenomena. During the PEF process, electroporation can promote pore formation, which causes the skin matrix to relax. When PEF is applied, the possible presence of trapped gas is expelled from the porous skin capillaries [8]. PEF treatment can penetrate those pores more effectively and hydrolyze the lipids localized in the skin [10].

PEF treatment may result in a decrease in protein and fat in tuna and prawns. However, the decrease in both protein and fat is not very significant compared to the food safety benefits obtained as a result of bacterial reduction. Medium-intensity PEF can be a fast bacterial inactivation technology but requires large-scale industries. In contrast, low-intensity PEF can be used as bacterial inactivation in small industries, although the treatment time must be increased for the desired effect to be achieved.

5. CONCLUSION

Both low-intensity and medium-intensity PEF can reduce bacteria and improve fisheries quality. However, medium-intensity PEF still has the risk of electrical backlash compared to low-intensity PEF, which is safer. The use of low-intensity PEF requires more time due to its effectiveness in inactivating bacteria compared to medium-intensity PEF, which can reduce more bacteria simultaneously. The effects on protein and fat between low-intensity and medium-intensity PEF are not too different, so low-intensity PEF is more feasible to consider regarding the safety of use.

REFERENCES

- [1] Van Impe J, Smet C, Tiwari B, Greiner R, Ojha S, Stulić V, et al. State of the art of nonthermal and thermal processing for inactivation of micro-organisms. *J Appl Microbiol* [Internet]. 2018;125(1):16–35. Available from: <http://dx.doi.org/10.1111/jam.13751>.

-
- [2] Luo Q, Hamid N, Oey I, Leong SY, Kantono K, Alfaro A, et al. Physicochemical changes in New Zealand abalone (*Haliotis iris*) with pulsed electric field (PEF) processing and heat treatments. *Lebenson Wiss Technol* [Internet]. 2019;115(108438):108438. Available from: <http://dx.doi.org/10.1016/j.lwt.2019.108438>.
- [3] Gudmundsson M, Hafsteinsson H. Effect of electric field pulses on microstructure of muscle foods and roes. *Trends Food Sci Technol* [Internet]. 2001;12(3–4):122–8. Available from: [http://dx.doi.org/10.1016/s0924-2244\(01\)00068-1](http://dx.doi.org/10.1016/s0924-2244(01)00068-1).
- [4] Faridnia F, Bekhit AE-DA, Niven B, Oey I. Impact of pulsed electric fields and post-mortem vacuum ageing on beef longissimus thoracis muscles. *Int J Food Sci Technol* [Internet]. 2014;49(11):2339–47. Available from: <http://dx.doi.org/10.1111/ijfs.12532>.
- [5] Bhat ZF, Morton JD, Mason SL, Mungure TE, Jayawardena SR, Bekhit AE-DA. Effect of pulsed electric field on calpain activity and proteolysis of venison. *Innov Food Sci Emerg Technol* [Internet]. 2019;52:131–5. Available from: <http://dx.doi.org/10.1016/j.ifset.2018.11.006>.
- [6] Alahakoon AU, Faridnia F, Bremer PJ, Silcock P, Oey I. Pulsed electric fields effects on meat tissue quality and functionality. In: *Handbook of Electroporation*. Cham: Springer International Publishing; 2017. p. 2455–75.
- [7] Oscillating Magnetic Field (Omf) And Pulsed Electric Field (PEF)-Assisted Supercooling For The Improved Shelf Life Of Fresh Salmon Fillets. Theses. University of Hawai'i at Mānoa Hamilton Library 2550 McCarthy Mall Honolulu. 2022.
- [8] Ahmad Shiekh K, Benjakul S. Melanosis and quality changes during refrigerated storage of Pacific white shrimp treated with Chamuang (*Garcinia cowa* Roxb.) leaf extract with the aid of pulsed electric field. *Food Chem* [Internet]. 2020;309(125516):125516. Available from: <http://dx.doi.org/10.1016/j.foodchem.2019.125516>.
- [9] Niu D, Zeng X-A, Ren E-F, Xu F-Y, Li J, Wang M-S, et al. Review of the application of pulsed electric fields (PEF) technology for food processing in China. *Food Res Int* [Internet]. 2020;137(109715):109715. Available from: <http://dx.doi.org/10.1016/j.foodres.2020.109715>.
- [10] Al-Hilphy AR, Abdulstar AR, Gavahian M. Moderate electric field pasteurization of milk in a continuous flow unit: Effects of process parameters, energy consumption, and shelf-life determination. *Innov Food Sci Emerg Technol* [Internet]. 2021;67(102568):102568. Available from: <http://dx.doi.org/10.1016/j.ifset.2020.102568>.
- [11] Pourzaki A, Mirzaee H, Hemmati Kakhki A. Using pulsed electric field for improvement of components extraction of saffron (*crocus sativus*) stigma and its pomace: Extraction of saffron components by pef. *J Food Process Preserv* [Internet]. 2013;37(5):1008–13. Available from: <http://dx.doi.org/10.1111/j.1745-4549.2012.00749.x>.
- [12] Crototova J, Tappi S, Genovese J, Rocculi P, Laghi L, Dalla Rosa M, et al. Study of the influence of pulsed electric field pre-treatment on quality parameters of sea bass during brine salting. *Innov Food Sci Emerg Technol* [Internet]. 2021;70(102706):102706. Available from: <http://dx.doi.org/10.1016/j.ifset.2021.102706>.
- [13] Onitsuka C, Nakamura K, Wang D, Matsuda M, Tanaka R, Inoue Y, et al. Inactivation of anisakis larva using pulsed power technology and quality evaluation of horse mackerel meat treated with pulsed power. *Fish Sci* [Internet]. 2022;88(2):337–44. Available from: <http://dx.doi.org/10.1007/s12562-022-01593-2>.
- [14] Ji F, Sun J, Sui Y, Qi X, Mao X. Microbial inactivation of milk by low intensity direct current electric field: Inactivation kinetics model and milk characterization. *Curr Res Food Sci* [Internet]. 2022;5:1906–15. Available from: <http://dx.doi.org/10.1016/j.crfs.2022.10.015>.
- [15] Olatunde OO, Shiekh KA, Ma L, Ying X, Zhang B, Benjakul S. Effect of the extract from custard apple (*Annona squamosa*) leaves prepared with pulsed electric field-assisted process on the diversity of microorganisms and shelf-life of refrigerated squid rings. *Int J Food Sci Technol* [Internet]. 2021;56(12):6527–38. Available from: <http://dx.doi.org/10.1111/ijfs.15355>.
- [16] Chotphruethipong L, Aluko RE, Benjakul S. Enhanced Asian sea bass skin defatting using porcine lipase with the aid of pulsed electric field pretreatment and vacuum impregnation. *Process Biochem* [Internet]. 2019;86:58–64. Available from: <http://dx.doi.org/10.1016/j.procbio.2019.08.012>.
- [17] SNI 01-2332.5-2006. Cara Uji vibrio parahaemolyticus. Jakarta: BSN; 2006.
- [18] Kempkes MA. Industrial Pulsed Electric Field Systems. Di Dalam: *Handbook of Electroporation*. Vol. 2. Springer International Publishing; 2017.
- [19] UU RI Nomor 1 Tahun 1970 Tentang Keselamatan Kerja. Undang-Undang Republik Indonesia Nomor 1 Tahun 1970 Tentang Keselamatan Kerja.
- [20] Peraturan Menteri Ketenagakerjaan Nomor 12 Tahun 2015 tentang Keselamatan dan Kesehatan Kerja Listrik di Tempat Kerja. Peraturan Menteri Ketenagakerjaan Nomor. 2015;12.
- [21] Badan Standarisasi N. Penjelasan Persyaratan Umum Instalasi Listrik tahun. In: *Standar Nasional Indonesia (SNI)* Jakarta: Yayasan PUIL. 2011.
- [22] M.S.Chandrasekar, M. Pushpavanam, Pulse and pulse reverse plating-conceptual, advantages and applications, *Electrochim. Acta*. 53 (2008) 3313–3322.
- [23] Pascual F. Accelerated life test planning with independent weibull competing risks. *IEEE Trans Reliab* [Internet]. 2008;57(3):435–44. Available from: <http://dx.doi.org/10.1109/tr.2008.928205>.
- [24] Acevedo PE, Jackson DS, Kotlowitz RW. Reliability growth and forecasting for critical hardware through accelerated life testing. *Bell Labs Tech J* [Internet]. 2006;11(3):121–35. Available from: <http://dx.doi.org/10.1002/bltj.20183>.
- [25] Jayatilleka S, Okogbaa G. Use of accelerated life tests on transmission belts for predicting product life, identifying better designs, materials and suppliers. In: *Annual Reliability and Maintainability Symposium, 2003*. IEEE; 2003.
- [26] M. Janicki, P. Janus, et al., Measurement and modeling of heat conduction in MEMS nanostructures, in: *17th IEEE ITherm Conference* vol. 17, 2018, pp. 196–202.
-

-
- [27] Gray JR. Environmental Instrumentation and Analysis Handbook. Lehr RD, editor. Wiley; 2004
- [28] Bockris JO, Reddy AK, Gamboa-Aldeco M. Modern Electrochemistry (2nd). Springer; 1998.
- [29] Odeyemi OA, Stratev D. Occurrence of antimicrobial resistant or pathogenic *Vibrio parahaemolyticus* in seafood. A mini review. *Revue de Médecine Vétérinaire*. 2016;6.
- [30] Newton A, Kendall M, Vugia DJ, Henao OL, Mahon BE. Increasing rates of vibriosis in the United States, 1996–2010: Review of surveillance data from 2 systems. *Clin Infect Dis* [Internet]. 2012;54(suppl_5):S391–5. Available from: <http://dx.doi.org/10.1093/cid/cis243>.
- [31] Liao X, Shen W, Wang Y, Bai L, Ding T. Microbial contamination, community diversity and cross-contamination risk of food-contact ice. *Food Res Int* [Internet]. 2023;164(112335):112335. Available from: <http://dx.doi.org/10.1016/j.foodres.2022.112335>.
- [32] Hackbusch S, Wichels A, Gimenez L, Döpke H, Gerdt G. Potentially human pathogenic *Vibrio* spp. in a coastal transect: Occurrence and multiple virulence factors. *Sci Total Environ* [Internet]. 2020;707(136113):136113. Available from: <http://dx.doi.org/10.1016/j.scitotenv.2019.136113>.
- [33] Kotnik T, Rems L, Tarek M, Miklavčič D. Membrane electroporation and electropermeabilization: Mechanisms and models. *Annu Rev Biophys* [Internet]. 2019;48(1):63–91. Available from: <http://dx.doi.org/10.1146/annurev-biophys-052118-115451>.
- [34] Luengo E, Raso J. Pulsed Electric Field-Assisted Extraction of Pigments from *Chlorella vulgaris*. In: *Handbook of Electroporation*. Cham: Springer International Publishing; 2017. p. 2939–54.
- [35] Demir E, Tappi S, Dymek K, Rocculi P, Gómez Galindo F. Reversible electroporation caused by pulsed electric field – Opportunities and challenges for the food sector. *Trends Food Sci Technol* [Internet]. 2023;139(104120):104120. Available from: <http://dx.doi.org/10.1016/j.tifs.2023.104120>.
- [36] Batista Napotnik T, Polajžer T, Miklavčič D. Cell death due to electroporation - A review. *Bioelectrochemistry* [Internet]. 2021;141(107871):107871. Available from: <http://dx.doi.org/10.1016/j.bioelechem.2021.107871>.
- [37] Fu S, Wang R, Zhang J, Xu Z, Yang X, Yang Q. Temporal variability of microbiome in the different plankton hosts revealed distinct environmental persistence of *Vibrio parahaemolyticus* in shrimp farms. *Microbiol Res* [Internet]. 2023;275(127464):127464. Available from: <http://dx.doi.org/10.1016/j.micres.2023.127464>.
- [38] Bhat ZF, Morton JD, Mason SL, Bekhit AE-DA. Current and future prospects for the use of pulsed electric field in the meat industry. *Crit Rev Food Sci Nutr* [Internet]. 2019;59(10):1660–74. Available from: <http://dx.doi.org/10.1080/10408398.2018.1425825>.
- [39] O’Dowd LP, Arimi JM, Noci F, Cronin DA, Lyng JG. An assessment of the effect of pulsed electrical fields on tenderness and selected quality attributes of post rigour beef muscle. *Meat Sci* [Internet]. 2013;93(2):303–9. Available from: <http://dx.doi.org/10.1016/j.meatsci.2012.09.010>.
- [40] Zhang Y, Wang R, Wen Q-H, Rahaman A, Zeng X-A. Effects of pulsed electric field pretreatment on mass transfer and quality of beef during marination process. *Innov Food Sci Emerg Technol* [Internet]. 2022;80(103061):103061. Available from: <http://dx.doi.org/10.1016/j.ifset.2022.103061>.
- [41] Karki R, Oey I, Bremer P, Silcock P. Understanding the effect of meat electrical conductivity on Pulsed Electric Field (PEF) process parameters and the ability of PEF to enhance the quality and shorten sous vide processing for beef short ribs. *Food Res Int* [Internet]. 2023;163(112251):112251. Available from: <http://dx.doi.org/10.1016/j.foodres.2022.112251>.
- [42] Warner RD, Wheeler TL, Ha M, Li X, Bekhit AE-D, Morton J, et al. Meat tenderness: advances in biology, biochemistry, molecular mechanisms and new technologies. *Meat Sci* [Internet]. 2022;185(108657):108657. Available from: <http://dx.doi.org/10.1016/j.meatsci.2021.108657>.
-

BIOGRAPHY OF AUTHORS**Arry Darmawan**

Academic Qualifications:

(BA) Chemistry Science from Yogyakarta State University, Indonesia

(BA) Food Science and Technology from Open University, Indonesia

(M.Sc) Industrial Engineering from Gadjah Mada University, Indonesia

(Doctoral candidate) Food Science from IPB University, Indonesia

Email: profarry@apps.ipb.ac.id

**Qirom**

Academic Qualifications:

(B.Ed) Electronics Education from Semarang State University, Indonesia

(M.Eng) Electronic Engineering from Islam Sultan Agung University, Indonesia

Email: qirom@poltektegal.ac.id

**Arum Restu Widyasti,**

Academic Qualifications:

(B.Ed.) in Chemistry Education from Yogyakarta State University, Indonesia

Doctoral Student at the Department of Chemistry, Faculty of Science, Chulalongkorn

University, Bangkok, Thailand (Now)

Email: aroemrestu@gmail.com
