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Minimum Intensity of Pulsed Electric Field for Deactivation of *Staphylococcus aureus*, *Salmonella sp*. and *Escherichia coli* Bacteria

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Abstract: Electric fields with high-intensity pulses can cause cell death in human organs. This study aimed to investigate the minimum intensity of pulsed electric fields that can still be used for bacterial deactivation, bacterial size effects on cell membrane permeability thresholds and effects of treatment to electric fields on protein and fat content. The study was conducted on a laboratory scale with samples of *Staphylococcus aureus*, *Salmonella sp*. and *Escherichia coli* bacteria grown in milk. The results showed that *Escherichia coli* bacteria needed an electric field with a minimum intensity of 3.5 kV/cm, while *Staphylococcus aureus* and *Salmonella sp.* required a minimum intensity of 4.0 kV/cm with a treatment time of about 25 minutes. The finding in this study was that larger size bacteria have a lower permeability threshold than small bacteria. The treatment by electric fields with minimum intensity did not reduce the protein content but decreased the fat content in milk. Decreased protein and fat content in milk was influenced by the intensity of the electric field used. The treatment of using pulsed electric fields has the potential to be used to process food products into low-fat foods.

Key words: Electric ・ Membrane permeability threshold ・ Bacteria

INTRODUCTION

Processing food products often cause contamination [1, 2], making food unsafe for consumption [3]. One way to deactivate bacteria but not produce heat on bacterial growing media is to use high intensity pulsed electric fields [4]. The interaction of the electric field with bacteria can result in irreversible electroporation of the bacterial cell membrane so that the bacteria die [5]. The same condition with bacteria can also occur in the body cells of workers who handle bacterial deactivation or sterilization if the security is lacking. The treatment uses an electric field with an intensity of 60 kV/cm and pulse duration of 60 ns, for one hour in human organs causing 10-20% of affected cells to die [6]. Treatment with a combination of 0.23 kV/m electric field with high dust concentration with an average duration of 6 hours/day causes complaints of facial skin [7]. Cell size increases when the electric field is 3.0 - 60 kV/cm with the duration of pulses greater than 60 ns charged [8]. An electric field with an intensity of 500 V/cm with pulse duration of 70 µs of 200 pulses can eliminate microcirculation in the treated area [9]. The research above shows that high-intensity electric fields and long pulse duration negatively affect the affected organ. Therefore deactivation of bacteria using a low intensity pulsed electric field is needed to minimize its impact on health.

Research on the deactivation of bacteria using electric field pulses has been carried out by Zhao *et al.* [10], Cortese *et al.* [11], Montfort *et al.* [12] and Pal [13]. Zhao *et al.* [10] conducted a study using electric fields with intensities of 18.1, 27.4 and 38.4 kV/cm, Cortese *et al.* [11] with an intensity of 35 kV/cm, Montfort *et al.* [12] with an intensity of 25 kV/cm and Pal [13], with intensity 10-80 kV/cm. Research generally uses high-intensity electric fields and does not report minimum intensity that can still be used, so it is necessary to investigate the minimum intensity of the electric field that can still be used to deactivate bacteria.

In order for the bacterial deactivation process to be safer for health, this study investigated the minimum intensity of pulsed electric fields that can still be used to deactivate bacteria and the duration of time required for deactivation. The results of this study are expected to be

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used as a reference for the deactivation of bacteria both for food sterilization or overcoming bacterial infections. The study also investigated the effect of bacterial size on cell membrane permeability thresholds, because contamination and infection can be caused by various types of bacteria. The study also investigated the effects of pulsed electric field treatment on protein and fat content in milk, because it has been previously reported that electric fields with pulses of 10-20 kV/cm reduce more than 40% α-casein and β-lactoglobulin in milk [14].

MATERIALS AND METHODS

Materials: Fresh cow's milk is taken from dairy farmers in the city of Batu-Malang, pure isolates of *Staphylococcus aureus, Salmonella sp*. and *Escherichia coli* bacteria from UIN microbiology laboratory, Nutrient Agar (NA), Nutrient Broth (NB) and Plate Count Agar (PCA) media.

Electric Field Generation: The pulsed electric field in this study was generated from a high voltage power supply connected to the switch. Switch output and ground power supply are connected to parallel plates of copper. The high voltage power supply used had an output voltage specification of 0 ± 20.0 kV direct current (DC), an output current of 0 to ± 20 mA DC. Switches were used to convert DC electric fields into pulses.

Sample Preparation: The research samples were sterilized (fresh cow's milk) then each of them was inoculated with *Staphylococcus aureus, Salmonella sp*. and *Escherichia coli* bacteria. Calculation of the number of bacterial colonies [15] was carried out before and after being treated using an electric field, where calculations before the next treatment were used as controls. Each treatment was carried out five repetitions. Measurement of protein and fat levels are carried out on fresh cow's milk without sterilization. Measurements are made before treatment and after treatment. Each measurement was carried out three times.

Sterilization and Measurement Processes: The treatment was carried out using an electric field of 2.0-4.0 kV/cm with pulse duration of 50 μs for 5-25 minutes and 4.5 kV/cm for 15 minutes. Treatment is carried out at an ambient temperature of 25°C and humidity of 60%. Milk that has been given bacteria was placed in a container made of glass. The number of bacterial colonies was calculated using a colony counter, while the protein and fat content was measured using Lactoscan MCC.

RESULTS

The treatment of using electric fields in milk causes the number of contaminant bacteria colonies to decrease. The logarithmic form of the decrease in the number of bacterial colonies was calculated using the equation:

$$Decrease\ (log) = -log\frac{N_t}{N_o} \quad (1)$$

where N_o. is the number of bacterial colonies before treatment and N_t is the number of bacterial colonies after being given an electric field treatment [16].

Electric Field Effect on Bacteria: The number of bacterial colonies before being treated using electric fields was 49 x 10^8, 89 x 10^8 and 215 x 10^7 Colony Forming Units per milliliter (CFU/ml) for *Staphylococcus aureus*, *Salmonella sp.* and *Escherichia coli* respectively. The number of bacterial colonies was used as a control in calculating the amount of bacterial decrease.

Fig. 1(a) shows that the electric field treatment of 2.0 - 3.5 kV/cm for 5 minutes reduced the number of bacterial colonies from 0.057 to 0.64 log so that it cannot be used to deactivate the *Staphylococcus aureus* bacteria. This is indicated by the same electric field treatment for 25 minutes decreasing the number of bacterial colonies that occur in the amount of 0.23 - 0.93 log. Treatment using a 4.0 kV/cm electric field for 5 minutes decreased the number of bacterial colonies that occurred by 1.06 log so that it can be used to deactivate *Staphylococcus aureus* bacteria. Deactivation using a 4.0 kV/cm electric field for 25 minutes decreased the number of significant bacterial colonies by 7.61 log, so it can be concluded that the deactivation of *Staphylococcus aureus* bacteria required a minimum electric field intensity of 4.0 kV/cm.

Figure 1(b) shows a graph of the effects of electric field treatment on *Salmonella sp.* Treatment with an electric field intensity of 2.0 - 3.5 kV/cm for 5 minutes reduced the number of bacterial colonies by 0.034 - 0.91 log, which showed that the intensity was not enough to be used to deactivate bacteria. The electric field ≤ 3.5 kV/cm has not been able to make the permeability of the bacterial cell membrane exceed the permeability threshold so that it has not caused damage to the cell membrane. Treatment using an electric field with an intensity of 4.0 kV/cm for 5 minutes reduced the number of bacteria by 5.45 log which means that the intensity can be used to deactivate *Salmonella sp.* bacteria. Therefore, it can be concluded that the deactivation of

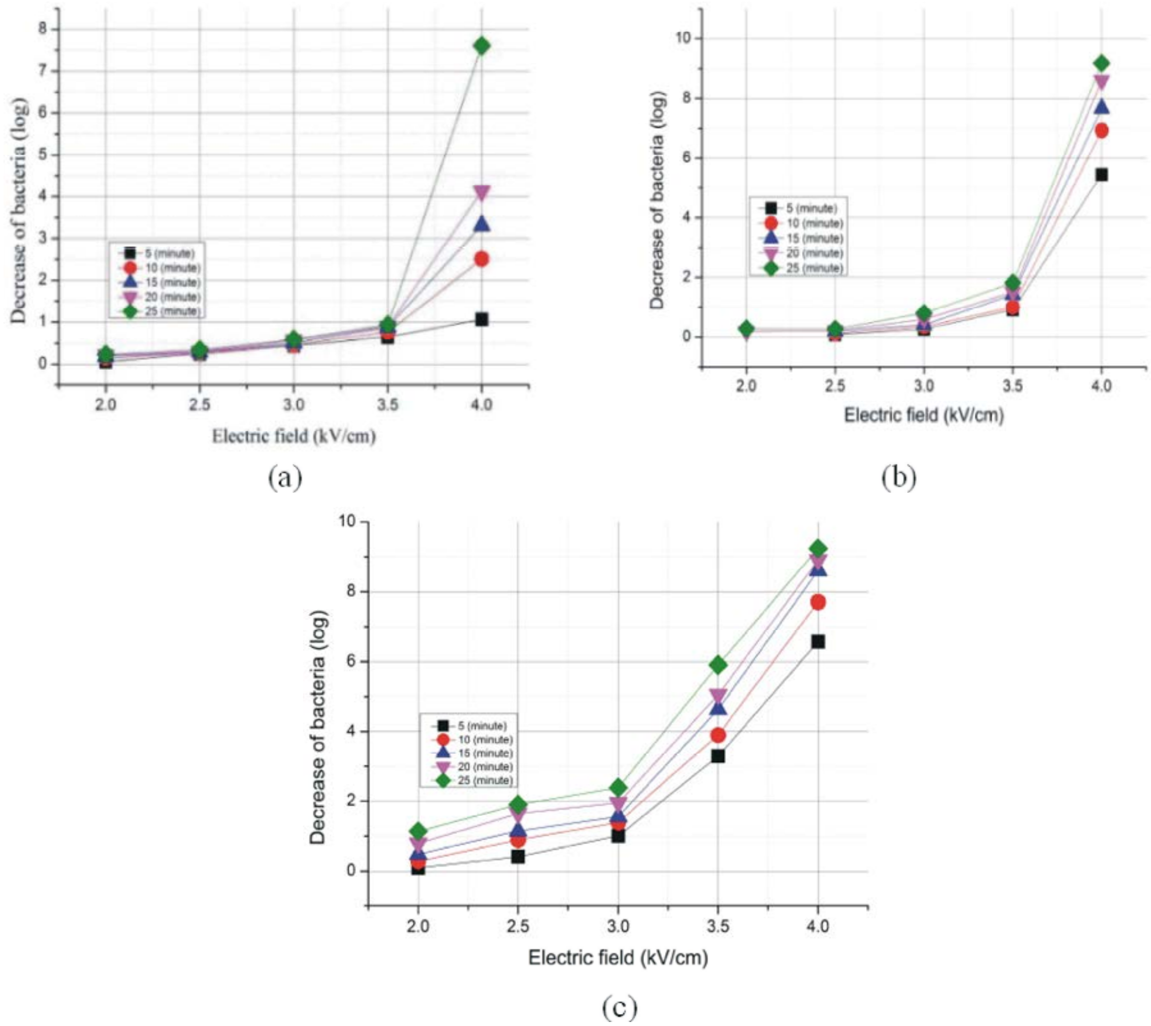


Fig. 1: Graphic electric field effect to the decreasing number of bacteria colony (a) *Staphylococcus aureus*, (b) *Salmonella sp* and (c) *Escherichia coli*

Salmonella sp. bacteria requires a minimum electric field intensity of 4.0 kV/cm, where treatment for 25 minutes decreases the number of bacterial colonies by 9.19 log.

The decrease in the number of *Escherichia coli* bacteria due to treatment to the electric field is shown in Figure 1(c). The figure shows that treatment using an electric field intensity of 2.0 - 3.0 kV/cm for 5 minutes decreases the number of bacterial colonies by 0.95 - 1.01 log so that it is not enough to deactivate *Escherichia coli* bacteria. This was proven that the addition of treatment time to 25 minutes decreased the number of bacterial colonies which occurred at 1.14 - 2.39 log. Meanwhile, treatment using an electric field of 3.5-4.0 kV/cm decreased the number of bacterial colonies by 3.29 - 6.57 log, allowing it to be used for the activation of *Escherichia coli* bacteria. With the same electric field, the addition of treatment time to 25 minutes decreases the number of bacterial colonies by 5.91 - 9.24 log. Therefore, it can be concluded that the deactivation of *Escherichia coli* bacteria requires a minimum electric field intensity of 3.5 kV/cm.

Increasing the intensity of the electric field after crossing the threshold of permeability causes a significant decrease in the number of bacteria. This condition can be seen in table 1, where the decrease in the number of bacteria due to treatment to the electric field 4.0 kV/cm compared to 4.5 kV/cm has a significant difference. Decreasing the number of bacteria due to treatment for 15 minutes with an electric field of 4.0 kV/cm was 3.31, 7.67 and 8.61 log for *Staphylococcus aureus*, *Salmonella sp.* and *Escherichia coli* bacteria respectively. When the electric field intensity is 4.5 kV/cm, there will be a decrease of 8.05, 8.65 and 8.96 log respectively.

Table 1: Bacteria treatment using an electric field for 15 minutes

	Decreasing number of bacteria (log)		
Field Intensity kV/cm	*Staphylococcus aureus*	*Salmonella sp.,*	*Escherichia coli*
2.0	0.19 ± 0.00309	0.11 ± 0.0024	0.49 ± 0.000890
2.5	0.29 ± 0.00085	0.19 ± 0.0063	1.15 ± 0.000061
3.0	0.51 ± 0.00187	0.40 ± 0.0071	1.56 ± 0.005900
3.5	0.88 ± 0.04090	1.42 ± 0.0440	4.63 ± 0.025000
4.0	3.31 ± 0.04782	7.67 ± 0.1776	8.61 ± 0.021000
4.5	8.05 ± 0.22421	8.65 ± 0.2120	8.96 ± 0.120000

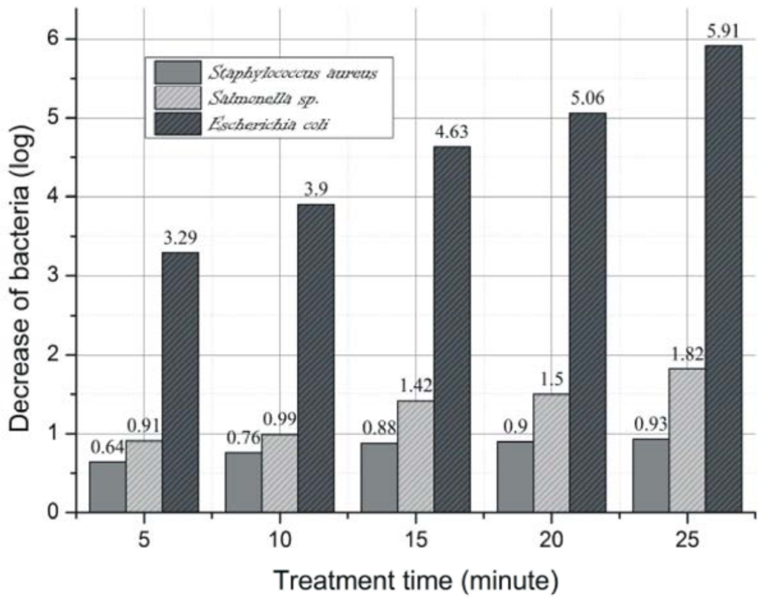
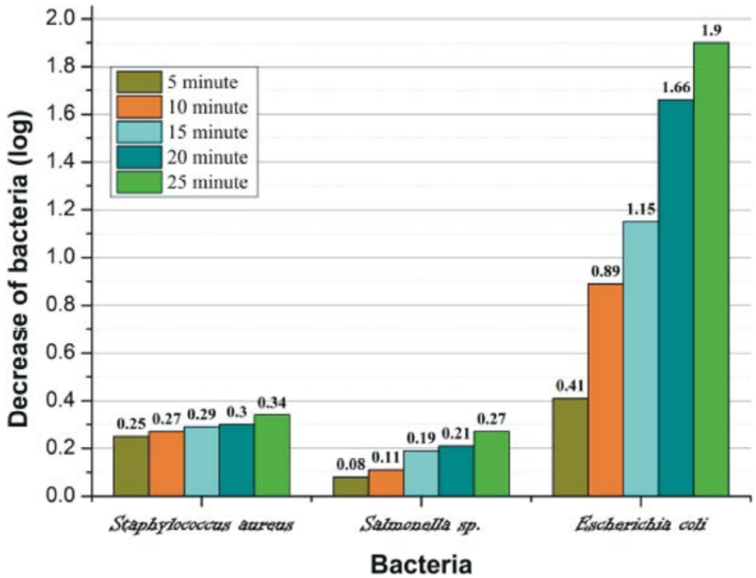


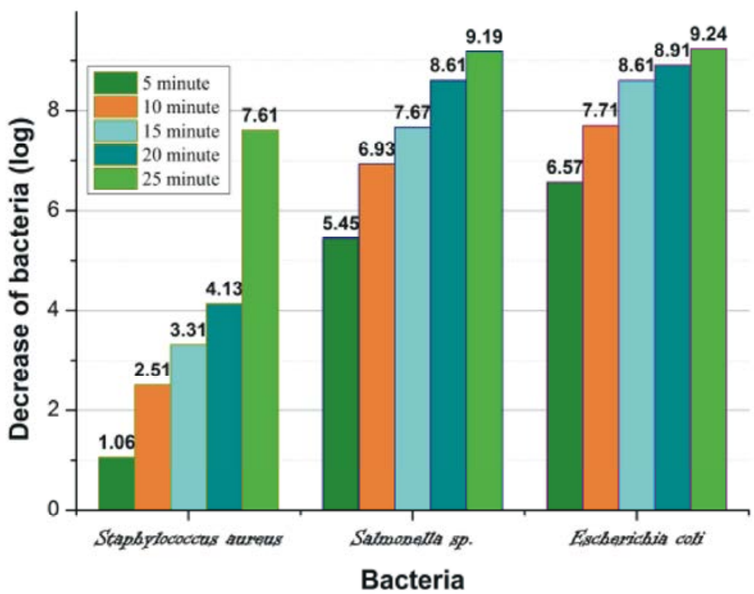
Fig. 2: Graph of the decrease in the number of colonies of *Staphylococcus aureus*, *Salmonella sp.* and *Escherichia coli* after being exposed to an electric field of 3.5 kV/cm

Effect of Bacterial Size: *Staphylococcus aureus* is a Gram-positive bacterium, which is coccus in shape with a diameter of 0.5 - 1.5 µm [17]. *Salmonella sp*. is a Gram-negative and rod-shaped bacteria with a size of 2 - 3 x 0.4 - 0.6 µm [18]. *Escherichia coli* is a Gram-negative bacterium with a size of ~ 1 µm with a length of ~ 2.5 µm [19]. Therefore, when viewed from its size, the bacteria *Staphylococcus aureus* < *Salmonella sp* < *Escherichia coli*. Treatment by the electric field against *Escherichia coli* bacteria caused a greater amount of reduction when compared with *Salmonella sp.* and *Staphylococcus aureus* bacteria. This condition occurs because the size of *Escherichia coli* bacteria is greater than that of *Salmonella sp.* and *Staphylococcus aureus* bacteria. Larger bacteria have a larger surface area so that treatment to the electric field causes electroporation in a larger area. Fig. 2. shows that the electric field treatment of 3.5 kV/cm for 25 minutes resulted in a decrease in the number of *Escherichia coli* bacterial colonies by 5.91 log, while *Staphylococcus aureus* and *Salmonella sp.* decreased by 0.93 and 1.82 log respectively. Thus it can be concluded that the deactivation of larger bacteria requires smaller energy compared to small-sized bacteria.

Long-Term Effects of Treatment on Bacteria: The decrease in the number of bacteria is also influenced by the duration of treatment [20]. The duration of treatment only plays a major role when the electric field used has exceeded the membrane permeability threshold. This condition can be seen in Fig. 3(a & b), where treatment using an electric field 2.5 kV/cm *Staphylococcus aureus* bacteria decreased by 0.25 log and 0.34 log for 5 minutes and 25 minutes treatment time, respectively. Meanwhile, treatment to an electric field was 4.0 kV/cm, *Staphylococcus aureus* bacteria decreased by 1.06 log and 7.61 log for 5 minutes and 25 minutes, respectively. Similar conditions also occur in *Salmonella sp.* and *Escherichia coli* bacteria, where treatment with electric



(a)

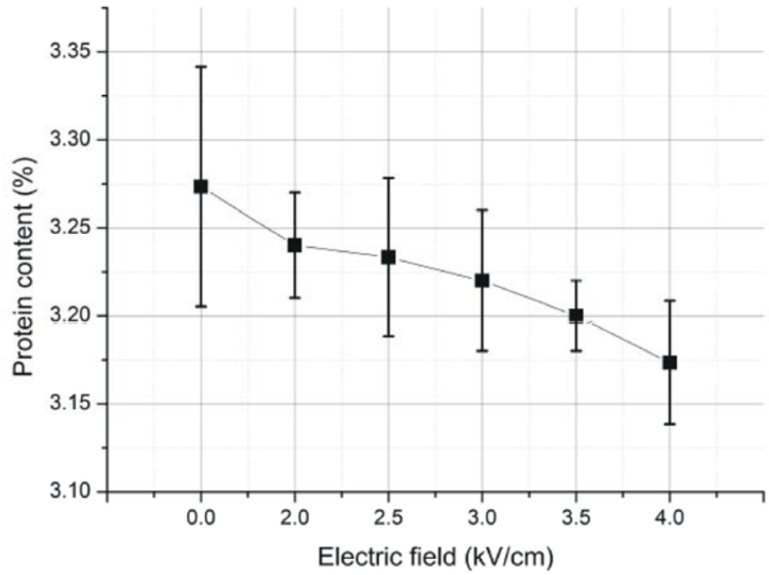


(b)

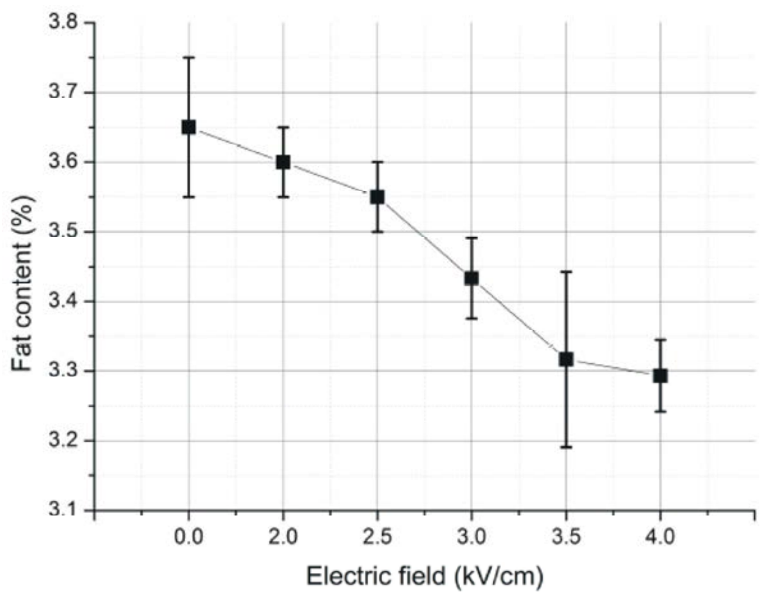
Fig. 3: Graph of the effect of time of treatment to a decrease in the number of bacterial colonies (a) treatment to an electric field of 2.5 kV/cm, (b) treatment to an electric field of 4.0 kV/cm.

field intensity of 2.5 kV/cm treatment time had little effect on decreasing the number of bacterial colonies, whereas at an intensity of 4.0 kV/cm treatment time had a large effect on decreasing the amount bacterial colonies.

Electric Field Effect on Protein and Milk Fat Content: Treatment to the electric field 2.0 - 4.0 kV/cm in milk causes a small decrease in protein content. Figure 4(a) shows that the milk protein content before being treated



(a)



(b)

Fig. 4: Effect of electric field on protein and fat content with a treatment time of 25 minutes (a) protein, (b) fat

with an electric field is 3.27% and after being treated with an electric field of 2.0 kV/cm for 25 minutes the protein content is 3.24%. Treatment to using a 4.0 kV/cm electric field for 25 minutes decreased the protein content to 3.17% or changed by 3.03% when compared to the initial content. The results of the statistical analysis concluded that the 4.0 kV/cm electric field treatment for 25 minutes did not affect the protein content in milk. Xiang B.Y. *et al*. [21] have reported that 20 kV/cm electric field pulses of 30 pulses cause a protein denaturation of around 43.7% and protein denaturation will increase with the increasing intensity of the electric field. Floury *et al.* [22] also reported that pulsed electric fields 45-55 kV/cm raised the temperature and affected food constituents including alkaline phosphatase, peroxidase, vitamin A and whey protein. Therefore, treatment using a lower intensity electric field has a smaller effect on protein content, according to Xiang B.Y. et al report.

The results of statistical analysis showed that the treatment using an electric field reduced the fat content in milk ($p < 0.05$). The fat content in the initial milk was 3.65% and after being treated using a 4.0 kV/cm electric field for 25 minutes, the milk fat content decreased to 3.29%, as shown in Figure 4(b). Gradient graph 4(b) shows that the change in the decrease in the greatest fat content occurs when the milk is treated with an electric field intensity of 2.5 - 3.5 kV/cm. In general, milk has a total fat content of about 3.4% and has the most complex fatty acid composition. As a result, of treatment to the electric field, there is an increase in cellular permeability, thus making fat more fluid [23]. As a result, there is a decrease in water content.

DISCUSSION

The outer bacteria cell membranes are the boundaries between the interior of the cell and the external environment. The main role of cell membranes is to regulate the transport of ions, nutrients, metabolites, peptides and proteins into and out of cells. A number of studies have been conducted to reveal the movement of important metal ions through transmembranes such as Na^+, Ca^+, K^+, Cl^- as well as organic nutrients such as amino acids, sugars and nucleotides [24].

Bacteria have non-isotropic properties [25] especially on bilayer lipid bacteria [26, 27] so that the microscopic optical properties depend on the direction. In addition, bacteria have electro-optical properties [28, 29]. Therefore, if the external electric field is applied to the bacteria, it will change the bacterial refractive index [30] especially on the cell membrane.

The treatment using an electric field of 2.0 - 3.5 kV/cm in the *Staphylococcus aureus* and *Salmonella sp.* bacteria caused a relatively small change in the refractive index so that the cell membrane permeability had not reached the threshold. Meanwhile, on the *Escherichia coli* bacteria, the 3.5 kV/cm electric field has been able to make the permeability of the cell membrane reach a threshold, so that there is an increase in the diffusion of water and ions in the cell membrane. Treatment by the electric field of 4.0 kV/cm and above causes a change in the refractive index which is able to make its permeability exceed the threshold. This condition causes the amount of diffusion of water and ions through the cell membrane to exceed the limit [31]. The increasing number of ionic streams in the cell membrane increases the conductivity of the cell membrane [32]. Due to the increased conductivity of cell membranes, it damages the membrane [33] finally cause bacteria to be inactive [5].

The decrease in the number of bacteria due to treatment to the electric field depends on the size of the bacteria, where bacteria with a large size of decline occur more than the small ones. *Escherichia coli* bacteria have a larger size compared to *Salmonella sp.* and *Staphylococcus aureus* bacteria, so to reduce the need for lower electric field intensity and shorter treatment time. This shows that the permeability threshold of bacterial cell membranes is influenced by cell size [34], the larger the cell size, the smaller the permeability threshold. Treatment to electric fields can cause electroporation of cell membranes so that permeability increases [35]. In large bacteria, the surface area of cell membranes is also large, thus accelerating the diffusion of water and ions which consequently accelerates membrane damage.

When the electric field has made the cell membrane permeability exceed the threshold, the length of treatment has an effect on decreasing the number of bacterial colonies. Where the addition of treatment time will extend the cell membrane in conditions with permeability exceeding the threshold so that damage to cell membranes and cytoplasm increases. Damage to cell membranes and cytoplasm makes bacteria inactive [5]. Meanwhile, the electric field with intensity below 3.5 kV/cm has not been able to make the cell membrane permeability exceed the threshold limit, so that the duration of treatment does not have a significant effect on cell membrane damage. This condition makes the addition of treatment time not increase the number of inactive bacteria.

The treatment of pulsed electric fields causes an increase in temperature [22], but other studies report that pulsed electric fields do not cause an increase in temperature [14]. We observed that the treatment of pulsed electric fields with an intensity of 4.0 kV/cm for 25 minutes caused an increase in the temperature of milk on average 3°C. The increase in temperature is caused by the effects of ohmic heating during treatment. The increase in temperature causes a decrease in protein content in milk. We observed that the treatment using an electric field with an intensity of 4.0 kV/cm for 25 minutes made the protein content in milk decrease by 3.03%. This result is identical to that reported by Xiang *et al*. [20], but the decrease is lower compared to what Xiang reported. In 2013 Lee *et al.* [14] reported that pulsed electric fields 10-20 kV/cm did not cause a decrease in protein content when tested using differential scanning calorimetry but decreased when tested using HPLC reverse phase. However, with lower electric field intensity, the decrease in protein content is lower. In general milk protein is divided into two namely casein micelles and whey protein [36]. Casein micelles are the main protein in cow's milk which is about 80% of the total protein content [36]. The treatment using electric fields can reduce both types of proteins, namely casein micelles [14] and whey protein [22]. The size of the decrease depends on the intensity of the electric field used for the treatment.

Milk fat is formed from globules emulsion between oil and water. Fat is composed of triglycerides which are a combination of glycerol and fatty acids. The electric field hitting the fat cause the increase of cellular permeability and results in reduced water content [23]. An increase in the electric field will induce an increase in cellular permeability so that the loss of water content increases. Therefore the fat content in milk decreases. Therefore treatment using an electric field is suitable for producing milk with low-fat content.

CONCLUSIONS

The minimum electric field intensity that could be used was 3.5 - 4.0 kV/cm depending on the size of the bacteria with a treatment time of more than 25 minutes. Small-sized bacteria require an electric field with higher intensity to reach the permeability threshold of cell membranes because the surface area of the cell membrane is smaller. The treatment using a 4.0 kV/cm electric field for 25 minutes caused a decrease in protein and fat content in milk, where the protein and fat content decreased by 3.03 and 9.77% respectively. Even though milk has been sterilized, storage for long periods of time requires low temperatures so that the milk color does not change much.

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Conflict of Interest: Authors declare no conflict of interest.

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