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ORIGINAL PAPER

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THE APPLICATION OF EXTREMELY LOW-FREQUENCY (ELF) MAGNETIC FIELDS TO ACCELERATE THE GROWTH OF *LACTOBACILLUS ACIDOPHILUS* BACTERIA AND THE MILK FERMENTATION PROCESS

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ABSTRACT

Background. The long fermentation process makes the nutritional content of milk decrease due to spoilage. One of the causes of spoilage is the growth of pathogenic bacteria in milk. In regard to this, *Lactobacillus acidophilus* bacteria have proven effective as an antibacterial pathogen. This study aims to determine the magnetic flux density (MFD) and exposure time that result in the optimum growth of *Lactobacillus acidophilus* bacteria and their effect on fermented milk's lactic acid content and pH.

Materials and methods. The research sample was *Lactobacillus acidophilus* bacteria grown in cow's milk. The exposure given used a time-changing magnetic field (MF) with a frequency of 50 Hz and a MFD of 0.1 mT, 0.2 mT, and 0.3 mT for 0–25 minutes, respectively.

Results. The results showed that the optimum bacterial growth occurred in samples exposed to a MFD of 0.2 mT for 5 minutes. Meanwhile, inhibition of bacterial growth began on the exposure to a MFD of 0.3 mT for 15 minutes. Moreover, it was found that the highest lactic acid content and the lowest pH were obtained in exposed milk with a MFD of 0.2 mT for 5 minutes.

Conclusion. Lactic acid content and pH were closely related to the number of *Lactobacillus acidophilus* bacteria colonies present in the milk.

Keywords: bacteria, fermentation, lactic acid, magnetic field, milk, pH

INTRODUCTION

Recently, there have been many studies aimed at studying the effects of exposure to magnetic fields (MF) on biological tissues. Most of these studies describe the effects of magnetic fields at the molecular level, where the MF can affect the biological function of organisms through changes in hormone secretion (Sun et al., 2010), enzyme activity, or ion transport by cell membranes (Cecchetto et al., 2015), and changes in the synthesis or transcription of deoxyribonucleic acid and others (Chow and Tung, 2000; Ruediger, 2009; Shabrangi et al., 2011). Several studies have also described the effects of magnetic fields on bacterial growth. Exposure to a MF reduces the viability of the bacteria, but the quantity of the effect depends on the strain (Fojt et al., 2004). Exposure to a weak static MF within a few hours makes a measurable change in the growth rate of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis* bacteria

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(Masood, 2017). The dynamic MF resonator is known to increase the growth rate of three species, namely *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Escherichia coli*, while reducing the growth of one species, *Serratia marcescens* (Tessaro et al., 2015).

Meanwhile, milk has been recognized as a natural food with complete nutrition (Park, 2009) and with essential functions for humans (Haug et al., 2007; Visioli and Strata, 2014). The problem is that the nutritional value of milk is easily spoiled, so it cannot be left for a long time. Milk stored at room temperature spoils after 14 hours (Richardson et al., 2019). Spoilage of milk can be described as a decrease in taste, texture, and color which makes it unfit for human consumption (Yellanki, 2017). Microorganisms are microscopic living things, and these are responsible for the heavy spoilage of milk and dairy products (Dhakane et al., 2019). Therefore, it is necessary to minimize spoilage by inhibiting the growth of pathogenic bacteria.

Previous studies have reported that *Lactobacillus acidophilus* bacteria in fermented milk have proven effective as an antimicrobial pathogen (Millette et al., 2007). *Lactobacillus acidophilus* is a probiotic that can significantly reduce food pathogens but not to very safe levels (Rosa et al., 2015). Rapid acidification in fermented milk will inhibit the growth of pathogens, specifically through the rapid production of lactic acid and the development of gel tissue through casein aggregation when the pH drops to the isoelectric point of casein (Damin et al., 2009; Rubin and Vaughan, 1979). Therefore, the short pH lag phase is of great interest to the industry to shorten the fermentation time, provided that there are no adverse effects on the sensory characteristics of milk (Chanos et al., 2020). One way to accelerate acidification is to accelerate the growth of lactic acid-producing bacteria. *Lactobacillus acidophilus* bacteria are lactic acid-producing bacteria (Linares et al., 2017), so by accelerating their growth, the pH drops faster.

Several earlier studies have also reported that most biological tissues are diamagnetic (Liu et al., 2017). The diamagnetic response to an external MF which strikes it produces magnetic induction in the opposite direction (Butler, 2014). In particular, a MF with a low MFD and frequency affects the movement of ions across the cell membrane (Wang and Hladky, 1994). Moreover, the MF also affects the conductance of K^+ channels on the cell membrane (Cecchetto et al., 2015). Changes in the conductivity of this cell membrane will later affect bacterial growth (Tirono et al., 2018), where small changes will accelerate bacterial growth, while significant changes will inhibit growth. In the exposure to a static MF with a MFD of 10 mT in *Bacillus licheniformis* bacteria after incubation of bacteria for 16 minutes, the number of bacteria increased by 36% compared to that without exposure to a MF (Mohtasham et al., 2016). Meanwhile, exposure to a static MF of 2.0–20.0 mT for 15–90 minutes also caused fluctuations in the growth of *Escherichia coli* bacteria (Mousavian-Roshanzamir and Makhdoumi-Kakhki, 2017). Magnetic fields are devices that can be used to accelerate the growth of bacteria (Masood, 2017). Therefore, a MF has the potential to be used to accelerate the milk fermentation process.

Based on previous research, it was found that *Lactobacillus acidophilus* bacteria are lactic acid-producing bacteria which can lower pH. Exposure to a MF with a low MFD can accelerate the growth of *Lactobacillus acidophilus* bacteria. Therefore, by growing *Lactobacillus acidophilus* bacteria in milk and exposing it to a MF with a low MFD, the bacteria will grow faster. Furthermore, the lactic acid content increases more rapidly, and the pH decreases more rapidly. A faster decrease in pH will inhibit the growth of pathogenic bacteria (Sánchez-Clemente et al., 2018), thereby minimizing damage to the milk and accelerating the fermentation process.

In this study, exposure to a MF changes along with a very low MFD and frequency. This study aims to determine the MFD and exposure time that contributes to the optimal growth of *Lactobacillus acidophilus* bacteria, lactic acid content, and milk pH so that the fermentation process can be faster. This study also aims to find the relationship between the number of *Lactobacillus acidophilus* bacteria and the lactic acid content and pH of milk

MATERIALS AND METHODS

Magnetic field generation

The MF used in the *acidophilus* milk exposure came from two Helmholtz coils arranged in parallel with a distance of 0.2 m. The Helmholtz coils were then connected to a sinusoidal electric current, resulting in an oscillating MF of 50 Hz.

Sample preparation

The research sample was the starter bacteria *Lactobacillus acidophilus*. The bacteria were grown in cow's milk which had a fat content of 5.66%. The *Lactobacillus acidophilus* bacteria used for this study were obtained from the Laboratory of Microbiology, Universitas Brawijaya – Indonesia, and the milk was obtained from the Village Cooperative of Dau-Malang, Indonesia.

The manufacturing process began with the sterilization of milk by heating it at a temperature of 135°C for 5 seconds. Next, the milk was allowed to stand until the temperature dropped to 37°C. After that, pure milk was mixed with the starter bacteria *Lactobacillus acidophilus* at a ratio of 100:1 to form *acidophilus* milk. This *acidophilus* milk was later allowed to stand for 2 hours before being exposed to an ELF MF.

Magnetic field exposure

Exposure was carried out 2 hours after mixing the starter *Lactobacillus acidophilus* with milk. Exposure to *acidophilus* milk using a magnetic field was carried out with magnetic flux densities of 0.1 mT, 0.2 mT, and 0.3 mT, each for 0–25 minutes. Next, in a measuring cup, *acidophilus* milk was incubated for 20 hours at a temperature of 37°C. After the incubation process was complete, the number of bacterial colonies was immediately calculated, and the lactic acid content and pH of the milk were measured.

Measurement

The number of bacterial colonies growing in the milk was counted using the total plate count method. The number of bacterial colonies was calculated 20 hours after the samples were exposed to a MF. The calculation was done by taking 1.0 ml of culture and then putting it in a bottle containing 9.0 ml of sterile distilled water. Next, 1.0 ml of culture was taken from a mixed culture bottle containing distilled water and was put in a bottle containing 9.0 ml of sterile distilled water. The process was continued until it was possible to calculate the number of bacterial colonies. After the dilution process had finished, 1.0 ml of culture was taken and spread on a petri dish that had been given liquid plate count agar (PCA) media, and then incubated for 20 hours at a temperature of 37°C. Subsequently, the number of bacteria was calculated using a Colony counter.

In addition, the pH of the milk was measured using a E521BNC pH meter, and the amount of lactic acid was tested using the titration method. The pH measurement was carried out 20 hours after the *acidophilus* milk was exposed to a MF. At the same time, 20 ml of the fermented milk sample was taken and put into the Erlenmeyer. Next, as many as 2.0 drops of 1% phenolphthalein indicator were put into the Erlenmeyer and titrated with 0.1 N NaOH solution until the color turned pink. The lactic acid content was calculated as a percentage using the equation:

$$\%\ \text{lactic acid} = \frac{\text{ml NaOH} \times \text{N} \times 90}{\text{gram example} \times 1000} \times 10$$

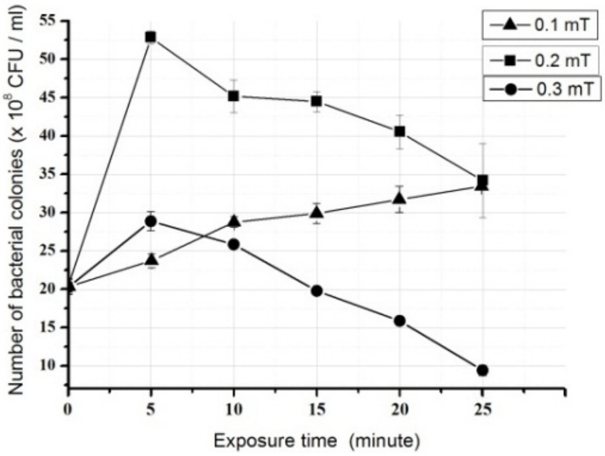
where:

N – the normality of the NaOH solution used as the titer.

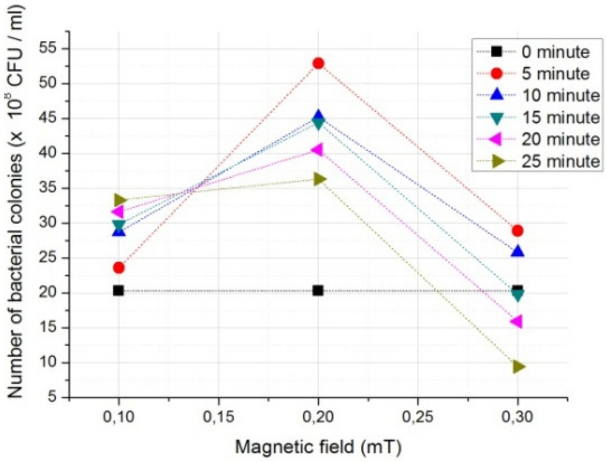
RESULTS

Number of bacterial colonies

Lactobacillus acidophilus are gram-positive, rod--shaped, non-motile, and non-sporing bacteria (Bull et al., 2013). Figure 1a shows the number of *Lactobacillus acidophilus* bacteria colonies present in fermented milk due to the exposure to magnetic fields. The exposure to the MF in this study used magnetic flux densities of 0.1 mT, 0.2 mT, and 0.3 mT for 0–25 minutes, respectively. The graph shows that exposure to a MFD of 0.1 mT and 0.2 mT for 5–25 minutes made the number of bacterial colonies higher compared to that without the exposure to a MF. Moreover, the exposure to a MFD of 0.3 mT for 5 and 10 minutes also resulted in a higher number of bacterial colonies than that without MF exposure, but the exposure time of 15–25 minutes made the number of bacterial colonies lower than without direction. Previous studies have revealed that exposure to a MFD of 2 mT and a frequency of 50 Hz to *E. coli* bacteria could cause a change in the characteristics of their growth curve (Segatore et al., 2012). Exposure to low-frequency magnetic fields with magnetic flux densities of 5 mT and 10 mT for 30 minutes is known to increase the bacterial growth by an average of nearly 68%, and exposure to magnetic flux densities of 10 mT and 20 mT with other exposure times was known to inhibit bacterial growth (Peña-Guzmán et al., 2019).



a



b

Fig. 1. The number of *Lactobacillus acidophilus* bacterial colonies in milk due to: **a** – changes in exposure time, **b** – changes in magnetic flux density

Figure 1b shows the effects of MFD on the number of bacterial colonies. The graph shows that exposure to a MFD of 0.2 mT for 5–25 minutes made the number of bacterial colonies in the milk greater than that in the exposure to a MFD of 0.1 mT and 0.3 mT. Exposure using a MFD of 0.2 m for 5 minutes resulted in the optimum number of bacterial colonies, which was 52.87 $\pm 0.85 \times 10^8$ CFU/ml.

Lactic acid content

Figure 2 shows the effects of exposure time to a MF on the lactic acid content in *acidophilus* milk. Exposure to the magnetic fields caused changes in the lactic acid content of the milk. It was found that the content of lactic acid changed with fluctuation depending on the MFD and exposure time. Exposure to a MFD of 0.1–0.3 mT for 5 minutes made the lactic acid content of the milk increase. The highest lactic acid content when exposed to a MFD of 0.2 mT for 5 minutes was 1.80 ±0.0114%. In addition, the exposure to a flux density of 0.3 mT for 15–25 minutes made the lactic acid content lower than that in the control.

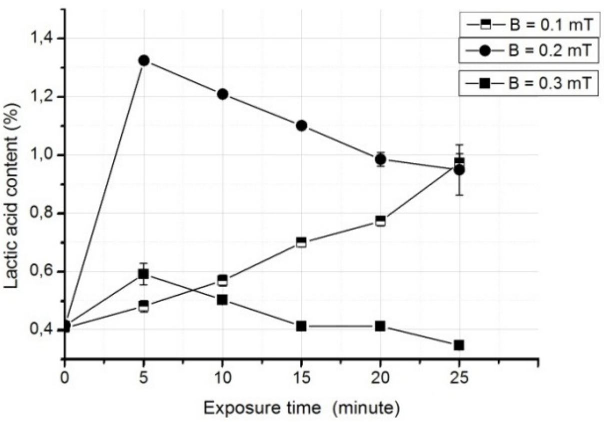


Fig. 2. Lactic acid content in acubate milk exposure to magnetic fields

pH of milk

Figure 3 illustrates the effects of exposure to magnetic fields on the pH of milk in which *Lactobacillus acidophilus* bacteria had grown. The graph shows that the exposure to magnetic flux densities of 0.1 and 0.2 mT caused a decrease in pH. Meanwhile, the exposure to a MFD of 0.3 mT decreased the pH at an exposure time of 5 and 10 minutes, and increased the pH at an exposure time of 15–25 minutes. In addition, exposure to MFD 0.2 and 0.3 mT with an exposure time of 10–25 minutes made the pH higher than that in the exposure time of 5 minutes. A previous study has also reported identical conditions where exposure to ELF magnetic fields with a flux density of 100 µT for 65 minutes lowered the pH of cheese cream from 6.7 to 5.55 after it had been stored for 30 hours (Kristinawati and Sudarti, 2017). In this present study, the lowest pH

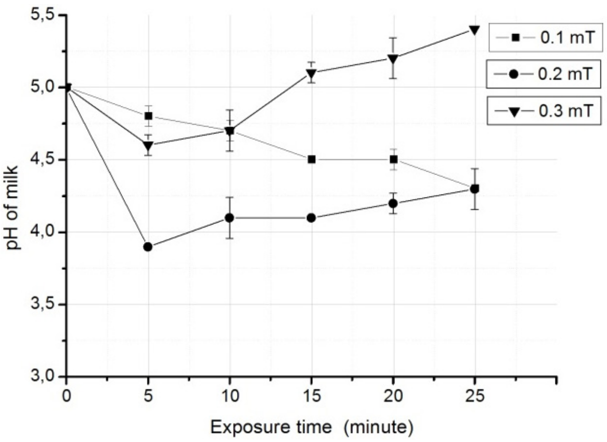


Fig. 3. The pH of milk due to the exposure to magnetic fields

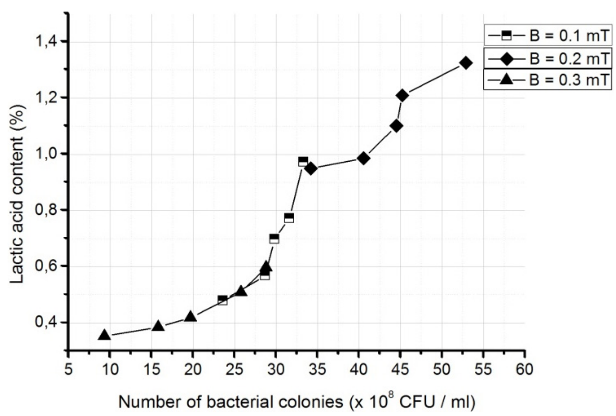


Fig. 4. The relationship between the number of *Lactobacillus acidophilus* bacteria colonies with the lactic acid content of milk

of milk was noted in the exposure to a MFD of 0.2 mT for 5 minutes, which was 3.90 ±0.00.

DISCUSSION

Exposure to ELF magnetic fields affected the growth of *Lactobacillus acidophilus* bacteria, milk lactic acid contents, and milk pH. The optimum number of bacteria and lactic acid content occurred in milk exposed to a MF with a MFD of 0.2 mT for 5 minutes. The minimum milk pH occurred in exposures using a MFD of 0.2 mT for 5 minutes. Optimum lactic acid contents and minimum pH are reached when the number of *Lactobacillus acidophilus* bacteria is 52.87 $\pm 0.85 \times 10^8$ CFU/ml.

The exposure to magnetic fields in *Lactobacillus acidophilus* bacteria caused changes in bacterial growth. The growth rate was different for each MFD and exposure time. Previous studies have reported that an exposure to a MFD of 10 mT and a frequency of 50 Hz for 24 minutes had different effects on bacterial growth (Strašák et al., 2005) as it could accelerate or inhibit it. An exposure to ELF magnetic fields with a MFD of 646.7 µT for 30 minutes reduced the number of *Salmonella* sp. bacteria in Gado-Gado (Sudarti, 2016). There have also been reports that exposure to the dynamic MF 'Resonator' increased the growth rate of *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Escherichia coli* bacteria, yet it slowed down the growth of *Serratia marcescens* (Tessaro et al., 2015). Therefore, it can be said that the effects of magnetic fields on bacterial growth were influenced by the MFD and exposure time. In this present study, the results showed that the optimum growth of *Lactobacillus acidophilus* bacteria was at a MFD of 0.2 mT and an exposure time of 5 minutes.

Moreover, it was found that exposure to magnetic fields in milk in which *Lactobacillus acidophilus* bacteria had grown changed the content of lactic acid. The pattern of changes in lactic acid content was identical to changes in the number of bacterial colonies. Therefore, the presence of *Lactobacillus acidophilus* bacteria in milk affected the production of lactic acid. Figure 4 shows the relationship between the number of *Lactobacillus acidophilus* bacterial colonies and lactic acid content. An increase in the lactic acid content followed an increase in the number of bacterial colonies. It has also been reported that the genus *Lactobacillus* sp., the homofermentative group, can break down sugar into lactic acid as the main product (Charteris et al., 2001). *Lactobacillus acidophilus* bacteria added to the food can produce lactic acid and bacteriocins (Anjum et al., 2014). Therefore, the lactic acid content in milk is influenced by the number of *Lactobacillus acidophilus* bacteria colonies.

An exposure to a MFD of 0.1–0.3 mT and an exposure time of 5–25 minutes in *acidophilus* milk caused a decrease in the pH of the milk, except for a MFD of 0.3 mT and exposure time of 15–25 minutes, which resulted in an increase. Previous studies have reported that the exposure to magnetic flux densities of 2900,

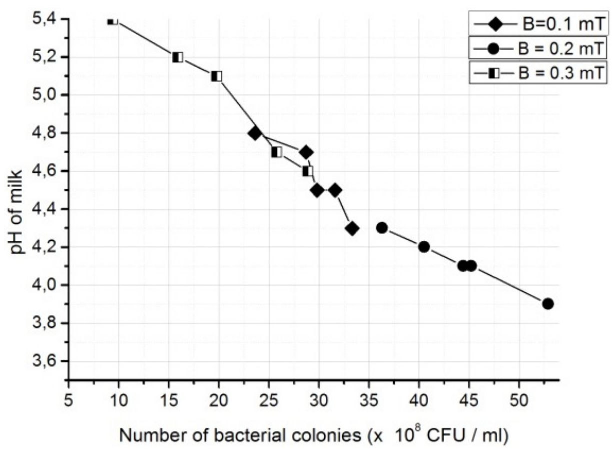


Fig. 5. The relationship between the number of *Lactobacillus acidophilus* bacteria colonies and the pH of milk

3300, and 5000 Gauss have led to an increase in the water pH (Amor et al., 2018). The exposure to magnetic flux densities of 500, 1000, 1500, and 2000 Gauss for 24 hours increased the pH of water from 6.4 to 6.8, 7.5, 7.9, to 8.6 respectively (Karkush et al., 2019). This condition contradicts the results of this present study. The difference in results is thought to be the effect of the *Lactobacillus acidophilus* bacteria being grown in milk. Figure 5 shows the inverse linear relationship between the number of bacterial colonies and pH in milk. The higher the number of bacterial colonies, the lower the pH of the milk. Previous research has also revealed that with an increase in the number of lactic acid bacteria and *K. marxianus*, the pH of goat milk decreases (Huang et al., 2020). An increase in the number of *Streptococcus salary* and *Lactobacillus delbrueckii* bacteria made the pH of milk decrease (Estrada et al., 2005).

CONCLUSION

Exposure to MFs in *acidophilus* milk affected the growth of *Lactobacillus acidophilus* bacteria. Its effects on bacterial growth depended on the MFD used and the exposure time. Optimum growth of bacteria occurred in exposure to a MFD of 0.2 mT for 5 minutes. Moreover, the number of *Lactobacillus acidophilus* bacteria in the milk affected the lactic acid content and pH of the milk. The higher the number of *Lactobacillus acidophilus* bacteria, the higher the lactic acid content and the lower the pH of the milk. The technique of accelerating the milk fermentation process using exposure to ELF MFs can be used in the commercial production of fermented milk.

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