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Fermentation Process of Dry Cocoa Beans through Extremely Low Frequency (ELF) Magnetic Field Exposure

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Abstract: Several studies have found and proved that exposure to Extremely Low Frequency (ELF) magnetic fields can cause the proliferation of cells or bacteria. Meanwhile, bacteria play an essential part in the fermentation process. Thus, this study aims to examine the fermentation process of non-fermented dry cocoa beans using Extremely Low Frequency (ELF) magnetic field exposure. The sample of this study was 30 kg of non-fermented dry cocoa beans divided into 10 sample bags of 3kg each and grouped into 4. The control group (K) consisted of 1 bag while the rest bags were divided into 3 sample bags exposed to an ELF magnetic field with intensities of 100 µT (E-100), 200 µT (E-200), and 300 µT (E-300). ELF magnetic field exposure was carried out at the beginning of the fermentation process with variation in the exposure time of 15 , 45 , and 75 minutes. During the fermentation process, temperature measurements were conducted. Moreover, moisture content measurements were also conducted during the drying process in the sun for up to 3 days. At last, measurements of pH and alcohol content were carried out on dried cocoa beans. The findings showed that there was no significant temperature change ($p > 0.05$) during the fermentation process. A decrease in the moisture content of cocoa beans during the drying process was proven to be faster in the sample group exposed to the ELF magnetic field compared to those in the control group. The alcohol and pH of dried cocoa beans from the sample group exposed to the ELF magnetic field were significantly higher than those in control ($p < 0.05$). The highest alcohol content was found in the sample group which was exposed to the intensity of the ELF of 200 µT for 45 minutes. Conclusion: Exposure to ELF magnetic fields can increase the fermentation activity of dry cocoa beans. This indicates that exposure to ELF magnetic fields in the fermentation process of cocoa beans might improve the quality of non-fermented dry cocoa beans.

Keywords: Extremely low frequency; pH; Moisture content; Alcohol content

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Introduction

The fermentation process is one of the post-harvest processing methods of cocoa beans that might improve the quality of cocoa beans. The price of Indonesian cocoa beans, especially those produced by the people in the international market, is still low for it is dominated by non-fermented beans. Only a small proportion of farmers carry out the post-harvest cocoa fermentation process. Thus, the production of Indonesian cocoa beans is dominated by non-fermented dry cocoa beans. However, dried cocoa beans have their disadvantage, which is that they have lost most of the water and substrate content. The moisture content is needed

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during fermentation for the enzymatic reaction process in the seeds and the growth of microbes in the pulp. Meanwhile, the substrate in the fermentation of cocoa beans is the sugar and citric acid contained in the pulp. A chocolate flavor might be enriched through the fermentation process since during the fermentation process an important precursor will be formed to support the chemical reaction of flavor formation (Barisic, 2019). Fermentation microbes will remodel the pulp into organic acids during the fermentation process. The acid will diffuse into the seeds and induce enzymatic reactions to form compounds for taste, aroma, and color (Afoakwa, 2014).

Several studies have been carried out with various methods of fermenting cocoa beans. Semi-mechanical fermentation method was proven to be faster and produced fermented beans with high chemical quality, which have high polyphenol, anthocyanin, reducing sugar, theobromine, and caffeine content (Pelaez et al., 2016). The addition of saccharomyces to the fermentation of cocoa beans had the potential to shorten the fermentation time of cocoa beans, with a higher fermentation index of 1.13 (Cempaka et al., 2014). Meanwhile, the addition of different yeast cultures in the fermentation process provided an opportunity to modulate the taste of chocolate (Meersman et al., 2016). The fermentation process of dried cocoa beans had been carried out by soaking the cocoa beans and then fermented by adding inoculum at the beginning and gradually during the fermentation process. Until the end of the fermentation process, the results show that index fermentation for dried cocoa beans is not met. This was presumably due to microbes that help fermentation in less quantity (Mulono et al., 2017). This proves that the fermentation process of dried cocoa beans is not as easy as the process of fermentation of fresh cocoa beans. Thus, it takes an effort to optimize the microbial propagation in the fermentation process of dried cocoa beans.

Experimental exposure to the Extremely Low Frequency (ELF) magnetic field in the fermentation process of non-fermented dry cocoa beans was conducted in this study as a solution to improve the quality of the fermentation process of non-fermented dry cocoa beans. Extremely Low Frequency (ELF) magnetic field is a component of ELF electromagnetic waves at frequencies less than 300 Hz. ELF magnetic fields can penetrate almost all material. However, the magnetic field includes radiation that cannot change generate ions and does not change temperature. The results prove that giving an ELF magnetic field with an intensity of 100 μT (50 Hz) does not interfere with DNA and is not behave mutagenic (Verschaeve et al., 2016).

Several findings from previous studies indicate that applying a low frequency ELF magnetic field (<500 μT) can significantly increase the number of S. thermophilus, L. lactis, and L. Acidophilus bacteria at an ELF magnetic field intensity of 100 μT for 5 minutes Sudarti, et al, 2018). Meanwhile, giving a magnetic field with an intensity of 100 μT for a duration of 15 minutes has been shown to increase the number of Lactobacillus casei bacteria during the milk fermentation process (Ridawati et al., 2017). ELF magnetic fields with an intensity of 250 μT have been tested to increase bacterial populations. However, the static magnetic field of 5000 G was not statistically significant (Tessaro et al., 2015). The application of an ELF magnetic field with an intensity of 300uT or 45 minutes in the fermentation process of coffee beans has been shown to optimize the proliferation of lactobacillus, which are important bacteria in the fermentation process (Sudarti et al., 2020). Exposure to magnetic fields with intensities of 400 μT, 600 μT, and 900 μT significantly increased mannatide and biomass during the fermentation process, but exposure to 1,200 μT and 1,500 μT significantly decreased the mannatide biomass. There were indications of decreased growth of Staphylococcus aureus Gram-positive and Escherichia coli Gram-negative after exposure to ELF-EMF, 50 Hz, 1000 μT for 2 hour (Oncul et al., 2016).

The results of those previous studies prove that exposure to ELF magnetic fields at low intensity (< 1 mT) has the potential to increase the breeding of bacteria. This study utilized the ELF magnetic field in the fermentation process, especially in dried cocoa beans. The intensity of exposure to the ELF magnetic field that was applied was 100 μT, 200 μT, and 300 μT. The ELF magnetic field in this study is derived from the ELF electromagnetic wave generator, Current Transformer (CT). The dominant ELF magnetic field was used as a treatment in the fermentation process of dried cocoa beans in this study by conditioning the instrument by minimizing the components of the electric field.

Method

This study was an experimental laboratory study aiming to optimize the fermentation process of non-fermented dry cocoa beans by giving treatment in the form of ELF magnetic field exposure with variations of 100 μT, 200 μT, and 300 μT and variations in exposure duration of 15 minutes, 45 minutes, and 75 minutes. The ELF magnetic fields came from an EM-ELF source, a Current Transformer (CT) machine capable of producing ELF electromagnetic wave exposure in the exposure chamber. This study was designed to enable the minimal electric field component by turning the voltage control knob to a value of 0 and the electric field exposure in the exposure room reaching about 5 mV/m (equivalent to the intensity of the natural electric field).

Amperage button settings to the condition were able to make the magnetic field exposure in the exposure chamber reached an intensity of 100 µT, 200 µT, and 300 µT for the needs of this study. The Current Transformer (ELF Electromagnetic Wave Source) instrument was visualized in the following figure.

Figure 1. Current Transformer (ELF Electromagnetic Wave Source)

The sample of this study was 30 kg of non-fermented dry cocoa beans. The dried cocoa beans were fermented by soaking the beans in the warm water for 3 hours. The non-fermented beans then divided into 10 groups of plastic bags. Furthermore, the beans were stored for 4 days in a fermenter box while the ELF magnetic field exposure was carried out around the 12th hour at the beginning of the fermentation process. Those 10 bags were divided into 4 groups which 1plastic bag in a control group, 3 plastic bags in the ELF magnetic field exposure of 100 µT, 3 plastic bags in the 200 µT ELF magnetic field exposure, and 3 plastic bags in the 300 µT magnetic field exposure. Each plastic bag in each treatment had a variety of exposure time of 15 minutes, 45 minutes, and 75 minutes. As an indicator of the optimization of the dry cocoa beans fermentation process, the temperature change was monitored every 12 hours during the fermentation process and laboratory checks of the moisture content during the drying process on day 1, day 2, and day 3. pH and alcohol content were also checked in cocoa beans after drying process. Following is the flow chart of this study.

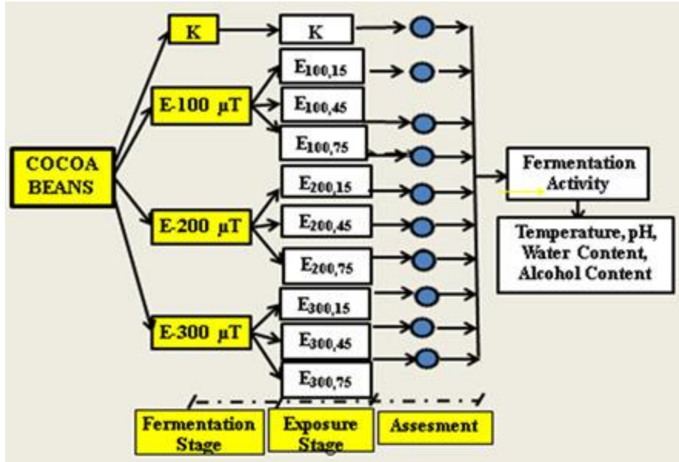

Figure 2. Research Design

Description:

S : Sample of non-fermented dry cocoa beans

K , : Sample in the control group was not exposed to the ELF magnetic field

$\mathbf{E_{100,15}}$,$\mathbf{E_{100,45}}$, $\mathbf{E_{100,75}}$: Samples in the group were given a 100 µT ELF magnetic field for the duration of 15 minutes, 45 minutes, and 75 minutes.

$\mathbf{E_{200,15}}$,$\mathbf{E_{200,45}}$, $\mathbf{E_{200,75}}$: Samples in the group were given a 200 µT ELF magnetic field for the duration of 15 minutes, 45 minutes, and 75 minutes.

$\mathbf{E_{300,15}}$,$\mathbf{E_{300,45}}$, $\mathbf{E_{300,75}}$: Samples in the group were given a 300 µT ELF magnetic field for the duration of 15 minutes, 45 minutes, and 75 minutes.

Fermentation temperature monitoring was carried out after the beans were exposed to the ELF magnetic field by measuring the temperature using a Celsius thermometer at 0 o'clock, 12 o'clock, at 24 o'clock, at 36 o'clock, and 48 o'clock during the fermentation process. Measurement of the water content of cocoa beans was carried out during the drying process on the 1st, 2nd, and 3rd day using the Cocoa test. Measurement of pH and alcohol content was carried out on all samples of dried cocoa beans using a pH-meter. Measurement of the amount of water content of cocoa beans used the gravimetric method while measuring the alcohol content was carried out by the gas chromatography method. The obtained data were then analyzed using ANOVA statistics.

Result and Discussion

Changes in Temperature of Fermentation of Dry Cocoa Beans

Measurement of temperature of cocoa beans during fermentation of dry cocoa beans at 0, 12, 24, 36, and 48 hours, both in the control group and the group that was given the ELF magnetic field. on the following graph.

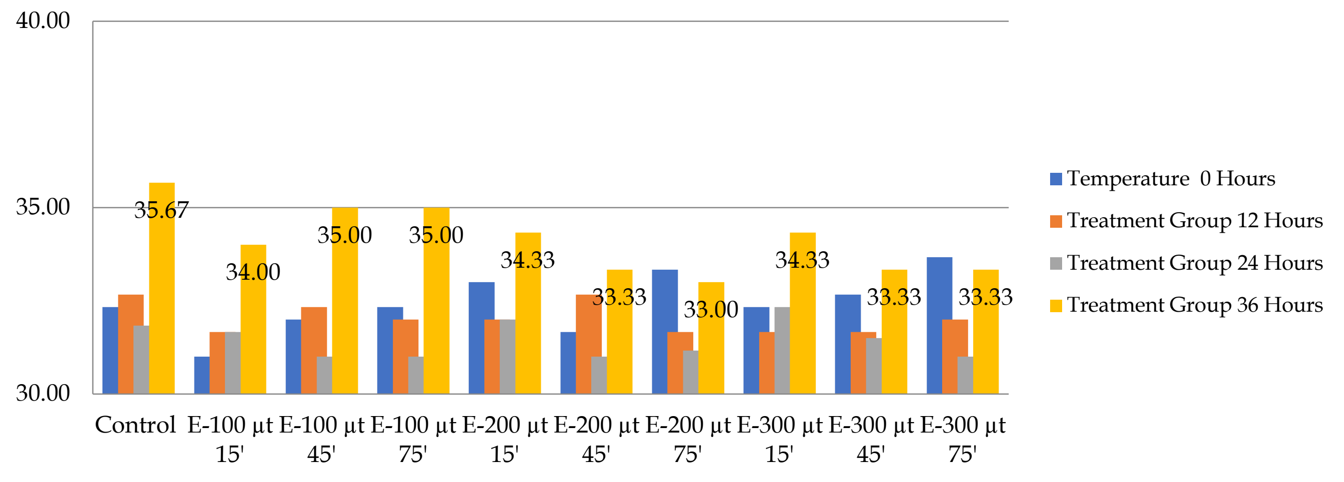

Figure 3. Temperature Fermentation

Figure 3 shows the temperature during the dry cocoa fermentation process after being exposed to the ELF magnetic field until the 48th hour. The results of temperature measurements during the fermentation process, at the 0th hour, 12th hour, and 24 hours showed no significant difference in temperature between groups ($p > 0.05$), with the average temperature of the cocoa beans during the fermentation process around 31 – 32.5°C. Meanwhile, the average temperature of cocoa beans during the fermentation process at the 36th hour and 48th hour showed a significant increase. However, there was no significant difference in the temperature of the cocoa bean sample group treated with ELF magnetic fields and the control group. The findings of this study prove that ELF magnetic field exposure does not affect temperature changes in the fermentation process of dry cocoa beans. This proves that the impact of ELF magnetic field exposure is non-thermal, or unable to increase the temperature of a material. There were not enough cocoa beans in each treatment variation. Since there was only 1 kg of cocoa beans each, thus the temperature during the fermentation process was stable. An increase in temperature at the 36th hour and 48th hour indicated an increase in the activity of fermented bacteria. However, there was no significant difference ($p > 0.05$) of the temperature of the cocoa beans during the fermentation process at the 36th and 48th hour between the sample groups exposed to the ELF magnetic fields and the control. This proves that the time needed for fermentation of cocoa beans is at least 2 days (48 hours) and the ELF magnetic fields do not affect temperature changes during the fermentation process.

Moisture content of Cocoa Beans

The results of measurements of moisture content carried out during the drying process in the sun on day 1, day 2, and day 3 both in the control group and the groups exposed to the ELF magnetic field were presented in Figure 4 as follows.

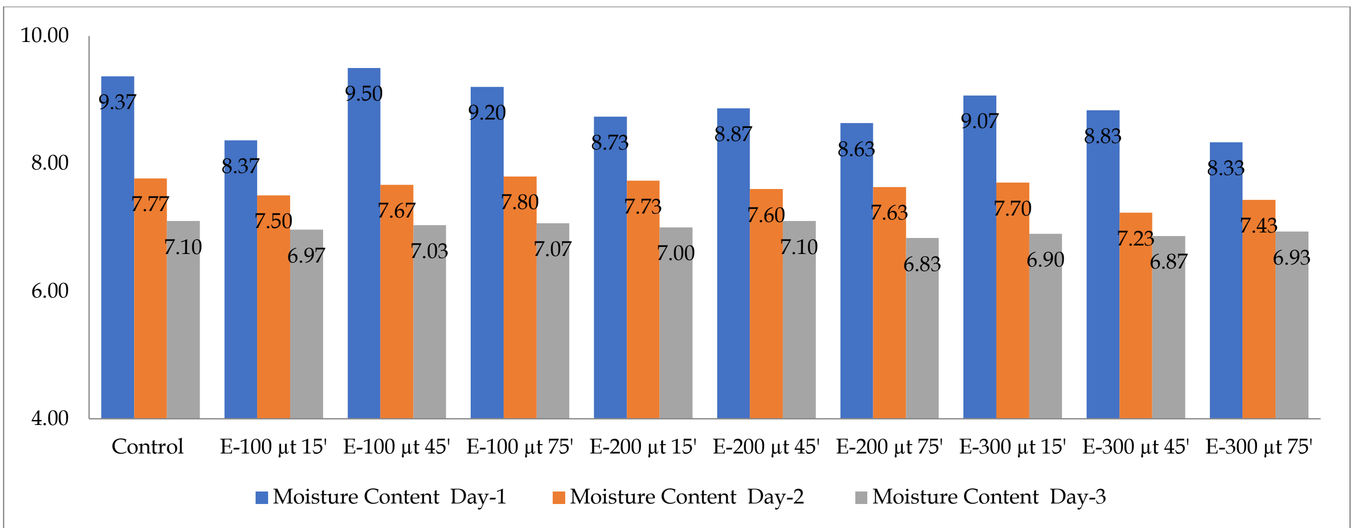

Figure 4. The moisture content of Cocoa Beas During the Drying Process

ANOVA analysis results on the moisture content of cocoa beans after the drying process in the first day showed that compared to control (9.337%), the decrease in the moisture content of cocoa beans was significantly faster ($p < 0.05$) in the sample groups exposed to the ELF magnetic field of 100 µT for 15 minutes up to 8.366%, 200 µT for 15 minutes up to 8.733%, 200 µT for 75 minutes up to 8.633%, and 300 µT for 75 minutes up to 8.333%. The amount of water content of cocoa beans after drying process on the second day compared to control (7.766%) showed that the decrease in moisture content of cocoa beans was significantly ($p < 0.05$) faster in the sample group subjected to the ELF magnetic field. 300 µT duration of 45 minutes to 7,233% and duration of 75 minutes to 7,433%. The amount of water content of cocoa beans after drying process on the third day was compared to the control (7.1% indicating that the decrease in moisture content of cocoa beans was much faster ($p < 0.05$) in the sample group subjected to a 200 µT ELF magnetic field for a duration of 75 minutes to 6.833%, while the sample group subjected to a 300 µT ELF magnetic field for 45 minutes duration reached 6.866% ($p = 0.087$).

The results of the above analysis prove that giving ELF magnetic fields with an intensity of 300 µT for 45 minutes gave a consistent effect of accelerating the decrease in moisture content significantly compared to control until it reached the lowest moisture content after drying process under the sun on the third day. Moisture content is an indicator of the quality of cocoa beans. According to the Indonesian National Standard (abbreviated SNI), moisture content that meets the requirements was < 7.5% (Mulyawanti et al., 2018). The results of this study prove that the amount of water content of fermented cocoa beans subjected to an ELF magnetic field with intensities of 100 µT and 200 µT has met SNI standards. The lowest water content was found in the samples of cocoa beans exposed to an ELF magnetic field with an intensity of 100 µT for 15 minutes and a group of samples subjected to a 200 µT ELF magnetic field for a duration of 75 minutes. Fermentation is the process of breaking down sugars and citric acid in the pulp to produce organic acids which is carried out by a microbial fermenter (Meersman et al., 2013).

The function of moisture in cocoa beans is to bring enzymes along with the substrate in the beans to enable the process of hydrolysis and oxidation of compounds that form the taste, color, and aroma of cocoa beans. The substrate in cocoa beans is sugar and citric acid which are located in the dregs and will be broken down by microbes during fermentation. Then the acid will diffuse into the seeds until an enzymatic reaction occurs to form candidate compounds for taste, smell, and color (Afoakwa et al., 2014).

The findings of this study indicate that applying an ELF magnetic field with an intensity of 300 µT for 45 minutes during the fermentation of dry cocoa beans can accelerate the drying process because the amount of water content of cocoa beans decreased significantly from day 2 and day 2. 3.

The pH level of Cocoa Beans

The results of pH measurements of dried cocoa beans after the fermentation process with the help of ELF magnetic field radiation are presented in Figure 5 below.

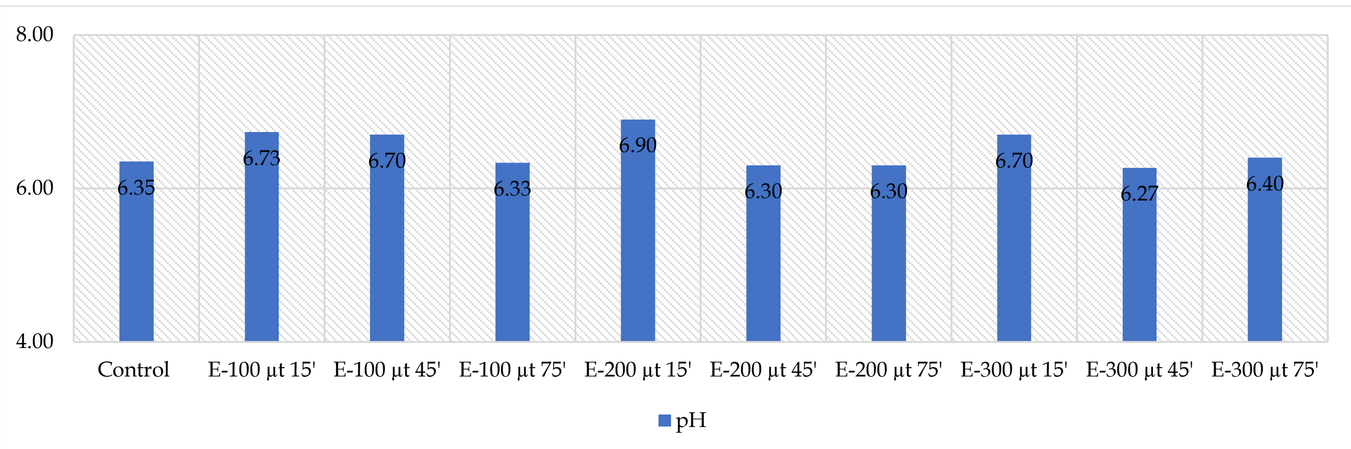

Figure 5. pH level of Cocoa Beans After Dry Fermentation.

ANOVA analysis results of cocoa beans compared to control showed a significant increase of pH ($p<0.05$) in the sample groups exposed to the ELF magnetic field of 100 µT for 15 minutes reached 6.7333, 100 µT for 45 reached 6.7, and the sample group exposed to the ELF magnetic field of 200 µT for 15 reached 6.9. One indicator of the quality of cocoa beans is pH. the changing of pH is determined by the fermentation process played by the activity of fermentation bacteria. Thus, the longer fermentation process might have an impact on increasing the acidity or decreasing the pH of the cocoa beans (Sabahannur, et al, 2016).

The increase in pH occurred in the sample groups exposed to the ELF magnetic field of 100 µT for 15 minutes reached 6.7333, and for 45 reached 6.7, as well as the sample group exposed to 200 µT for 15 minutes, reached 6.9 presumed to be caused by citric acid from the overhaul of the pulp which diffused into the cocoa beans experienced obstacles. Meanwhile, the decrease in pH occurred in the sample group exposed to ELF magnetic field of 100 µT for 75 minutes reached 6.33, as well as the sample group exposed at 200 µT for 45 minutes and 75 minutes reached 6.3. It might be caused by the citric acid resulting from the overhaul of the pulp to diffuse more easily into cocoa beans. Those findings prove that the acidity (pH) of cocoa beans is a parameter of good quality cocoa. The effect of fermentation of cocoa beans is that it can change the pH value (Meryandini, et al, 2019). Meanwhile, organic acids will trigger enzymatic reactions in the beans that allow biochemical modifications to create compounds that give the cocoa aroma, taste and color (Mulono, et al, 2017). The increase in pH during the fermentation process occurs for the existing citric acid that was used up (Wahyuni, et al, 2018). Whereas, the pH value of good cocoa beans is near neutral (pH>6) to make typical chocolate compounds can be formed intensively (Sabahannur, et al, 2017). Therefore, based on the results of this experiment, the application of an ELF magnetic field with an intensity of 200 µT for a duration of 15 minutes can increase the pH value of cocoa beans until it reaches 6.9 after fermentation. Meanwhile, giving an ELF magnetic field with an intensity of 100 µT with a duration of 15 minutes and 45 minutes can increase the pH value to be 6.7333 and 6.7.

Alcohol Content of Cocoa Beans

The results of measuring the alcohol contained in cocoa beans in the control group and sample groups exposed to ELF magnetic fields are presented in Figure 6 below.

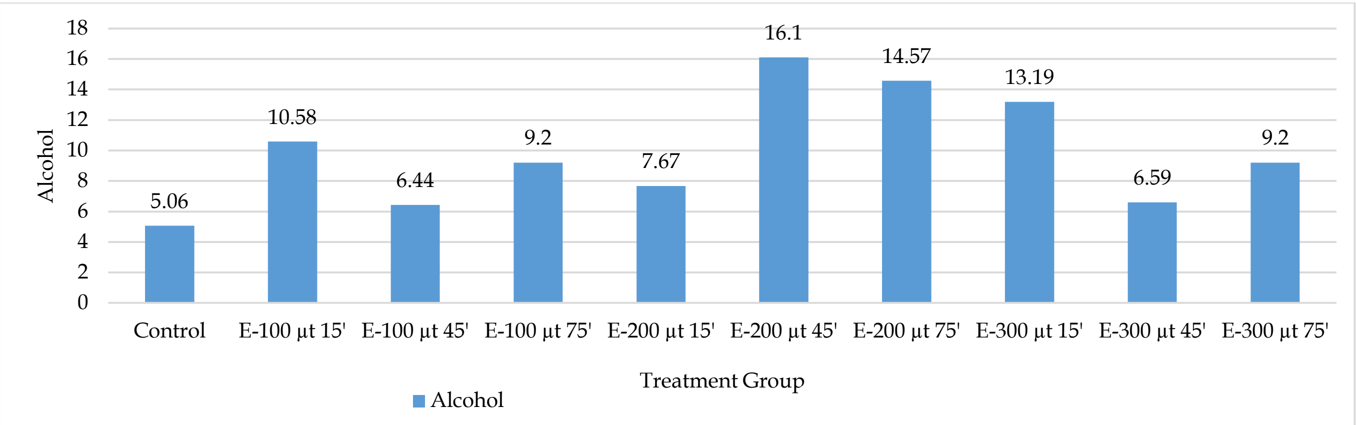

Figure 6. Cocoa Beans Alcohol Content.

A significant increase in alcohol content ($p < 0.05$) occurred in the dry fermented cocoa bean sample group after being exposed to a 100 µT ELF magnetic field for 15 minutes, and in dry fermented cocoa beans, the sample group after being exposed to a 200 µT ELF magnetic field for 45 minutes duration and 75 minutes compared to controls. The highest alcohol content was found in the sample group subjected to the ELF 200 µT magnetic field for 45 minutes duration. Fermentation is a method to increase the quality level of cocoa beans through a biochemical process due to the performance of microbes in the cocoa fermentation process (Sudarminto, et al, 2017). The cocoa fermentation process involves yeast, lactic acid bacteria, and acetic acid bacteria (Ho VTT, et al, 2014).

There are 3 stages of the fermentation process. 1) The anaerobic stage occurs at the beginning of the fermentation on the 2nd and 3rd day when yeast converts sugar into alcohol under conditions of low oxygen and low pH below 4. 2) The next step, when lactic acid bacteria are most prevalent on the 4th and 5th days of fermentation. Lactic acid bacteria break down sugars and some organic acids to give birth to lactic acid. 3) The third step or the step of acetic acid bacteria where the presence of acetic acid bacteria occurs during the fermentation process, but its presence becomes very significant until the end of the fermentation process where there is an increase in aeration (Beckket, et al, 2009). Acetic acid bacteria play a role in converting alcohol into acetic acid causing the temperature to increase to 50°C. Besides pH, the alcohol content in cocoa beans is considered to contribute to the quality of taste. During the fermentation process, the fine aroma precursors produced in the pulp might migrate into the cocoa beans and then be maintained during the drying phase (Castro- Alayo, et al, 2018).

The increase in alcohol content in the sample group that was given an ELF magnetic field of 200 µT intensity for 45 minutes and 75 minutes was thought to be caused by the spread of alcohol into the cocoa beans becoming easier at lower pH conditions. Therefore, the presence of an ELF magnetic field during the fermentation process can be used as an alternative solution in improving the taste quality of non-fermented dry cocoa beans.

Conclusion

Based on the results of statistical analysis, it can be concluded that ELF magnetic field exposure intensity of 100 µT - 300 µT in the fermentation process of cocoa beans has the potential to improve the quality of non-fermented dry cocoa beans. It might increase the quality of non-fermented dry cocoa beans indicated by an increase in pH in the cocoa beans after the fermentation process exposed to the ELF magnetic field at an intensity of 200 µT for 15 minutes reaching 6.9. It is proven that at low pH conditions, it will be easier for alcohol to diffuse into cocoa beans. Thus, the measured alcohol content in cocoa beans increases.

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