

Optimizing lactobacillus growth in the fermentation process of artificial civet coffee using extremely- low frequency (ELF) magnetic field

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Abstract: This study aimed to analyze the fermentation process of Civet Coffee through Extremely Low Frequency (ELF) magnetic field radiation. The research method used a Completely Randomized Design (CRD) experimental. The sample of high quality pulped coffee that fermented by adding α - amylase and is grouped in seven. The six groups are exposed by ELF magnetic field in 100 μ T and 300 μ T for 15 minutes, 30 minutes, and 45 minutes, and one group is served as the control. The result indicated that the population of Lactobacillus in the experiment groups increases significantly compared to the control. The exposed sample achieves the highest population in 300 μ T intensity for 45 minutes. Also, the total of pH during the fermentation process did not show any significant changes. ELF magnetic field exposure in 300 μ T optimized Lactobacillus proliferation drastically in the fermentation process of artificial Civet coffee. This research can contribute to developing fermentation technology based on ELF magnetic field radiation as well as improving the quality of artificial Civet coffee.

1. Introduction

Coffee is one of the most significant commodities in Indonesia and has a more favorable prospect to compete in the national or international marketplace. Indonesian coffee has the 4th position in international exportation after Brazil and Colombia. Most of the Indonesian exporting coffee is robusta, which made up 94%, and the rest is Arabic. Nevertheless, the problem is that Indonesian robusta mostly considered to have a low quality that affects the price [1], [2]. Indonesian civet coffee is the world's most expensive coffee, which costs almost US\$ 100 for every 100-gram coffee beans. Moreover, civet coffee is the most significant product of Indonesia, taken from partially digested coffee beans and excreted by the Asian Civet. This coffee is exotic and has a different taste, smell, and quality of other coffee because of the fermentation process in the civet digestion and excretion [3]. Unfortunately, some people considered that civet coffee has less hygiene and is not a halal product.

One alternative to this problem is to make civet coffee with an artificial fermentation process. The fermentation process allows Luwak coffee production to be made easier and cheaper. There have been many studies on artificial fermentation of civet coffee but has not been any artificial coffee with the same flavor as the original civet coffee obtained from civet droppings [4]. The similarity score based on the cup test is 84,83 by adding α - amylase enzyme [5]. Some ways to optimize the fermentation



process of coffee beans, mostly use a probiotic microbe from civet or using isolated bacteria from the civet feces. The taste of fermented Arabica using probiotic bacillus from civet digestive reach 79,92 for the Cup test total score [6], [7]. Also, Usman et al. Indicated the fermented robusta from three isolated bacteria taken from civet feces proved that the long-time fermentation decrease the level of caffeine, the weight of coffee beans, and the liquid pH [8]. Therefore, the method of adequate hygiene, safety, and halal fermentation are required.

Extremely Low Frequency (ELF) magnetic field radiation is one of the electromagnetic wave spectra that has a frequency of around 0 - 300 Hz. The energy of ELF is too tiny, and it has ionizing radiation and a nonthermal effect. The small amount of energy contained in the ELF magnetic that the interaction with biologic material cannot make the charge or molecule of ionizing [9]. ELF magnetic field exposure is not poisonous and has potential as a cell proliferation stimulant [9], [10]. Also, the ELF magnetic field can pierce almost all materials or unobstructed more safety and cheaper than gamma rays. This research aims to prove that ELF magnetic field exposure can improve the fermentation activities in the artificial civet coffee by indicating the growth of the *Lactobacillus* population and the total pH.

2. Method

This research focuses on laboratory experimental by using a Completely Randomized Design (CRD). Firstly, a freshly pulped robusta coffee beans are fermented by adding α - amylase enzym around 0,0465 gram per 14 kg. Furthermore, after 2 hours of fermentation. The coffee bean then is split into seven groups contain 2 kg coffee beans for each. One group served as the control. Six groups are exposed by ELF magnetic field in 100 μ T and 300 μ T intensity for 15 minutes, 30 minutes, and 45 minutes respectively (figure1).

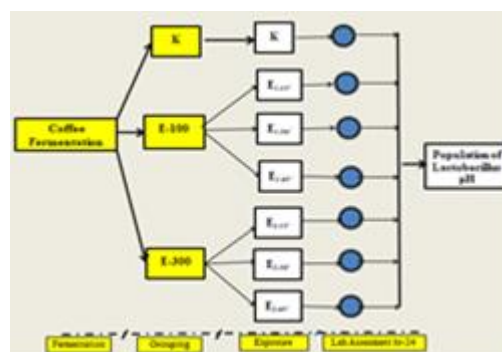


Figure 1. Figure research design

This research focused on analyses of the ELF magnetic field effect after adjusting the voltage and input stream 100 μ T and 300 μ T in the natural intensity of electric field 4 V/cm. The laboratory test is conducted to measure the population of *Lactobacillus* bacteria and the total pH produced in artificial civet coffee during the fermentation process. The *Lactobacillus* is measured by total plate count, while the pH is marked by pH meter.



Figure 2. Figure EMF sources exposure

3. Result

The result of the *Lactobacillus* population test during the fermentation of artificial civet coffee after exposed by ELF magnetic field is illustrated in figure 3 and figure 4:

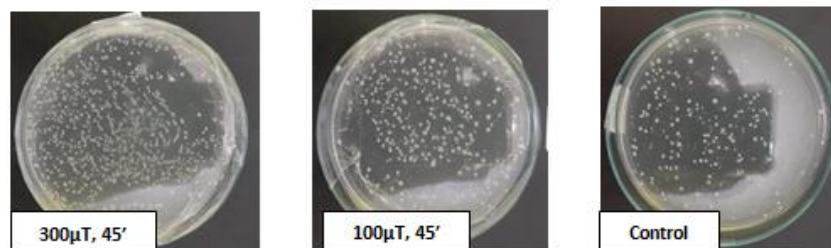


Figure 3. Figure the growth of *Lactobacillus* bacteria

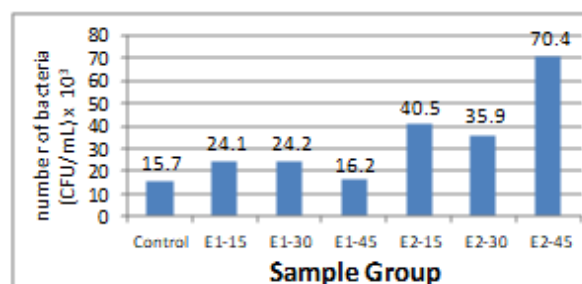


Figure 4. The graph of the *Lactobacillus* bacteria population

Figure 4 illustrated that the number of *Lactobacillus* in a group sample exposed by the ELF magnetic field is higher than the control group. The highest population is perceived by the sample population that was given 300 μ T intensity in 45 minutes. Nevertheless, the number of *Lactobacillus* in the group exposed by the ELF magnetic field in 100 μ T in 45 minutes is almost the same with the control. Also, The average of *Lactobacillus* population for each group diverse significantly (sig. = 0,000 < 0,05) (table 1). Therefore, the intensity and the high ELF magnetic field radiation effect on the *Lactobacillus* bacteria population is determined by the LSD test (table 2).

Table 1. Analysis of the *Lactobacillus* population

	df	Mean Square	F	Sig.
Between Groups	6	219852.413	9.480	.000
Within Groups	35	23190.629		
Total	41			

Table 2. LSD Test Result

(I)	(J) Group	Sig.	95% Confidence Interval
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Group			Lower Bound	Upper Bound
Kontrol	100 μ T, 15 menit	.350	-261.8237	95.1570
	100 μ T, 30 menit	.340	-263.4904	93.4904
	100 μ T, 45 menit	.961	-182.8237	174.1570
	300 μ T, 15 menit	.008	-425.8237	-68.8430
	300 μ T, 30 menit	.028	-379.8237	-22.8430
	300 μ T, 45 menit	.000	-725.1570	-368.1763

The result of LSD analysis showed that the population of *Lactobacillus* bacteria in 300 μ T intensity for 15 minutes, 30 minutes and 45 minutes significantly ($\text{sig.} = 0.000 < 0.05$) are higher than the control group. The highest population of *Lactobacillus* for the sample group of coffee fermentation was exposed 300 μ T for 45 minutes. Otherwise, the lowest population of *Lactobacillus* bacteria is presented by the ELF magnetic field in 100 μ T for 45 minutes.

Furthermore, the result of the pH test during the artificial civet coffee fermentation after exposed by the ELF magnetic field was presented in figure 5 and table 3.

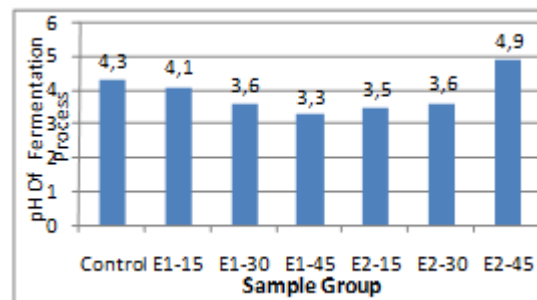


Figure 5. The graph of *Lactobacillus* Population

Table 3. Significance Analysis of pH

	df	F	Sig.
Between Groups	6	2.091	.079
Within Groups	35		
Total	41		

Table 3 indicated that the average pH of artificial civet coffee fermentation did not show a significant distinction ($\text{sig.} = 0.079 > 0.05$) for each group. Moreover, the LSD analysis that mostly affects the pH score during the fermentation process presented in table 4.

Table 4. LSD Test result

			95% Confidence Interval	
(I) Group	(J) Group	Sig.	Lower Bound	Upper Bound
Kontrol	100 μ T, 15 menit	.718	-.9158	1.3158
	100 μ T, 30 menit	.211	-.4158	1.8158
	100 μ T, 45 menit	.068	-.0825	2.1491
	300 μ T, 15 menit	.154	-.3158	1.9158
	300 μ T, 30 menit	.191	-.3825	1.8491
	300 μ T, 45 menit	.310	-1.6825	.5491

The result of LSD analysis reported that there is not any significant distinction of the pH score during the process of artificial civet coffee fermentation sample in 100 μ T or 300 μ T intensity and the control sample. Meanwhile, the highest pH score is produced in 300 μ T for 45 minutes, even though

there was not quite significant ($p = 0,310 > 0,05$). Also, the low pH is observed in 100 μT for 45 minutes and no significance ($p = 0,068 > 0,05$).

4. Discussion

The fermentation process is needed to develop the quality of coffee, even though some restrictions caused imperfect fermentation. Hence, it is required to produce technological innovation regarding the fermentation process. In this study, the results indicated that the ELF magnetic field exposure in 300 μT improves the activity of artificial civet coffee beans fermentation through the growth of the *Lactobacillus* population that classified into lactic acid. Lactic acid bacteria, such as *Lactobacillus Plantarum*, *Lactobacillus Brevis*, and *E. Casseliflavus*, are essential to the process of coffee beans fermentation, especially coffee fermentation by using a wet method or partially damp method.[8] The α - amylase enzyme, which is added during the proses of fermentation, aims to hydrolysis the starch component and pulp of coffee so that it can optimize the role of microbe during the fermentation process [2].

An organic acid is a metabolite substance that creates bacterial metabolisms like lactic acid and acetate acid. The accumulation of organic acids will decrease pH or proton H^+ since the decomposition of amino acids. Frequently, the more increase the acid substance, the pH has been more decrease. In contrast, the pH condition, which improves, is produced by the instability of microbe performance in weaving environment temperature. The carboxylicesters, such as methyl butanoate, Asam cyclohexanol, and organic substance that contain sulfur, caused stinker defect for coffee beans and increased the total pH [2]. In line with this research, the *Lactobacillus* examination raised, and the total pH in the group sample exposed by the ELF magnetic field in 300 μT for 45 minutes increased also. Meanwhile, the decreasing population of *Lactobacillus* is also followed by the decrease of pH in the sample group exposed by the ELF magnetic field in 100 μT for 45 minutes. The fermentation process of *Lactobacillus* can change caffeine to be an ester substance. Caffeine is part of alkaloids that decomposed as ester like chlorogenate acid through esterification. The mechanism of esterification separates the complex material in caffeine to become chlorogenate acid. However, the molecular size and weight fall off. It is more comfortable to diffuse in a cell wall and water-soluble. The release of chlorogenate acid from caffeine and its decomposition to be other organic substance is soluble in the fermentation mechanisms. The lengthy soluble process makes the soluble chlorogenate acids raise — the decrease of caffeine in coffee directly proportionate to the fermentation time.

The ELF magnetic field can directly interact with the *Lactobacillus* membrane. The magnetic force work in each ion and molecules of cell components. In this process, the polarization of the cell will occur. The cell encountered the growth process and cell cycle; it will affect the whole molecular process of the cell cycle. In the cell cycle process, ion calcium (Ca^{2+}) has a vital role as the second messenger in the molecular process that affected the survival and cell proliferation [11], [12]. The ELF magnetic field exposure has no ruined effect. The magnetic field exposure in the frequency of 50Hz MF by 2000 μT intensity does not give ruin DNA or abnormal cellular function in the neurogenic cell [13]. The intensity and frequency EMF effect depends on the duration of exposure, as well as the species character. The intensity and frequency EMF effect depends on the duration of exposure, as well as the species character [14]. The ELF can trigger the proliferation of *Lactobacillus* in the fermentation process of robusta coffee beans.

5. Conclusion

The ELF magnetic field exposure in 300 μT can improve the growth of *Lactobacillus* that has an essential role in the fermentation process of artificial civet coffee. The total of pH during the fermentation process did not show any significant changes. This research can be used as the basic idea to improve the fermentation technology of coffee based on ELF magnetic field radiation.

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