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Optimization of the fermentation process through ELF magnetic field radiation and its effect on Cascara Caffeine

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ABSTRACT

Coffee is in high demand on the global market due to its distinctive scent. Part of the coffee is coffee waste which needs be processed to increase its economic worth. One of the efforts made is to vary the fermentation process with the ELF magnetic field on cascara. Thus, the purpose of this study was to optimize the effect of the ELF magnetic field fermentation process on cascara robusta coffee to reduce caffeine content. We utilized an experimental study. The results demonstrated that a magnetic field affected the levels of caffeine, trigonellin, and bacteria during the fermentation process. Caffeine and trigonelin test results for each treatment in the control and treatment groups.

Keywords: Optimization of cascara fermentation, ELF Magnetic field, Effect of caffeine content.
Article type: Research Article.

INTRODUCTION

Robusta coffee is an important plantation commodity within the global economy. The widespread rapid growth of coffee shops increases the demand for coffee beans. Increasing coffee consumption makes waste from coffee processing a concern to the environment (Ruta & Farcasanu 2021). Cascara, a by-product of coffee production, can be employed as an effective product. Cascara is a health-promoting substance with functional properties. Cascara is composed of caffeine, tannins, trigonellin, and polyphenolic chemicals (Heeger *et al.* 2017). The development of cascara is enabled by its nutrient- and antioxidant-rich composition. One of the cascara processing methods that has been created is kombucha cascara, which has a unique flavour with a sour taste, sweetness with a distinctive cascara colour (red brown), and a mild bitterness due to the presence of flavonoids (Nurhayati *et al.* 2020), therefore it is not popular with the general public. In order for the flavour to be favourable welcomed by the community, the fermentation process should be improved. Fermentation has numerous human applications, including industrial production, waste treatment and environmental management, food preservation as well as health care (Xiang *et al.* 2019). Understanding the process of using microbes in food or beverages can improve flavour, nutrition, and metabolites (Butt *et al.* 2019). The application of modernization technology creates new opportunities for fermentation engineering, including the production of cascara herbal tea. In Los Angeles, cascara is brewed with 6.5 g L^{-1} of water, soaked for 6.5 minutes at 90 ºC, with the addition of 7.1 g sugar and 5.7 mL lime juice (Heeger *et al.* 2017). A production process that is not too complicated yet rich in the benefits of cascara tea can be developed by coffee farmers, so that this commodity continues to grow. However, fermented cascara tea is not optimal due to the fact that *Lactobacillus* sp. bacteria do not reproduce optimally. In order for bacteria to multiply optimally, technological innovations are required. Bacterial biological responses under the impact of magnetic fields are particularly intriguing. On every living item, the consequences of exposure to a magnetic field

with a given intensity and different strains of bacteria vary. Numerous elements, such as environmental conditions and chemical compounds, influence bacterial growth. Thus, understanding the interplay between biological systems and the magnetic field intensity that affects them is important (Fijałkowski *et al.* 2013). According to the findings of the study Kristinawati & Sudarti (2016), exposing cheese to a 100 T magnetic field for five minutes activated mesophilic and thermophilic bacteria, so that the fermentation process operated optimally. Based on results of Sudarti *et al.* (2018), exposure to the ELF magnetic field with an intensity of 100 µT for 5 minutes can activate the growth of *Streptococcus thermophilus*, *Lactococcus lactis*, and *Lactobacillus acidophilus* bacteria. According to the results of Sadidah *et al.* (2015), exposure to 500 µT and 300 µT ELF magnetic fields led to alterations in the number of microbes and pH in the tape fermentation process. The results of Sudarti *et al.* (2020) showed that exposure to a magnetic field of 300 µT for 45 minutes increased the *Lactobacillus* population and increased the quality of artificial civet coffee. Extremely Low Frequency (ELF) magnetic fields have been utilized extensively in food fermentation processes. However, the application of ELF magnetic field exposure on Robusta coffee cascara fermentation is currently limited. Several enzymes, specifically pectinases and amylases, contribute in the coffee fermentation process. In addition, aerobic bacteria, Enterobacteriaceae, yeast, filamentous fungi, and lactic acid bacteria (LAB) such as *Lactobacillus plantarum*, *L. brevis*, and *Enterococcus casseliflavus* play a role in the coffee fermentation process (Nasanit & Satayawut 2015). Giving exposure to the ELF magnetic field is expected to optimize the work of microorganisms in the cascara robusta coffee fermentation process by carrying out cell proliferation, so that the substance content obtained in cascara robusta coffee can reach optimal levels. Using microorganism culture is recommended to improve the physical quality and taste of coffee. The addition of microbe cultures to coffee fermentation will alter the population balance of microorganisms involved in the fermentation process, hence altering the fermentation process and its outcomes (Ruta & Farcasanu 2021). Therefore, this study was conducted to examine the impact of exposure to the ELF magnetic field on the coffee cascara fermentation process. Based on the description above, the authors are interested in conducting study on "optimizing the fermentation process through ELF magnetic field radiation and its effect on caffeine levels in cascara."

MATERIALS AND METHODS

An experimental study with a completely randomized design was employed. The treatment was done in the form of an ELF magnetic field with an intensity of 300 T. The samples in this study were 32 kg fresh cascara, 2 kg as a control group not exposed to magnetic fields and not fermented, as well as 30 kg fresh cascara divided into 5 groups of 6 kg each, fermented with variations of soaking coconut water (E1), sugarcane juice (E2), lemon juice (E3), distilled water (E4) and enzymes (E5). Variations in several treatments for cascara can be seen in Fig. 1. Each treatment group was divided into 3 groups, group 1 with 2 kg weight was fermented with cascara without a magnetic field, group 2 cascara with a magnetic field of 300 µT for 15 minutes and group 3 with cascara at 300 µT for 45 minutes. The data were analysed using MANOVA analysis and supported by descriptive analysis. The tools in this study included plastic trays, plastic boxes, an analytical balance, a thermometer, an ELF magnetic field and EMF field tester, a stir bar, a test tube, a test tube rack, a beaker glass, a pH meter, aluminium foil, a measuring cup, a petri dish, a heating stove, a bunsen, a micropipette, a tip, and a Liquid Chromatography Mass Spectrometry (LC-MS). Meanwhile, the materials used in this study were cascara robusta coffee, liquozyme, coconut water, lemon juice, sugarcane juice, distilled water, labels, *Lactobacillus* MRS agar, fial bottles, syringes, and hydrophilic polyvinydene flouride (PVDF) acrodisc. Acidity test was done using a pH meter, and caffeine test used LC-MS. The stages of the study were carried out in several stages including identification and sample preparation, preparation of medium, isolation of bacteria, and enumeration of bacteria, caffeine, as well as trigonelin. The sample identification of this study started with determining the sampling location of the to-be-harvested coffee cherries, manually sorting the coffee cherries to separate the red coffee cherries from the remaining impurities, washing the selected coffee cherries under running water, and placing the remaining coffee cherries in an open container. The water was wasted and dried. The next stage was do the pulping process manually with the help of a knife, so that the resulting cascara was not crushed and its contents were not lost due to impact if the coffee was ground with a pulper machine. Then, the cascara was sorted, separated with silver skin and collected in a closed container for processing. The fermentation of this study was carried out. The wet cascara was divided as much as 500 g in a container according to treatment. There were two treatments carried out, including variations in immersion water and variations in the magnetic field. The first variation was cascara soaked in sugarcane juice, soaked in coconut water, soaked in lime juice, soaked in enzymes, and as a control cascara

soaked in distilled water. In each treatment there were 3 containers for further treatments, including one cascara sample for control, one was exposed to a 300 T magnetic field for 15 minutes, and one was given a 300-T magnetic field for 45 minutes. Thus, the total sample becomes 15 containers. The wet cascara was placed into a closed container and left for 6 hours. Thereafter, the cascara was given a magnetic field according to its intensity, the sample which has been given a magnetic field was left for another 6 hours, dried on a clean tray with the help of sunlight until the water content was ± 7%, packed according to the treatment, labelled and stored in cool place.

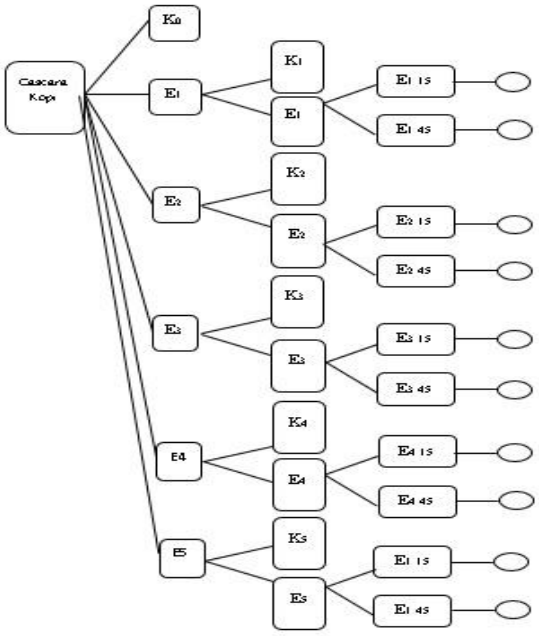


Fig. 1. Variation of treatment on cascara.

Making the media began with preparing the necessary tools and materials. The tools needed were a beaker glass, a test tube, a test tube rack, a petri dish, a stir bar, an electric stove, a bunsen lamp, a micropipette, a tip, an analytical balance, aluminium foil, and a measuring cup. Meanwhile, the materials needed were: coffee husks (cascara), Methicillin-Resistant Staphylococcus Aureus (MRSA), distilled water, spirits, alcohol, labelled paper, and plastic wrap. The steps started from placing the petri dish in the Laminar Air Flow (LAF) to prepare the media container, weighing the MRS media as needed. In this study, the calculation was:

MRSA Conditions → 65.13g L^{-1}
1 cup = 15 mL
45 cups = 15 × 45 = 675 mL (rounded to 700 mL)
Calculation → 700 × 65.13/ 1000 = 45.5/700 mL

On the next process, we added 700 mL distilled water into a 1000 mL beaker glass, then 45.5 g MRSA. It was stirred until well mixed. The mixture of the two was placed on the electric stove and stirred until it boiled. The media was poured into the petri dish in the LAF which has been prepared for 15 mL each and waited until the media solidifies. The bacterial isolation stage started from preparing 5 test tubes for each treatment. Each test tube was filled with 9 mL distilled water. We prepared 75 test tubes, each tube filled with 9 mL distilled water. One gram of cascara sample was taken and poured into a small beaker glass mixed with 10 mL distilled water. It was stirred until evenly distributed. The same things were done for all samples. In the LAF, 1000 µL of a homogeneous cascara was taken and distilled water mixture using a micropipette that has been tipped. It was placed in test tube 1, made a dilution by taking 1000 µL and poured it in the second tube. The same procedures were done until the 10^{-5} dilution. In the 5^{th} test tube, 100 µL of sample was taken. The spread plate method was used to grow microorganisms on agar media by pouring the bacterial culture stock or removing it on solid agar media. The plastic wrap was installed so that the media was airtight and away from contamination. The process of counting bacteria began with counting bacteria directly by estimating the number of bacterial colonies in the petri dish. This calculation required high accuracy because the calculation was carried out on live bacteria and followed by

testing caffeine and trigonellin. Starting with preparing 1 g of cascara sample, adding 50 mL distilled water, heating the sample at 100 ºC for 5 minutes while stirring on a magnetic stirrer. We also filtered it to take the filtrate, poured the sample into the fial bottle using a 2-3 mL syringe, and filtered using a PVDF acrodisc. The sample was placed into the LCMS tool, opened the lab solution application and carried out the running process according to the desired test. All stages of the research can be seen in Fig. 2.

RESULTS AND DISCUSSION

The following are the results of effects of variations on fermentation using soaking water that are different from the duration of exposure to magnetic fields on caffeinated cascara robusta coffee in the control and experimental groups. Data on the calculation of the number of bacteria can be seen in Table 1.

Table 1. Data from the calculation of the number of bacteria in fermented and non-fermented cascara

No	Test Type	Exposure time		Sample Code				
				E1	E2	E3	E4	E5
1	Cascara Fermentation	-	24 hours	61	43	250	201	125
		15'		35	5	196	119	390
		45'		40	48	31	9	61
		-	48 hours	84	46	202	210	141
		15'		43	5	40	96	398
		45'		36	53	40	20	93
2	Non Fermented Cascara		24 hours	96	53	15	45	103
			48 hours	102	80	30	100	303

Based on Table 1, the number of bacteria in the treatment with different immersions shows a different number of bacteria. Likewise, when compared with the length of exposure, the results are also different. This is in accordance with that of Fijałkowski *et al.* (2013) who reported that exposure to magnetic fields with different intensities will show different responses. It depends on the bacterial strain. Bonaventura *et al.* (2014) also reported that ELF-MF exposure significantly increased bacterial growth and affected biofilm formation and viability. Exposure to ELF-MF 250 T significantly increased the growth rate of three species, Staphylococcus epidermidis, Staphylococcus aureus, and Escherichia coli (Lucas *et al.* 2017). Tessaro *et al.* (2015) also stated that exposure to ELF-EMF (250 μT) significantly increased the growth rate of three species (Staphylococcus epidermidis, Staphylococcus aureus, and Escherichia coli), while slowing the growth of one species (Serratia marcescens). Verschaeve *et al.* (2016) stated that a magnetic field of 100 T (50 Hz) does not damage DNA and is therefore not mutagenic in this assay nor does it affect the DNA damage capacity of the mutagen used. However, exposure to a 1 mT ELF magnetic field for 2 hours caused changes in the physicochemical properties of Gram-positive and Gram-negative bacteria, and a slight decrease in bacterial growth was found (Oncul *et al.* 2016).

		
Sorting coffee cherries for grinding.	Separating cascara from the coffee beans.	Cascara was ready to be fermented with a magnetic field.

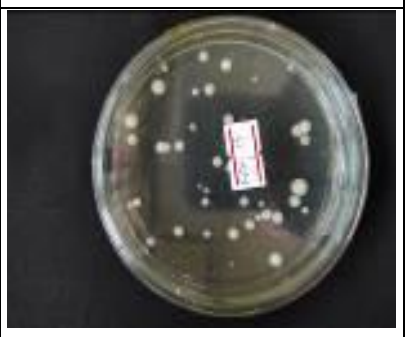
		
Setting up the magnetic field machine.	Coffee cherries peeled in the sun.	Making agar media for bacteria.
		
Pouring the media in a petri dish.	Growing bacteria on agar media.	Counting the growing bacteria.
		
Putting the sample into the stirrer bottle.	Entering the sample into the LCMS.	Data analysis using the Lab Solutions application.

Fig. 2. The documentation of implementing caffeine analysis research on cascara.

A study of the ELF magnetic field on bacteria reported that exposure to 100 T intensity for 5 min led to effective in elevating the proliferation of *S. thermophilus*, *L. lactis*, and *L. acidhopilus* bacteria in the cheese cream-making process (Sudarti *et al.* 2018). This fact strengthens the evidence that exposure to an intensity of 100 T for 5 min is effective in upraising the quality of cream cheese, with an indicator of the decreased water content of cream cheese (Andika & Sudarti 2016). Meanwhile, the exposure to an intensity of 500 T for 30 min was able to suppress microbial growth in sticky tape (Sadidah *et al.* 2015). Exposure to an ELF magnetic field intensity of 300 μT for 5 and 15 minutes in a cowshed with a milk fermentation process was proven to be able to maintain the pH value (Ridawati *et al.* 2015, 2017). Many factors affect the growth of bacteria such as environmental factors and chemical conditions. Prolonged exposure to cascara does not kill bacteria. Also, bacterial cells do not die under high magnetic fields (Mazzafera &, Robinson 2000). In this study, the coffee was exposed to a magnetic field at the start of fermentation to induce acetic acid bacteria (AAB) and lactic acid bacteria which encouraged the conversion of pulp substrates into ethanol, lactic acid and acetic acid (Pereira *et al.* 2016). The results of the study also found media contaminated with fungi (See Fig. 3). Data from the analysis of caffeine and trigonellin in each sample analysed by LCMS can be seen in Table 2. Based on this Table, the order of caffeine content from high to low in fermented cascara without a magnetic field included cascara with lemon immersion at a concentration of 0.095, cascara with distilled water soaked at 0.089, cascara without immersion at 0.078, cascara with sugarcane

juice soaked at 0.044, and the last is cascara with enzyme immersion at 0.035. Meanwhile, the order of caffeine content in cascara which was fermented with a magnetic field for 15 min from high to low included: cascara with distilled water at 0.091, cascara with soaking lemon juice with 0.064, cascara without soaking at 0.059, and cascara with immersion of enzymes at 0.046. Finally, fermented cascara with an exposure time of 45 minutes after sorting from high to low included cascara not soaked at a rate of 0.095, cascara with distilled water soaked at 0.085, cascara with lemon soaked at 0.053, cascara with sugar cane soaked at 0.036, while the lowest was cascara with enzyme bath at 0.034. Unfermented cascara has a relatively high caffeine content compared to fermented cascara, which is 0.443.

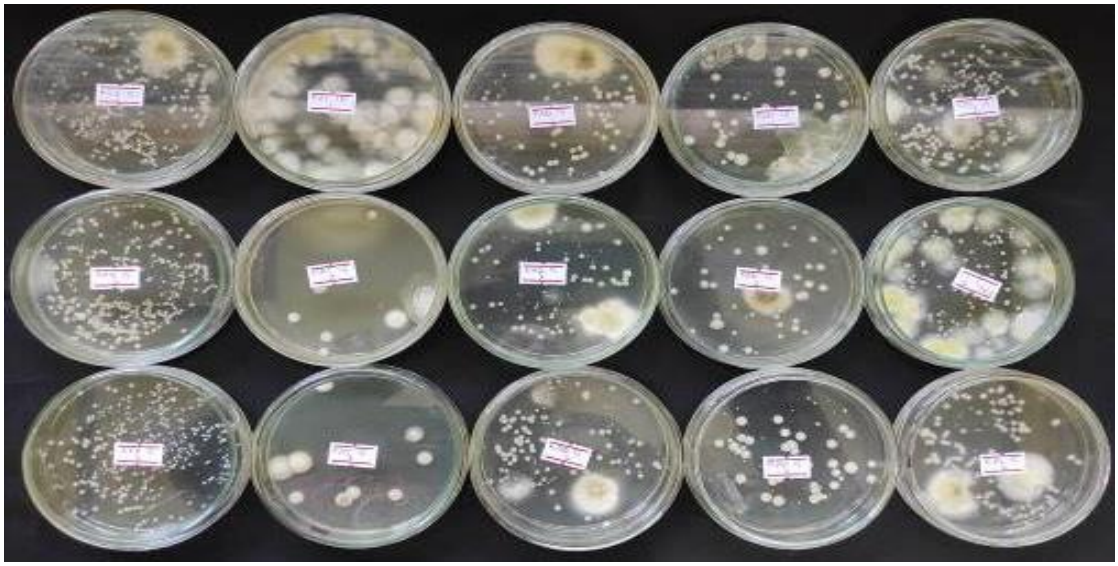


Fig. 3. Mushroom contaminated media.

The order of trigonellin levels from high to low in fermented cascara without a magnetic field included cascara with immersion of sugarcane juice with a content of 0.096, cascara without immersion with 0.050, cascara with enzyme immersion at 0.048, cascara with distilled water soaked at 0.040, and finally cascara with lemon juice at 0.035. While the trigonellin content in cascara was fermented with a magnetic field for 15 minutes when sorted from high to low, cascara by immersing sugarcane juice with a concentration of 0.07, cascara without immersion with 0.053, cascara by immersing aquadest with 0.036, cascara by soaking enzymes with cascara content by soaking in lemon water at 0.025. The trigonelin levels in cascara fermented with a magnetic field for 45 minutes were sorted from high to low, including cascara with sugarcane juice immersion at 0.078, cascara without immersion at 0.051, cascara with aquades immersion at 0.032, cascara with soaking lemon juice at 0.021 and cascara with an enzyme bath at 0.012. Unfermented cascara has a relatively low trigonellin content compared to fermented cascara of 0.012.

Table 2. Results of analysis of caffeine and trigonellin levels.

No	Test Type	Exposure time	Sample Code				
			E1	E2	E3	E4	E5
1	caffeine	-	0.078	0.44	0.095	0.089	0.035
		15 minutes	0.059	0.057	0.064	0.091	0.046
		45 minutes	0.095	0.036	0.053	0.085	0.034
2	Trigonelline	-	0.050	0.035	0.035	0.040	0.048
		15 minutes	0.053	0.046	0.025	0.036	0.026
		45 minutes	0.051	0.034	0.021	0.032	0.014
3	Non Fermented Caffeine		0.443				
4	Trigonelline Non Fermentation		0.012				

Based on these results, it can be concluded that cascara processed with enzymes and sugarcane contained the least amount of caffeine. The lowest quantities of trigonelin were found in the fermentation process using soaking water, including lemon and enzyme immersion. Enzymes can expedite the fermentation process by accelerating the response rate. The results of the examination of caffeine content in fermented and unfermented cascara differ significantly when compared as a whole. Unfermented cascara contains a large amount of caffeine, while fermented cascara contains far less. This is inversely related to non-fermented cascara's trigonelin levels, which are lower than fermented cascara. In terms of the duration of exposure, fermented cascara with a 45-min exposure intensity had a lower caffeine concentration than fermented cascara with a 30-min exposure intensity. The longer the fermentation period, the lower the coffee's caffeine content. This is because in the fermentation process caffeine is degraded into uric acid, 7-methylxanthine, and xanthine (Anal 2019). The amount of yeast increased during 24 hours of fermentation and produces pectinolytic enzymes which have the ability to produce a special aroma by degrading the pulp and mucus layer to produce acids and other metabolic compounds which diffuse into the seeds (11). Long fermentation would cause coffee acidity to increase due to the formation of aliphatic acids which will turn into carboxylic acid esters which can cause defects and bad taste (Bonaventura *et al.* 2014).

CONCLUSION

Analysis of caffeine and trigonellin in cascara has been done in this study. The results for caffeine and trigonellin levels were reversed. Caffeine concentration is lower in fermented cascara, whereas trigonelline output is lower in unfermented cascara. The number of bacteria contained in each treatment varied. This is dependent on the treatment, with various soaks revealing varying amounts of bacteria.

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