



# Volatile Compounds Changes in Unfermented Robusta Coffee by Re-Fermentation Using Commercial Kefir



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**Submission:** December 7, 2018; **Published:** April 23, 2019

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## Abstract

Robusta coffee usually has a low quality because the formation of flavored precursors is imperfect. The efforts to form a flavored precursor of unfermented Robusta coffee can be done by re-fermenting the coffee using reproduction which is able to help the formation of flavored precursors such as lactic acid bacteria found in kefir. The appropriate temperature condition and time period are needed to be considered in re-fermenting the unfermented Robusta coffee. This study aimed to determine the effect of temperature and time on the volatile compound's changes in unfermented Robusta coffee by commercial kefir. This study used two factors, including temperature (27, 37 and 47 °C) and re-fermentation time (6, 12 and 18 hours). The results showed that duration and temperature of re-fermenting could improve the aroma characteristics and volatile compounds. Re-fermentation of Robusta coffee bean can increase total of volatile compounds at 37 °C for 18 hours. The increasing volatile compounds of acid, alcohol, aldehyde and acetate groups contributed to give the pleasant aroma as follows acidity, fruity, nutty and caramelly aroma.

**Keywords:** Unfermented Robusta coffee; Re-fermentation; Temperature; Time of fermentation; Volatile compounds

## Introduction

Robusta coffee is the most widely cultivated coffee in Indonesia with a production of 465,614 tons [1]. Robusta coffee has a lower quality than Arabica coffee [2]. One of the cause lower quality in coffee flavor was dry way processed generally used. Unfermented coffee is a kind of coffee beans produced by very simple methods and facilities. Aklimawati et al. [3] reported that the taste of unfermented robusta coffee in the slope area of Tambora has a final score of cup test among 45-70. The low quality will cause the unfermented coffee has a low economic value.

Some efforts to improve the quality of unfermented robusta coffee which has been done by Aklimawati et al. [3] were grading, with separating unfermented robusta coffee beans into two groups based on Indonesian National Standard [4]. This study was carried out to improve the quality of unfermented robusta coffee on coffee processing in wet manner using re-fermentation stages with commercial kefir.

Kefir was fermented milk using kefir grain were contains complex microbes, consist of bacteria (species of *Lactobacillus*, *Lactococcus*, *Leuconostocs* and *Acetobacteria*) and yeasts [5]. Lactic acid bacteria are microorganisms which have the largest population in kefir.

Fermentation of coffee beans can improve flavor because of the metabolites produced by microbes [6]. During fermentation, bacteria will breakdown the complex compounds into short chain compounds such as reducing sugars, and metabolites product such as organic acids [7]. All these compounds play a role in aroma formation during coffee roasting.

Re-fermentation is a processing technology that has been applied on unfermented cocoa beans to improve cocoa beans quality [8]. The factors affected the fermentation must be considered to get a good coffee flavor. The right starter, the appropriate and controlled fermentation were important things. Over fermented

can become a problem during fermentation [9]. As well as we know, there is limited information about unfermented robusta coffee re-fermentation, specially using commercial kefir. For this reason, this study was conducted to determine the effect of temperature and duration re-fermentation on coffee flavor and the volatile compounds changes in unfermented robusta coffee by commercial kefir.

## Materials and Methods

### Plant materials

The materials used were unfermented robusta coffee harvested from Sidomulyo Village, Jember Regency, Indonesia and commercial kefir "Kefir Jember". The materials used for analysis were MRSA (DeMann Rogosa Sharpe Agar),  $\text{CaCO}_3$ , NaCl, and alcohol 70% were purchased from Merck. The tools used in this study were fermentor height 130cm, width 40cm (CV. Asmak Kopi, Indonesia), roaster capacity 10kg (AR, Indonesia), grinder, cupstest assessment forms, pH meters, SPME (Solid Phase Microextraction) 3 phases (dicinylbenzene/carboxen/polymethylsiloxane) (supelco), GC-MS (Agilent 7890 A-5975) Alkanes standard were purchased from Aldrich (Steinhein, Germany).

### Unfermented robusta coffee re-fermentation

Unfermented robusta coffee was sorted from damaged beans and foreign objects. Robusta coffee (1000g) was soaked in 2L distilled water for 2 hours then drained and added 2% lactose and 1% kefir of the coffee beans weight. Re-fermentation was carried out for 6, 12 and 18 hours using controlled temperatures (27 °C, 37 °C, and 47 °C with two replicates). The next step was washing robusta coffee beans using water and dried in the sun drying (T: 26-28 °C; RH: 80-85%) for 2 days until the moisture content became 13%.

### Lactic Acid Bacteria Population

The lactic acid bacteria population were calculated using BAM method [10]. The coffee samples (5g) diluted with 45mL sterile distilled water. Dilution was carried out up to 10<sup>-7</sup>. Dillution results were taken 1mL from the last three dilution levels and poured into a petridish then added with MRSA and  $\text{CaCO}_3$ . Then, it was incubated at 37 °C for 48 hours.

### pH Value of Dry Re-fermented Asalan Robusta Coffee Beans

Measurement of pH value using pH meter [11]. Dry coffee was dissolution in a ratio of 1:3 coffee beans and distilled water.

### Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis was carried out with GC-MS Agilent 7890-5975C. Chromatographic separation was performed with an RTX-5MS column internal diameter and Helium as carrier gas at a constant flow of 1.0mL/min. Injector temperature 250 °C. Ion and interface temperature were 300 °C and 275 °C. The oven

initial temperature was 40 °C (5 mins), increased to 180 °C with 3 °C/min rate, and then increased to 250 °C (5 mins) with 10 °C/min rate. The GC injector was set in splitless mode.

Volatile compounds identification was based on comparison of their mass spectra and that presented in NIST 2,0 database and confirmed by comparison of their retention indexes with the published references. Linear retention indexes (RI) were calculated using the retention data of linear alkanes solution in n-hexane [12].

## Results and Discussion

### Population of lactic acid bacteria during fermentation

There were various types of microorganisms in robusta coffee beans fermentation using kefir starter, but the microorganism observed in this study was lactic acid bacteria. The population of lactic acid bacteria during re-fermentation of unfermented Robusta coffee beans using kefir starter can be seen in Figure 1.

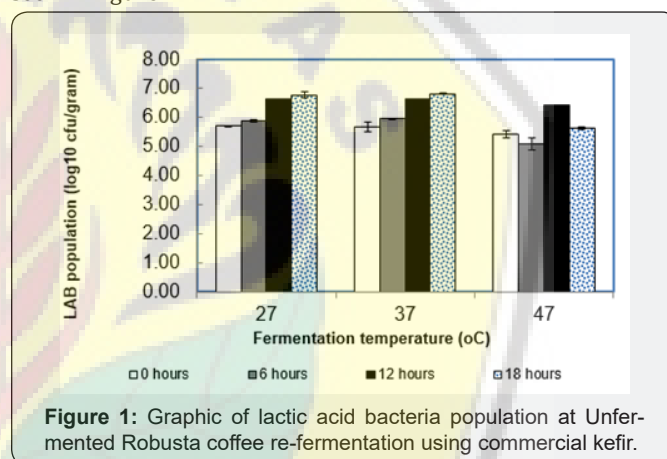


Figure 1: Graphic of lactic acid bacteria population at Unfermented Robusta coffee re-fermentation using commercial kefir.

Three different temperature levels were affected the growth of lactic acid bacteria. Coffee fermentation at 27 and 37 °C temperatures during 0, 6, 12 and 18 hours were increase the total population of lactic acid bacteria, while at 47 °C there was decrease bacteria within the 6 and 18 hours. According to Khalid [13], the optimal temperature in lactic acid bacteria growth was in 10-45 °C temperature. Fermentation using 27 °C and 37 °C increases the population of lactic acid bacteria along with the longer fermentation. A slight increase in population was expected because microbes are still in the lag phase. Rolfe et al. [14] reported that the lag phase is defined as the initial period in the bacterial population life when the cell adapts to the new environment before starting the exponential phase.

The death phase in this study occurred at 45 °C fermentation from 12 to 18 hours. At the 12 hours, the number of lactic acid bacteria was 5.90 log cfu/g and decreased to 5.64 log cfu/g at the 18<sup>th</sup> hour. According to Sarah et al. [15], the meaning of the "death phase" is a phase where the number of dead cells is more than the number of new cells.

## pH value of dry coffee

The pH value of unfermented Robusta coffee beans has the highest value (6.2), compare to Robusta coffee re-fermentation using the commercial kefir, and as can be seen in Table 1.

**Table 1:** Changes in the pH of Robusta coffee beans from dry re-fermentation.

Temperature (°C)	Fermentation Period (hour)			Control (unfermented Robusta coffee beans)
	6	12	18	
27	5.8	5.6	5.5	6.2
37	5.6	5.3	5.3	
47	5.9	5.8	5.8	

Based on the table, it can be seen that the existence and longer re-fermentation processed will reduce the pH value of unfermented robusta coffee beans. According to Suprihatin [7], coffee fermentation produces both primary and secondary metabolism, for example organic acids which will reduce the pH value. Fermentation temperature affects the pH of coffee beans. Fermentation using 37 °C has the lowest pH value compared to unfermented robusta coffee beans using 27 °C and 47 °C temperatures whereas the unfermented robusta coffee bean which was fermented in 47 °C temperature has the highest pH value compared to other fermentation. This result accordance with Wardani et al. [16] were using 30, 34, 37 and 40 °C temperatures on milk fermentation, the results showed that pH value of 40 °C fermentation temperature was the highest while 37 °C temperature fermentation was the lowest pH value. This due to lactic acid bacteria population was growth directly comparable to the acid production and pH value decrease. The fermentation temperature (37 °C) was the optimal temperature for the lactic acid bacteria growth, so acid production and decline of pH takes place quickly. The pH reduction indicated that there was a breakdown of macro compounds by microbes. The broken compound would be a precursor flavor.

## Coffee volatile compounds

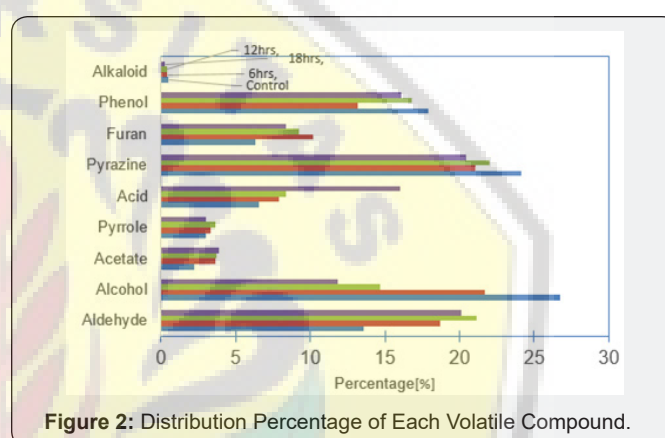
Totally 17 volatile compounds were identified in unfermented robusta coffee beans (control) while those of re-fermentation treatment using commercial kefir at 37 °C temperature were increase with the longer fermentation period. Totally 21, 22 and 23 volatile compounds were identified in re-fermentation of unfermented robusta coffee beans using commercial kefir for 6, 12 and 18 hours.

The volatile compounds that have been identified can be grouped into 9 classes, including acids, pyrazine, furan, phenol, aldehyde, alcohol, acetate, pyrrole and alkaloids as can be seen in Figure 2.

Based on these data, volatile compounds were classified as acid compounds including, acetic acid, anhydride with formic acid and pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester. According to Caporaso et al. [17], Robusta coffee

generally has high concentrations acetic acid compounds. Acid group contribute to acidic aromas in coffee [18]. The acidic group showed increasing in peak area with longer fermentation. Bressani et al. [19] stated that lactic acid bacteria produce acid as a result of its metabolites.

Other groups of volatile compounds that have high percentage area were alcohol group. Pyrazine group is volatile compounds formed by coffee roasting. The aldehyde group was one of the important compounds in coffee aroma. The figure shows the distribution of the compound when the volatile organic compound is taken as 100% at each fermentation time. From the control fermentation greatly reduced alcohol, pyrazine and phenol, in the other hand increased aldehyde, furan and acid. From this, we can see that the sour and nutty odors were strengthened (Figure 2).



**Figure 2:** Distribution Percentage of Each Volatile Compound.

Changes in each compound associated with fermentation are shown in Figure 3. It is indicated by relative evaluation based on the control ( $t = 0$ ). By fermentation, 5-methyl-2-furan-carboxaldehyde was increased about 1.5 times that of control in furans. Phenols overall are decreased. However, in proportion 2-methoxy-4-vinylphenol increased about 2-fold. Furan compounds identified were 5-methyl-2-furancarboxaldehyde and  $\alpha$ -furfurylidene- $\alpha$ -furylmethylamine compounds. 5-methyl-2-furancarboxaldehyde compounds contribute to the burnt aroma, sweet and spicy [20]. These furan compounds were formed by the thermal degeneration of carbohydrates, ascorbic acid, or unsaturated fatty acids during coffee roasting [21]. The spicy aroma identified in this study is not only produced by furan group but also affected by the presence of phenol group compounds, namely 2-methoxy-4-vinylphenol [22] (Figure 3).

The compounds included in the phenol group in this study were 4-ethyl-2-methoxy phenol; 2-methoxy-4-vinylphenol; butylated hydroxytoluene and 2-methoxy phenol. Based on the research conducted by Lee et al. [9], guaiacol (2-methoxy phenol) and p-vinylguaiacol (2-methoxy-4-vinylphenol) compounds have increased by the existence yeast fermentation p-vinylguaiacol compounds was formed when enzymatic decarboxylation occurs in ferulic acid compounds while guaiacol is formed due to redox reaction, decarboxylation oxidative and non-oxi-

datave compounds -vinylguaicol. In the phenol group, there were 2-methoxy-4-vinylphenol compounds that contribute to the spicy aroma [22]. The area of the 2-methoxy-4-vinylphenol

compound increases along with the longer fermentation. So, it can be interpreted that the longer the fermentation of the spicy aroma in coffee, the aroma will become stronger.

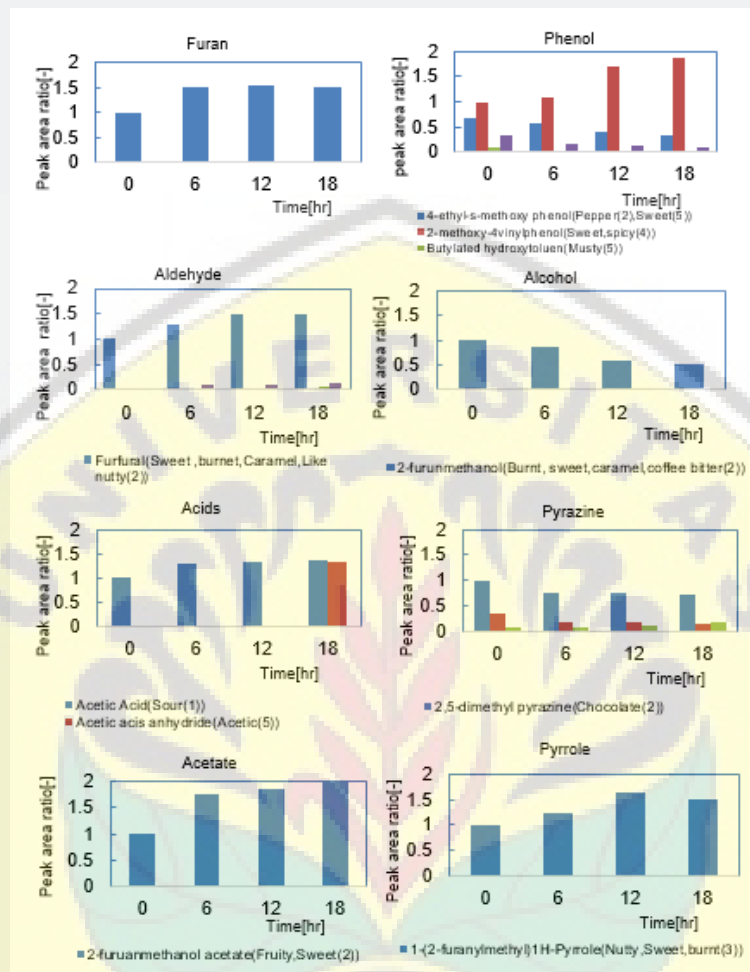


Figure 3: Change of Each Compound during Fermentation.

2-furanmethanol as alcohols decreased about till 60% of control, while Furfural in aldehydes increased by 1.5 times. The compounds included in alcohol group in this study were 2-furanmethanol. According to Caporaso et al. [22], Robusta coffee generally has 2-furanmethanol compound with high concentrations. These compounds contribute to burnt, sweet, caramel, coffee and bitter aroma in coffee [23]. Some volatile compounds included in aldehyde group in this study were furfural compounds; benzeneacetaldehyde; nonanal and 2-nonenal. These compounds contribute to sweet, burnt, caramel, nutty, fruity, and fatty aromas [20,24]. This aldehyde group was increases along with the length of fermentation. According to Dan et al. [25], aldehyde was the main volatile compounds found in fermented milk products that can increase volatility.

Benzene acetaldehyde compounds have an increasing in area along with the length fermentation. This considered as the cause that aldehyde compounds can be produced directly

from ethanol by alcohol dehydrogenase activity [25]. Benzene acetaldehyde compounds identified in the re-fermentation of Robusta coffee beans contribute to give sweet and fruity aroma [20]. It is considered that the re-fermentation of 18-hour unfermented Robusta coffee beans in commercial kefir have a fruity aroma.

Acetic acid in Acids, 2-furanmethanol acetate in Acetates and 1-(2-furanylmethyl)1H-pyrrole were increased 1.5-2 times, while Pyrazines were decreased overall. The acetate group can be identified in coffee re-fermentation was 2-furanmethanol acetate. This compound has increased along with the longer fermentation. This acetate 2-furanmethanol compound has the same contribution to the benzene acetaldehyde compound which can give the characteristics of fruity aroma [24]. According to Caporaso et al. [22], pyrazine compounds which generally identified in Robusta coffee beans were a 2-methyl-pyrazine compound; 2,6-dimethylpyrazine; 2,5-dimethylpyrazine; ethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyra-

zine and 3-ethyl-2,5-dimethyl pyrazine. According to Lee et al. [9], pyrazine compounds are formed from amino acid precursors which occur during caramelization on roasting. Methyl pyrazine compounds; 2,5-dimethyl pyrazine; 2-ethyl-6-methyl pyrazine; 2-ethyl-3,5-dimethyl pyrazine and 3-ethyl 2,5-dimethyl pyrazine contribute in aroma nutty, roasted, cocoa, chocolate and coffee in coffee [24]. The pyrrole compounds were can identified in this study were 1-(2-furanylmethyl) 1H-pyrroles and 1-(1H-pyrrol, 2 yl) ethanone. Pyrrole compounds was formed from the amino acid's degradation during the roasting process [26]. These pyrrole compounds contribute to the aroma of nutty, sweet and burnt [23]. Caffeine compounds were identified and belong to the alkaloid class. Based on the area, this compound has decreased due to the breakdown of caffeine compounds into other compounds such as caffeic acid [9]. According to Burdock [20], caffeine is a volatile compound that does not contribute to the aroma of coffee compounds. Thus, the decrease of this compound has no effect on the aroma of coffee produced [27-29].

## Conclusion

Totally 17 volatile compounds were identified in unfermented robusta coffee beans. Re-fermentation of coffee bean can enhance the pleasant aroma until 23 compounds in 37 °C for 18 hours. Some compounds group including of acid, alcohol, aldehyde and acetate groups were contributed to acidity, fruity, nutty and caramelly aroma.

## Acknowledgment

I am deeply indebted to my supervisor, Professor Hiroyuki Harada for warm support, thoughtful guidance, and for financial funding this publication.

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DOI: [10.19080/NFSIJ.2019.08.555745](https://doi.org/10.19080/NFSIJ.2019.08.555745)

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