Fermentation of Arabica coffee seeds (*Coffea arabica*) using probiotic bacteria from domestic chickens *Gallus domesticus*

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**Abstract.** The research about fermentation of coffee *Coffea arabica* using a consortium of probiotic bacteria which is a collection from the microbiology laboratory of the Faculty of Mathematics and Natural Sciences, Hasanuddin University has been carried out on. This study aims to determine the flavor of arabica coffee *C. arabica* and its chemical composition after the fermentation process. The length of fermentation time was divided into 2 times, namely 24 hours and 36 hours, fermentation using a consortium of probiotic bacteria that had been rejuvenated on coffee peel media for 2 x 24 hours. Organoleptic testing was carried out to see the panellists preference for the taste, color and aroma of coffee after fermentation. The results showed that coffee with a 24-hour fermentation time with the addition of probiotic bacteria was the most liked by the panellists with the category slightly less acidic taste, slightly black color, and normal aroma. The GC-MS test results showed organic compounds formed after the fermentation process. In the 24-hour control sample there were 14 types of organic compounds, the sample with the addition of a consortium of probiotic bacteria contained 11 types of organic compounds, the 36-hour control sample contained 11 types of organic compounds, and samples with the addition of a consortium of probiotic bacteria contained 13 types of organic compounds.

1. Introduction

Post-harvest management of coffee can be carried out in three ways, namely, wet processing, dry processing, and semi-wet processing. Wet coffee treatment has better quality than dry and semi-wet coffee. Both wet and dry processing undergo a fermentation process. Fermentation can affect the taste of coffee by the presence of microbial activity in the fermentation process that occurs [1].

The coffee bean fermentation process is a process of breaking down complex compounds in coffee beans into simpler compounds involving several microorganisms which aim to help release the mucus layer that still covers the coffee beans. This fermentation process affects the quality and taste of coffee and reduces the caffeine content of the coffee beans. In the fermentation process, microorganisms are used and one example of bacteria that can be used is probiotic bacteria. Probiotic is a term used for microorganisms that can have an effect either on other organisms or their host. One example of probiotics is Lactic Acid Bacteria which is the most widely used bacteria in the fermentation of food and beverages.

Several fermentation studies on coffee such as Robusta coffee conducted by [2] prove that using...
probiotic bacteria for fermentation in coffee will get better coffee flavor results. Fermentation carried out on Arabica coffee using lactic acid bacteria is also able to produce better taste and quality of coffee products [3]. 241 isolates from the genus Enterobacteriaceae and 46 isolates of gram-positive bacteria were found in the coffee fermentation process. In addition, 237 isolates were found from several types of lactic acid bacteria, most of which were from the Lactobacillus group [4]. Some of these studies prove that the use of a consortium of probiotic bacteria can be used to obtain a distinctive taste and aroma in coffee. Based on this, this research was conducted to determine the effect of fermentation using a consortium of probiotic bacteria from chicken intestines.

2. Methods

2.1 Arabica coffee fermentation
A total of 1.5 kilograms of coffee cherries were added to the bacterial consortium starter as much as 5% of the coffee weight. Fermentation lasted for 24 hours and 36 hours at room temperature 25 ℃ with a pH condition of 6. After the fermentation is complete, the coffee cherries are peeled and dried in the sun for 3 days. Then, the coffee bean horn skin is peeled using a huller machine. The coffee beans are then roasted at a light roast level for about 15 minutes, then mashed using a grinder.

2.2 Total organic acids test
Analysis of organic acid content using the Gas Chromatography-Mass Spectrometry (GCMS) method. The GC-MS test was carried out by means of a 0.5 ml isolate pipette and put it in a 50 mL measuring flask and diluted it with acetone then crush it to the limit mark. Pipette as much as 3 mL and put it in the vial. Instrument conditions GC-MS injector temperature 250 ℃ with the splitless method, pressure 76.9 kPa and a flow rate of 14mL / min and a ratio of 1:10. Ion source and interface temperatures 200 ℃ and 280 ℃, solvent cut time of 3 minutes, 400-700 m / z. Column type SH-Rxi-5Sil MS column length 30 m with inner diameter 0.25 mm. The initial temperature of the column is 70 ℃ with a holding time of 2 minutes and the temperature is increased to 200 ℃ at a rate of 10 ℃ / min and the final temperature is 280 ℃ with a holding time of 9 minutes at a rate of 5 ℃ / min so that the total analysis time is 36 minutes. The chromatogram data obtained were read using the NIST and Wiley 9 libraries.

2.3 The taste of Arabica coffee after fermentation test
The taste test was conducted at Just Kafe with the cuptest method. A total of 8.5 grams of ground coffee was brewed with boiling water as much as 150 cc. This test is carried out by panelists with a maximum test of 36 glasses. The taste test parameters are divided into 3 categories, namely taste, aroma, and color.

3. Results and discussion

3.1 Arabica coffee fermentation
In the fermentation results, it can be seen that there is a difference between each treatment. In the 24-hour control, the color of the coffee skin did not change and still smelled of fresh coffee, while in the fermentation with the addition of a consortium of probiotic bacteria 24 hours, it was seen that the coffee skin turned brown, softened and smelled of alcohol. At 36 hours of control, coffee skin color turned brown, softened and smelled of alcohol. Meanwhile, the fermentation with the addition of a consortium of probiotic bacteria for 36 hours showed that the coffee skin turned brown and softened but had a stronger alcohol odor.

This indicates that fermentation with the addition of bacterial isolates occurred faster than the control. This happens because bacteria help the fermentation process by turning the sugar in the coffee skin into a food source. During the fermentation process, lactic acid bacteria catabolize the pentose and hexose found in coffee meat into a large number of final metabolites such as lactic acid, acetic
acid, CO2, and ethanol, through phosphocytolase or the pentose phosphate pathway [5].

3.2 Total organic acids test
Based on the GC-MS test, these are the compounds found as shown in table 1.

| No | Compounds       | The number of gram |
|----|-----------------|--------------------|
| 1  | Furan           | 0.11               |
| 2  | Caffein         | 0.42               |
| 3  | Linoleic acid   | 0.04               |
| 4  | Stearic acid    | 0.03               |
| 5  | Acetic acid     | 0.06               |
| 6  | Chlorogenic acid| 0.11               |
| 7  | Palmitic acid   | 0.01               |
| 8  | Diamantane      | 0.03               |
| 9  | Pyrolo          | 0.003              |
| 10 | Tocotrienol     | 0.006              |
| 11 | Phenol          | 0.005              |

Table 2. 36-hour Gas Chromatography-Mass Spectrometry (GC-MS) Compound Test Result.

| No | Compounds       | The number of gram |
|----|-----------------|--------------------|
| 1  | Furan           | 0.07               |
| 2  | Phenol          | 0.008              |
| 3  | Caffein         | 0.61               |
| 4  | Linoleic acid   | 0.04               |
| 5  | Stearic acid    | 0.02               |
| 6  | Pyrolo          | 0.004              |
| 7  | Acetic acid     | 0.05               |
| 8  | Tocotrienol     | 0.005              |
| 9  | Palmitic acid   | 0.004              |
| 10 | Chlorogenic acid| 0.02               |
| 11 | Hydrocarbon     | 0.02               |
| 12 | Essential oil   | 0.03               |
| 13 | Ascorbic acid   | 0.08               |

In fermentation with the addition of a consortium of probiotic bacteria 24 hours there are 11 types of organic acids. Meanwhile, the 24-hour control contained 14 types of organic compounds. Furans, phenols, caffeine, linoleic acid, stearic acid, pyrolo, acetic acid, tocotrienols, and palmitic acid are compounds present in both samples. Hydrocarbons, essential oils, pyrazine, ascorbic acid were compounds that were only found in 24-hour control samples. Also, diamantane compounds were only found in samples with the addition of a 24 hour consortium bacteria.

In fermented coffee with the addition of a consortium of probiotic bacteria in 36 hours, there are 13 types of compounds. While the 36 hours control contained 11 types of compounds. Furans,
chlorogenic acid, caffeine, linoleic acid, stearic acid, acetic acid, and palmitic acid were the types of compounds found in both samples. Propionic acid and diamantane compounds were found in the control sample for 36 hours. Meanwhile, phenolic compounds, pyrrolo, hydrocarbons, essential oils, and ascorbic acid are the types of compounds found in the sample with the addition of 36 hours of probiotic bacteria consortium.

The differences in the compounds found in the two samples could occur due to the addition of the consortium bacteria and from the coffee roasting process. The fermentation process forms compounds such as chlorogenic acid, acetic acid, caffeine and phenol. Thus, the addition of a consortium of probiotic bacteria and the length of fermentation time caused a difference in the number of compounds formed. The coffee roasting process also affects the amount of compounds formed. During this process, the Maillard reaction or non-enzymatic browning reaction occurs so that the longer the roasting time and temperature of the coffee, the more compounds will evaporate [6].

3.3. The taste of Arabica coffee after fermentation test

3.3.1. Taste. The results of the assessment by the panelists, in the 24-hour control sample, there were 7 panelists who chose a slightly sour taste, 1 panelist chose very less acidic taste, 4 panelists chose a slightly less acidic taste, 1 chose normal taste, and 1 chose very sour whereas, in the sample with the addition The 24-hour bacterial consortium contained 5 panelists who chose a slightly less sour taste, 5 chose slightly less acidic, 2 chose slightly sour, and 2 chose very sour. In the 36-hour control sample, there were 7 panelists who chose a slightly sour taste, 3 chose a slightly less acidic taste, 3 chose normal taste, and 1 chose very sour, while in the sample with the addition of a bacterial consortium there were 5 panelists who chose a slightly sour taste, 1 chose very less acidic, 1 chose slightly less acidic, 4 chose normal taste, and 3 chose very sour.

The sour taste of coffee is influenced by the number of organic compounds formed during fermentation and during the roasting process. Acid compounds formed are acetic acid, linoleic acid, stearic acid, chlorogenic acid, palmitic acid, and ascorbic acid. The amount of content of several acidic compounds then creates a sour taste in the ground coffee sample. In addition, compounds such as furan, pyrazine, and pyridine also affect the acidity of coffee [7].

3.3.2. Flavour. In the 24-hour control sample there were 5 panelists who chose normal coffee aroma, 1 chose very less sharp, 4 chose slightly less sharp, 3 chose slightly sharp, and 1 chose very sharp while in the sample with the addition of a 24-hour bacterial consortium there were 7 panelists who chose normal coffee aroma, 1 choose very less sharp, 1 choose a little less sharp, and 5 choose a bit sharp. In the 36-hour control sample, there were 6 panelists who chose normal coffee aroma, 4 chose a bit less sharp, 1 chose slightly sharp, and 3 chose very sharp, while in the sample with the addition of a 36-hour bacterial consortium there were 5 panelists who chose a slightly sharp coffee aroma, 4 4 chose a slightly less sharp scent, 4 chose normal aroma, and 1 chose very strong scent.

The aroma of arabica coffee is known to be stronger, so that the aroma that is formed is sharper when compared to Robusta coffee. The sharp aroma of coffee in Arabica coffee gives pleasure to the connoisseur [8]. After fermentation was carried out with the addition of a consortium of probiotic bacteria at 36 hours, the aroma parameter that most panelists chose was a slightly sharp coffee aroma while in the control sample the aroma was normal. In the addition of a consortium of probiotic bacteria 24 hours, the aroma parameter that was most preferred was normal coffee aroma and control samples also had normal aroma. So, it can be seen that the fermentation time can affect the aroma that is formed in steeping Arabica coffee. Aroma is one of the important things in assessing the quality of coffee. The better the quality of coffee, the better the coffee aroma will be [9]. The aroma of coffee that is captured by the sense of smell is the result of the evaporation of volatile organic compounds [10].

3.3.3. Colour. The results of the assessment by the panelists, in the 24-hour control sample, there were 10 panelists who chose a slightly less black color of coffee, 1 chose very less black, and 1 chose very black while in the sample with the addition of a 24-hour bacterial consortium there were 7 panelists
who chose a slightly less black color, 3 chooses normal black, 3 chooses slightly black, and 1 chooses very black. Whereas in the 36-hour control sample, there were 7 panelists who chose a slightly less black color, 4 chose normal black, and 2 chose slightly black. In the sample with the addition of a bacterial consortium, there were 8 panelists who chose a slightly less black color, 4 chose normal black, and 2 chose slightly black. 

The change in the color of the coffee beans to brown begins when the coffee beans are roasted at temperatures above 100°C, there is pyrolysis of organic compounds in the coffee beans. In this study, a temperature of 198°C was used with a light roasting level so that the coffee color that was formed was brown [11]. So that the color parameter most chosen by the panelists is a slightly less black color, which indicates that the color of the coffee matches the color formed by the light roasting process. The roasting process of coffee beans affects the color of the coffee produced [12]. The longer the time and the higher the temperature used, the blacker the coffee color will be.

3.3.4. Panellist acceptance. The results of the assessment by the panelists. The results showed that coffee with a 24-hour fermentation time with the addition of probiotic bacteria was the most liked by the panelists with the category slightly less acidic taste, slightly black color, and normal aroma. Coffee samples with the addition of a consortium of probiotic bacteria 24 hours were the most preferred coffee by the panelists compared to the controls. It is characterized by a slightly less acidic taste, normal aroma, and a slightly less black color. Taste, color, and aroma greatly influence the panelist's preference for coffee. The level of consumer preference is influenced by the color, taste and aroma of coffee [13].

4. Conclusion
Based on the research it can be concluded that there are 17 types of organic compounds were found using GC-MS test and the fermentation process with the addition of a consortium of bacteria had an effect on changing the taste of Arabica coffee beans.

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