Test the quality of robusta coffee probiotic snail through different time fermentation

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Abstract A research has been conducted test the quality of robusta coffee snails probiotic through different time fermentation. The research was carried out at Gunung Kutul area Pucaksari village sub district of Busungbiu Buleleng regency Bali province on April till November 2018. The research used random samples RAL with 4 treatments and 5 replications with 20 samples. Parameters observed included physical and taste test including physical quality of water content defect content of defective value and coffe flavour, aroma, body acidity, flavour. The result show that increasing time fermentation can reduce caffeine levels and increase the acidity of coffee beans and reduce the weight of coffee beans. Rendement coffee robusta bean of wet seeds was 52.85% still relatively high and there was no contamination in the levels of impurities, foul odors and fungi. The colour of coffee beans becomes darker along with the length of time fermentation due to the increasingly optimal microbial work at the time of fermentation. In coffee fermentation treatment for 3 days appeared the most prominent formation of oleic acid as much as 50,148 ppm followed by palmitic acid as much as 36,943 ppm and stearic acid as much as 17,112 ppm where coffee fermented with 3 days was classified as specialty coffee with score 83.50 % if compare with fermentation 0 day appeared formation as oleic acid as much as 45,142 ppm, palmitic acid as much as 34,047 ppm, stearic acid as much as 15,153 ppm and value score 82.83 %. Coffee fermentation with snail microbe contain miristat acid in coffee beans. Miristat acid has anti-oxidant, anti-carcinogenic properties, source energy, decrease level cholesterol

Keywords: Coffee Beans, Fermentation, Snail Inoculant

1. Introduction
In Indonesia coffee is the second major export commodity after crude oil, where in international trade, coffee is traded in almost all countries of the world which have an important role for the economy of the community [1]. Coffee commodities are able to contribute substantial foreign exchange. According to the chairman of the coffee and industrial compartment, the Indonesian exporters and coffee industry association (AEKI), Pranoto Soenarto, Indonesian coffee production annually can reach 400,000 tons, and the income earned from coffee exports can reach USD 1.3 billion or around Rp 17 trillion [2]. Indonesia is an important coffee producing country, where BPS data in 2013 [3] shows that the area of coffee plantations in Indonesia managed by large companies is only around 47,000 ha, while the area of community coffee plantations reaches 1.2 million ha. This commodity is also very important for the lives of people in rural areas where more than 1.84 million rural families earn their living as coffee farmers. In addition, coffee commodities also provide employment for Indonesian people in the fields

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of industry and trade [4]. Commercially there are two types of coffee produced in Indonesia, namely arabica coffee and robusta coffee (Association of Indonesian Coffee Exporters and Industries (AEKI). Robusta coffee is a coffee plant that can grow at a lower altitude than the height of Arabica coffee planting, ie at an altitude of 400-800 m above sea level (Association of Indonesian Coffee Exporters and Industries [5]. Robusta coffee has seed characteristics including; coffee beans are rather round and larger, thicker seed arches, midline from top to bottom almost evenly, for seeds that have been processed, there is no epidermis in the curve [6].

Various types of processed coffee products that have been circulating in the community and are quite popular with coffee lovers, one of which is civet coffee products, but along with the development of modern biotechnology, gradually the presence of civet coffee will experience fading. On the other hand, behind the rampant tips of civet coffee connoisseurs, now for coffee connoisseurs, you can also enjoy the taste of quality coffee with specific flavors and distinctive aroma according to the taste of coffee connoisseurs, namely probiotic snail coffee. Snail is Mollussca group (soft body) has a latin name: (Achatina fulica) belonging to the tribe (Achatinidae), which is a group snails land (snails) that feed on young leaves in various types of plants and ornamental plants so that for the plant enthusiasts, the snail is considered one of the plant pests. Snails are one of the common land slugs that have a habit of living in a humid and active place at night (nocturnal). Nocturnal properties are determined by dark factors at night while also determined by the temperature and humidity factors of the environment. These soft-bodied animals forage at night by relying on ocular tentacles containing chemoreceptor cells and have light receptors in the form of ocelli located on the head used as a detection device for the environment, temperature and even in food searches.

Snail mucus contains various fatty acids including omega 3 fatty acids in the form of linoleic lemal acid as much as 30.4%, omega 6 fatty acids in the form of linolenic acid as much as 31.2% and omega 9 fatty acids in the form of Oleic fatty acids as much as 7.3% where the three types of fatty acids are very good for the health of the body [7]. In the digestive tract in the form of the liver and pancreas of this animal there are several types of microbes that can produce enzymes that have a role in the digestive system which can be used as probiotic coffee.

The aim of this research is test the quality of robusta coffee probiotic snail through different time fermentation.

2. Materials and Methods

2.1. Time and location
The research was conducted in Bali from April to November 2018 at Subak Abian Gunung Kutul, Pucaksari Village, Busungbiu Sub-District, Buleleng Regency off Robusta coffee which included: preparation, field observation, coffee harvesting and selection, post-harvest (skin stripping, fermentation, drying and turning)), rendemen test, test the physical quality and taste and analysis of fatty acid content. The harvesting and selection of raw materials in the form of Robusta logs were carried out in Pucaksari Village, Busungbiu District, Buleleng Regency. While for skin stripping activities, fermentation and yield measurements were carried out at Balitbangtan BPTP Laboratory Bali (Denpasar). To test the physical quality and taste of coffee carried out at the Coffee and Cocoa Research Center - Jember, while the analysis of fatty acids was carried out at the Testing Laboratory of the Post Harvest Center - Bogor.

2.2. Research methods Fermentation techniques
In general, fermentation is carried out on all coffee (both fermented without conch microbes and fermented with snail microbes). The raw material of the fruit (coffee) used is a type of robusta coffee which is harvested (picked) red from the selection results strictly, then peeled. Wet coffee beans that have been peeled off (at the latest 2 hours after skin release are treated with fermentation with probiotic microbes. Treatments given are follows: - k0; Wet coffee beans are fermented for 1 day without using microbes snail – k3; Wet coffee beans are fermented for 3 days with snail microbes – k4; Wet coffee
beans are fermented for 4 days with snail microbes – k6; Wet coffee beans are fermented for 6 days with snail microbes. After the fermentation ends according to the time, the coffee beans are dried in the sun to dry (moisture content < 12 %), then stripping the skin is done so that it produces dried coffee beans without epidermis (OSE).

2.3. Measurement of Management
The sought-after yield is HS / hard skin (with the epidermis) and dry beans separated from the green bean. Besides that, it also measured the yield of wet coffee beans to determine the volume of inoculant fluid used in the coffee.

2.4. Physical and Flavor Quality Tests.
The dried coffee beans produced were tested for yields, as well as the physical quality and taste which included: water content, defect value, aroma, flavor, thickness, bitterness, and acidity. In the taste test, in addition to the coffee and cocoa research center - Jember, taste tests are also carried out by coffee drinkers in order to obtain direct assessment from coffee drinkers, even if only with qualitative and subjective values related to taste. In the yield study using a completely randomized design (CRD) with 4 (four) treatments with 5 replications as 20 samples for each replication to measure the yield of green beans from wet coffee. The parameters observed in physical and taste tests include; [1] Physical quality includes water content, impurities, defect values, etc. [2] Coffee flavor includes; aroma, acidity, body, flavor etc.

2.5. Test for fatty acid content
The test series was carried out at the Laboratory of Assessment of Post - Harvest Large Crops in Bogor to determine the types and levels of fatty acids contained in fermented coffee beans with conch probiotics such as palmitic acid, oleic acid, and linoleic acid.

3. Results and discussion

3.1. Fermentation of Robusta Coffee Beans
One of the stages of the primary processing that greatly determines the quality of steeping coffee beans is fermentation [8] where at the fermentation stage a chemical event will occur which is very useful in the formation of flavor characters, namely the formation of precursor compounds of taste, such as organic acids, amino acids, and reducing sugars [9;10]. Besides that, in the fermentation process, coffee beans will occur a rendement of dried seeds process of decomposition of complex compounds in coffee beans, into compounds that are simpler by involving several microorganisms / enzymes originating from organs in living things, especially digestive organs, sewers, genital pathways, intestine pathways and respiratory pathways in animals including soft-bodied animals / molluscs / snails [11]. The role of enzymes in the digestive tract of animals is able to produce coffee with a distinctive taste and aroma [6].

Besides that with increasing fermentation time in Robusta coffee beans can reduce caffeine levels. Where according to [12], caffeine is a chemical compound alkaloid which is naturally contained in more than 60 types of plants, especially in coffee plants. [13] stated that the increase in fermentation time, the caffeine content in coffee beans will decrease. This occurs as a result of the activity of proteolytic bacteria that produce a high enough protease enzyme. [14] states that protein breakdown causes reduced caffeine levels in coffee. In addition, the ability of cellulolytic bacteria and xylanolytic bacteria inoculums derived from microbes from snails to degrade cellulose and hemicellulose contained in coffee, resulted in the breakdown of sugar content which can later affect the organic acid content in coffee beans. According to [15] the results of the sugar breakdown process are lactic acid and other acids, namely ethanol, stearic acid, and palmitate, oleic acid and linoleic acid.

On the outside of coffee beans that are like gel or mucus which consists of 80% pectin and 20% sugar [16]. The mucus layer of coffee beans containing sugar is used by the inoculum / microbe as the substrate. The reduced mucus layer causes water to enter the coffee beans more easily through the pores.
on the skin of the horn. The entry of water into coffee beans causes caffeine to dissolve. This is caused by the nature of caffeine which is easily soluble in water, according to the statement of \[17\] where caffeine is soluble in water. Caffeine dissolves in water because it can bind one water molecule. The fermentation process can also reduce the weight of robusta coffee beans. The weight of coffee will decrease both after stripping and after the fermentation process.

3.2. Rendemen of Wet Coffee Beans
From the results of the coffee fruit picking carried out in August, the yield of wet coffee beans was 51.50%, 52.55 %, 53.30 % and 54.05 % respectively, which earned an average of 52.85%. shows that the yield of wet coffee beans is influenced by the type and time of harvest where the harvest time together with the rainy season / rainfall in the busungbiu region is relatively high, causing the water content in the coffee fruit to be higher and the water deposited more in the flesh. This results in the composition of the coffee beans being smaller when compared to the coffee fruit which harvests in the dry (dry) month.

The fermentation treatment will have an effect on the yield of dried coffee beans (post-treatment). Where the coffee beans are fermented for a long time, the dry seed yield decreases even though the difference is not real. This is allegedly because in the fermentation process there is a process of decomposition of some food substances in coffee beans and some of them become simple compounds and easier to experience evaporation (table 1).

| No | Code | Dimensions of spindles (%) | Coffee Powder |
|----|------|-----------------------------|---------------|
|    |      | Wet beans                   | Dry beans with skin (HS/ hard skin) | Dry beans with skin (ose) | Dry Roasted Seeds |
| 1  | k0   | 56.75                       | 26.37 a       | 19.45 a                | 15.10 a            | 13.10 a           |
| 2  | k3   | 56.75                       | 26.34 a       | 19.43 a                | 15.01 a            | 13.05 a           |
| 3  | k4   | 56.75                       | 26.29 a       | 19.40 a                | 14.92 a            | 13.02 a           |
| 4  | k6   | 56.75                       | 26.26 a       | 19.30 a                | 15.12 a            | 13.01 a           |

From the table above shows where the composition of the skin and the water content in the fruit flesh and the skin are much higher. On the other hand, with a higher water content, the fermentation process becomes easier and rather heavy. But the yield of coffee beans will tend to be lower due to the fermentation process.

3.3 Physical Quality Test of Robusta Coffee
During the fermentation process there is an increase in the level of dissolved protein in coffee and as time increases the fermentation given causes the color of Robusta coffee beans to become increasingly darker this occurs as the impact of microbial performance in penetrating the coffee beans will be stronger with increasing length of fermentation \[18\]. Water content for treatments k0, k3, k4 and k6 were 11.8%, 12.5, 12.3% and 12.7% respectively. But in general the water content found in dried beans of Robusta coffee is still too high (above 12%). This shows that in sun-drying on Robusta coffee takes a longer time (over 12 days), as a result of robusta coffee beans larger and thicker. For the defect values obtained for k0, k3, k4 and k6 respectively 12.5 mg, 35.8 mg, 44.2 mg, 65.5 mg, while the total impurities are 0 %, or in other words there is no contamination in coffee beans (table 2). Likewise in all coffees there is no foul smell and mold.
Table 2. Physical Quality Test Results in Robusta Coffee

| No | Parameter                  | k0   | k3   | k4   | k6   |
|----|---------------------------|------|------|------|------|
| 1  | Water content (%)         | 11.8 | 12.5 | 12.3 | 12.7 |
| 2  | Impurities (%)            | 0%   | 0%   | 0%   | 0%   |
| 3  | Dejective value (mg)      | 12.5 | 35.8 | 44.2 | 65.5 |
| 4  | Pass the sieve 6,5 mm (%) | 0%   | 0%   | 0%   | 0%   |

In the size of the 6.5 mm sieve, none of the seeds escaped, this indicates that the seed size is relatively uniform from k0 to k6 and all of them belong to the large seed category. Result of the study showed that fermented coffee with snail microbe didn’t seem to effect the physical changes in coffee beans (coffee bean size) so that all fermented coffee escaped past the 6,5 mm sieve size, this is accordance with statement of [8] where the fermentation process doesn’t have a significant influence on changes in some physical properties of coffee beans, one of which is the size of the seeds. However, on the other hand, size of the seeds gives a significant influence on the loss of roast, the density of the coffee cage roast, pH of steeping, total acidity, and the characteristics of steeping body [19].

3.4. Test of Taste

From the taste test results showed that probiotic snail coffee treated with fermentation with a time of 3 days (k3) had the highest total score compared to other fermented or control coffee (k0). The total score obtained by k0 is 82.83 lower than k3 which has a score of 83.50 (table 3). This shows that coffee beans (k3) are of high quality and can be categorized as specialty coffee. SCAA [20,21] determines special coffee limits if the total score of taste results is cupping test > 80.00. While the total score in k4 and k6 coffee is 78.75 and 79.00, respectively. This indicates that along with the increase in fermentation time there will be a tendency to decrease the quality of the taste for the coffee treatment and coffee flavor score this treatment has not been classified as specialty coffee because the score is still < 80.00. These results indicate that from the test results the taste of K3 treatment gave the highest score among the treatments, this means that the time of fermentation with probiotic microbes from enzymatic from the optimal snail is 3 days. This indicates that within a maximum limit of 3 days the fermentation process will result in more intensive chemical changes, causing more organic acid, amino acid, and reducing sugar precursor compounds to form. As a result, more volatile and non-volatile flavor compounds are formed through the Maillard reaction during the roasting process so that it can influence the flavor and aroma of brewed coffee. The roasting indicates where the release of volatile compounds will occur in building the fragrance of the coffee itself [22].

From the results of laboratory analysis, the coffee and cocoa centers in East Java, Jember, show where in K3 coffee the fermentation time of 3 days arises the taste and aroma; Cocolaty (chocolate), fragrant Pandan Herbal, Caramel and Basmatik Rice, in K0 taste and aroma appear; Cocolaty, Fragrant and Flowery Herbal Pandanus, in K4 Cocolaty taste and aroma, Herbal Astringen, Coffe Pulp, Fruty and Caramel appear while in K6 taste and aroma appear; Cocolaty, Herbal Astringen and Bad After Taste. From the results of the analysis, it shows where the tastes and aromas that are most favored by consumers / coffee drinkers are in K3 coffee due to the taste and aroma that appears quite strong and distinctive taste.

Table 3. Test Results for Robusta Conch Probiotic Coffee Flavors

| No | Characteristics   | k0 (%) | k3 (%) | k4 (%) | k6 (%) |
|----|-------------------|--------|--------|--------|--------|
| 1  | Fragrance         | 7.92   | 7.75   | 7.33   | 7.50   |
| 2  | Flavour           | 7.92   | 8.00   | 7.33   | 7.25   |
| 3  | Drink After the taste | 7.75 | 7.75   | 7.25   | 7.00   |
| 4  | Acidity           | 7.83   | 7.875  | 7.17   | 7.00   |
| 5  | Body              | 7.92   | 8.00   | 8.00   | 8.00   |
| 6  | Uniformimity      | 10.00  | 10.00  | 10.00  | 10.00  |
| No | Characteristics  | k0 (%) | k3 (%) | k4 (%) | k6 (%) |
|----|------------------|--------|--------|--------|--------|
| 7  | Balancetaste     | 7.83   | 8.125  | 7.25   | 7.25   |
| 8  | Clean Cup        | 10.00  | 10.00  | 10.00  | 10.00  |
| 9  | Sweetnes         | 7.83   | 7.875  | 7.17   | 7.50   |
| 10 | Overalltaste     | 7.83   | 8.125  | 7.25   | 7.00   |
| 11 | Taint / Defect   | 0.00   | 0.00   | 0.00   | 0.00   |
|    | Final Score      | 82.83  | 83.50  | 78.75  | 79.00  |

Description:
- k0: Fermentated coffee a day without probiotics
- k3: Coffee fermented 3 days with probiotics snails
- k4: Coffee fermented 4 days with probiotics snails
- k6: Coffee fermented for 6 days with snail probiotics

On the other hand, from the results of the taste test (test) by coffee drinkers, it shows that the coffee fermented for 3 days (k3) has the highest value, followed by k0, k4 and k6. It may take several repetitions to ensure the quality level, or indeed until now there is no standard for the quality of probiotic snail coffee flavor.

4.6 Organic Acid Content

Organic acid content in coffee beans is very important because of its role as a precursor compound that contributes to shaping the quality of taste, especially for acidity components in steeping coffee [23]. In addition, organic acids also play a role in the formation of ester compounds, namely volatile compounds that form the aroma of coffee [24]. Fatty acids function as nutrients, and are a component of food, giving flavor to food. Fats provide flavor from a combination that gives a soft, delicious taste, shape and aroma to food. Fat is also a carrier of compounds of lipophilic taste caused by the presence of precursors for the formation of tastes [23].

From the results of analysis tests where the content of organic acids and fatty acids in probiotic snail coffee was detected the presence of several types of organic acids such as myristic acid, stearic acid, palmitic acid, oleic acid and linoleic acid. But overall, the acid content - the most prominent fatty acid is oleic fatty acid. The highest palmitic acid content was seen in coffee with a fermentation time of 3 days (k3), which was equal to (36, 943 ppm), followed by other fermented coffee k0, k3 and k4, each of 34,047 ppm, 35,545 ppm and 34,076 ppm. However, this result seems to be much better in 3 day (K3) coffee treatment, where the coffee fermented for 3 days oleic acid content appears to be much greater at 50.148 ppm. Where oleic acid in coffee is thought to increase the taste and aroma of coffee. These results indicate that the shorter the fermentation time given can increase the oleic acid content consumed by consumers resulting in avoidance of the risk of narrowing of arteries or coronary heart disease [7]. Besides that, the coffee beans also contain myristic acid, palmitic acid, stearic acid. This shows that there is a tendency for fermentation time to also cause a decrease in palmitic acid, stearic acid (see table 4), as well as the linoleic acid contained in coffee beans the composition of the is also affected by the time of fermentation.

### Table 4. Organic Acid Content in Fermented Robusta Coffee Beans.

| No | Types of organic acids | Content (ppm) |
|----|------------------------|---------------|
|    |                        | k0    | k3    | k4    | k6    |
| 1  | Miristat               | -     | -     | 0.105 | -     |
| 2  | Palmitat               | 34.047| 36.943| 35.545| 34.076|
| 3  | Stearat                | 15.153| 17.112| 15.743| 12.641|
| 4  | Oleat                  | 45.142| 50.148| 40.884| 45.076|
| 5  | Linoleat               | 0.657 | 0.583 | 0.402 | 0.181 |

Coffee beans naturally have precursor compounds in the form of trigonellins, chlorogenic acids, lipids, and peptides [25; 26], but at the fermentation also chemical events that are very useful in the formation of flavor characteristics, namely the formation of flavor precursor compounds such as reducing sugar are also formed, amino acids and organic acids [27]. The fermentation time can also cause
the highest formation of stearic acid in k3 treatment when compared with other treatments whose value is still below it. Where the formation of stearic acid increases with increasing time of fermentation. There is a tendency that the high stearic acid content can be considered positive considering stearic acid can play a role in the body's metabolic processes. It turns out that stearic acid is formed by adding fermentation time but in k4 and k6 coffee the composition of the acid content actually decreases due to excessive fermentation time. This is presumably because the fermentation time is too long. The results of the analysis also show that the formation of palmitic acid will be higher with the increase in fermentation time given.

An interesting thing the coffee fermentation with snail microbe contains miristat acid (0,105 ppm) has functions as an anti-oxidant which can prevent inflammation of the intestine and suppress stress so the presence of this acid can be positive and decrease level cholesterol [28]. Miristat acid is formed from the breakdown of carbohydrates by microbes in the fermentation process, especially by the ability of cellulolytic bacteria and xylanolytic bacteria inoculums derived from microbes from snails to degrade cellulose and hemicellulose contained in coffee [14]. According to [29] One of the functions of linoleic acid is to reduce the risk of stroke, reduce the risk of heart disease and diabetes mellitus. The low composition of linoleic acid is thought to be due to relatively short fermentation time, further research is needed to further determine the effect of fermentation on the content of these fatty acids. so that it can provide more relevant arguments

4. Conclusions and recommendations.

a. Rendement coffee robusta wet bean was 52.85 % still relatively high and there was no contamination in the levels of impurities, foul odors and fungi.

b. Fermentation greatly affect the yield of OC coffee beans and ground coffee but the effect is not noticeable.

c. The colour of coffee beans becomes darker along with the length of time fermentation due to the increasingly optimal microbial work at the time of fermentation.

d. Coffee fermentation 3 days appeared the most prominent formation of oleic acid as much as 50.148 ppm followed by palmitic acid as much as 36.943 ppm and stearic acid as much as 17.112 ppm where coffee fermented with 3 days was classified as special coffee with 83.50 % score when compared fermentation 0 day appeared formation of oleic acid as much as 45,142 ppm, palmitic acid as much as 34,047 ppm, stearic acid as much as 15,153 ppm and a score of 82.83 % which results are still below it.

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