Microorganism growth profiles during fermentation of Gayo Arabica wine coffee

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Abstract. Coffee is a non-alcoholic beverage that is consumed globally due to its specific flavour and functional properties. Nowadays coffee is diversified based on its coffee varieties, brewing methods, and bean processing techniques. Wine coffee is a commercial name for fermented coffee, a new coffee diversification product. Wine coffee is produced by fermenting coffee cherries for 30 to 60 days. As a new product, the process is not well studied. This research aims to explore the microorganisms’ activity and its profile growth during 30 days of fermentation. The documentation of pH and temperature, and microbial sampling during coffee cherry fermentation were conducted seven times, started at 0 days; 2 days; 4 days; 6 days; 8 days; 10 days; 12 days; 14 days; 16 days; 20 and 30 days. The results showed that pH decreases and temperature increases during fermentation. pH started from 5.0 and down to 3.9 when fermentation is terminated after 30 days, whilst the temperature slightly changes from 25°C to 30°C. The microorganism’s population shows the presence of yeast, lactic acid (LAB) and acetic acid bacteria (AAB) in wine coffee production. The yeast population increases in the mid fermentation as the cherries are damaged and provide suitable nutrition for the yeast. LAB also co-exists at a similar stage. Further research should be done, especially to understand the interaction mechanism between yeast and yeast, yeast and LAB, and yeast and AAB.

1. Introduction
Coffee is a term used to define a plant, bean, and a brewed beverage [1]. As a green bean, coffee is one of the global trade commodities from agriculture except for cocoa bean, palm oil, and tobacco. Therefore, coffee holds a strategic economic position for a national coffee producer such as Indonesia, as the green bean trade impacts national income. Based on ICO [2], Indonesia coffee production in 2020 was 12,000 (in thousands 60 kg bags green bean) and it increased up to 5.8% from 2019. As production rose, the amount of green bean exported was also significantly higher than the previous year. In 2020, Indonesia exported up to 6,727 (in thousands 60 kg bags) of green beans in all forms of varieties [3] and consumed coffee up to 5,000 (in thousands 60 kg bags) [4]. Coffee could be consumed in many ways based on its varieties, processing methods, and also its serving techniques.

Wine coffee is one of the coffee diversification products based on its processing method. To obtain wine coffee, coffee cherries are fermented for up to 30 days and they are dried with indirect sunlight during daylight and placed in a plastic bag for the rest of day. The drying is continuously repeated until the cherries have a moisture content of 20%, then it is hulled to separate the husk and re-dried until the green bean has moisture contents around 12-14% [5]. The term of wine coffee was used to describe the
wine-like aroma of its brewed drink, as resulted from its cherries fermentation. This coffee is also known as fermented coffee and it has a niche market due to its unique flavour, high prices, and uncertain processing difficulties [6]. In Gayo Highland, wine coffee used coffee cherries harvested from area 1300-1500 masl since these cherries produce wine coffee with intense quality [7]. The higher elevation level is reported to produce bigger coffee cherries and a higher amount of mucilage in coffee pulp [8]. Since wine coffee fermentation occurs without pulping the pulp, the amounts of the pulp might influence towards cupping quality of wine coffee. However, the main influence factor of wine coffee quality is the length of fermentation and the microorganism activities during the fermentation.

Coffee fermentation is a metabolic process that uses sugar or any carbohydrate sources from coffee pulp either with or without oxygen. During fermentation, natural enzymes in pulp and microorganisms are acquired from the surrounding environment. The microorganisms such as bacteria, yeast, and mould degrade the mucilage and produce enzymes, alcohols, and organic acids. Many reports stated that different coffee fermentation methods influence metabolic reactions in coffee fruit, which contribute chemical contains and cupping quality of coffee beans [9,10]. Bressani [11] stated that the use of microbial starters in wet process-controlled fermentation improves the cupping quality of coffee.

Coffee fermentation in a wet, dry or semi-dry process is usually done in 24-48 hours as spontaneous and traditional fermentation. The metabolism relies on natural enzymes and microorganisms such as yeast, lactic acid bacteria, and acetic acid bacteria [10,12]. Then during fermentation, these microorganisms grow sequentially as fit as the presence of nutrition in the surrounding environment. However, in wine coffee, since the fermentation process is different, the phenomenon that occurs might be different and it is not adequately studied yet. The information related to the types and numbers of active microorganisms during wine coffee fermentation is not found yet. Since the types and number of actives microorganisms are very related to present and local microorganisms, an intensive study related to the fermentation of Gayo arabica wine coffee should be done with aims to fulfil the information gap and to understand the microbial activity during wine coffee fermentation. Another objective of this study is to study the influence of different lengths of coffee cherries fermentation on the green bean quality of wine coffee.

2. Materials and methods
The research took place in the Laboratory of Industrial and Food Microbiology and Laboratory of Food and Agricultural Product Analysis, Universitas Syiah Kuala, Banda Aceh, Indonesia started from May to July 2021. The research was sequentially divided into three stages; started from 1) coffee cherry preparation and fermentation; 2) sampling during fermentation and 3) analysis of microbial activity during fermentation by measuring pH, temperature and, the population of microorganisms.

This research was conducted as an explorative experiment with the length of fermentation as an independent variable. Coffee cherries were fermented in 30 days and the observation was taken on designed dates.

2.1. Materials
The required materials were classified as research and analysis materials. Multi-cultivar of Gayo arabica cherry from Takengon, Central Aceh with elevation area circa 1100-1300 masl was used as materials in this research. For total cell count analysis, media MRSA (de Man Rogosa Sharpe) by Merck, GYC (glucose yeast extract calcium carbonate), MEA (malt extract agar) by Oxoid, peptone, aquadest, mannitol, glucose, lactose, sorbic acid, penicillin, chloramphenicol, alcohol 70% were used. Then the utilities were digital balance, plastic bag for coffee cherry fermentation, micro pipett, cortex, laminar flow, colony counter, oven pH, thermometer, incubator, needles and common glassware for laboratory activities.
2.2. Coffee cherry preparation and fermentation
Gayo arabica red coffee cherries were picked manually in Central Aceh Takengon. The cherries were packed in plastic bags then transported by bus to Banda Aceh approximately arrived around 7-8 hours later. The cherries then floated, sorted, cleaned from green and defect cherries, traces, leaves, and other foreign matters. The observation was done for four repetitions and it represent as sample. Each sample was weighed as 5 kg and, placed in an airtight plastic bag, and put in a dark place. When the fermentation length was fulfilled, the coffee cherries were dried and process to the green bean. The drying process of wine coffee in this research refers to Dairobbi et al [5]

2.3. Sampling during fermentation
The documentation of pH and temperature during coffee cherry fermentation were done seven times, started at 0 days; 2 days; 4 days; 6 days; 8 days; 10 days; 12 days; 14 days; 16 days; 20 and 30 days. Samples for pH and total colony measurement were obtained directly after temperature checking. Sixty g of coffee cherries were taken randomly from 4 different places in a plastic bag without stirring. Samples were placed in aluminium foil closed beaker glass for further analysis.

2.4. Analysis of microbial activity during fermentation

2.4.1 Temperature. For temperature and sampling collection, the plastic bag was opened based on the observation dates. As stirring was avoided, the temperature was recorded by placing the thermometer 4 times in different places without stirring. The result was calculated and the mean value was used as the temperature of coffee cherries during fermentation. The procedure was redone based on treatment levels until 30 days of fermentation [13].

2.4.2 pH. At every designed observation date, from 10 up 15 coffee cherries were taken from samples. The pulp of each coffee cherry was peeled and mixed with 100 ml distilled water. The liquid was mixed for 15 minutes and left for another 10 minutes. pH was measured by the pH meter with duplo replication for every sample [13].

2.4.3 Total cell counts. The existence and number of microorganisms in coffee cherry fermentation could be obtained by total cell counts. The microorganisms were placed and grew in different media. The procedure was referred with modification to [14, 15] as explained. 20 g of coffee cherries were mixed with 180 ml peptone 0.1%. These combined materials were homogenized with vortex for 3 minutes. One ml sample was then diluted with radiant dilution in peptone liquid 0.1%. Then each 0.1 ml aliquot was placed and streaked in specific media, where lactic acid bacteria (LAB) were placed in media de Man Rogosa Sharpe (MRS) agar and added 0.2% sorbic acid and 100 mg/l cycloheximide. Acetic acid bacteria (AAB) was streaked in Glucose Yeast Extract Calcium Carbonate (GYC) media which contained 0.1% cycloheximide and 50 g/l penicillin. As for the yeast, it was placed in Malt Extract Agar (MEA) under anaerobic condition. The inoculated media was then incubated at 37°C for 48 hours and then counted for its total cell counts. The steps were repeated three times for each observation a sample.

3. Results and discussion

3.1. Changes in temperature and pH during fermentation
Temperature and pH could be used as an indicator of microbial activity during fermentation. Muzaifa et al [13] explained that the changes in pH during fermentation could be caused by microbial activity and could influence the quality of the fermented product. The observed pH and temperature in 20 days of coffee cherries fermentation are presented in Figure 1.

The observed pH of coffee cherries in 30 days fermentation was started at 5.0 in the beginning and slightly downed to 3.9 in the final days. pH was observed to has a steady decline started from the first
days to 12 days of fermentation at the level of 3.9. But then it slightly climbed to 4.1 on the twentieth day before recorded back to 3.9 on the thirty days of fermentation. The declining trend of pH or acidity levels explains that the microorganisms' presence and degrades the coffee pulp. Silva et al. [16] confirm that microorganisms ferment the pectinaceous sugars of coffee fruits into ethanol and several types of carboxylic acids such as lactic, butyric, and acetic. Pulp degradation, as well as microbial metabolites, are commonly present in form of acid compounds [10], which makes the downtrend of pH during the first 10 days of fermentation. Later, Coughland and Mayer [17] also mention that several types of enzymes are generated by Bacillus species, which possibly participate in cellulose and pectin degradation in coffee cherries. Therefore, Bacillus species might be present in this wine coffee presentation and should be further investigated.

As there are clear differences between fermentation in wine coffee processing and wet or semi-wet processing, the phenomenon during fermentation should be different as well. In wet or semi-wet processing, the pulp is removed before fermentation and the fermentation is done approximately 24 to 48 hours. On the other hand, in wine coffee processing, there is no pulp removal prior to fermentation and the fermentation could be prolonged from 30 to 60 days. Therefore, in wine coffee processing, the microbial activity might be slower than wet processing since the pulp is present in whole fruits and has not been damaged. As predicted, as pectinaceous sugars present in larger amounts, the yeast and acid bacteria have a dominant contribution in this research.

Figure 1. Change of temperature and pH during 30 days coffee cherries fermentation in wine coffee processing.

The observed temperature in wine coffee processing started at 28°C where the plastic bags are placed at room temperature. The temperature steadily increased to 30.58°C on the sixth day before down to
29°C on the next two days. Then from eight to sixteen days, the temperature is steady at 29°C before it climbed to 30°C on twenty and thirty days of fermentation. During the first 6 days of fermentation, the temperature trend increases due to the yeast is started to break down the sugar in coffee fruits and changes it into alcohol. Then the presence of alcohol promotes the growth of lactic acid bacteria. As Abubakar et al [18] state that increasing fermentation led to increasing the rate of fermentation. This phenomenon is commonly signed by increasing temperature.

From 8 to 16 days of fermentation, the yeast and lactic acid bacteria are significantly increased roughly in similar numbers. Both of these microorganisms might have antagonistic interactions toward each other. As the pulp degrades the yeast also increases and produces more alcohol [19]. Alcohol then contributes to the growth of lactic bacteria but acts as a limiting factor of the yeast itself [13]. Therefore, the rate of fermentation is declining during these days but then start to working again on the sixteen days onward as the fermentation temperature is climbing up again.

3.2. Total population of microorganisms during fermentation
Information related to types of active microorganisms during fermentation is important to understand the activities and their impact on product quality. Fermentation in wine coffee is classified as traditional and spontaneous fermentation since no starter is added. Therefore, only three common microorganisms active in microbial fermentation were counted its growth during fermentation, which is LAB, AAB, and yeast as presented in Figure 2.

![Figure 2](image-url) Change of microorganism’s population during 30 days coffee cherries fermentation in wine coffee processing. LAB= Lactic Acid Bacteria; AAB: Acetic Acid Bacteria.

The pattern of yeast growth profile in wine coffee fermentation is very distinctive since the fermentation occurs in the forms of coffee cherries. Dairobbi et al [5] stated that coffee cherries fermentation is contributed to the formation of a wine-like aroma. During wine coffee fermentation, yeast degrades sugar in coffee fruits and changes it into alcohol. The growth was up and down during 30 days of fermentation. At the beginning of fermentation, yeast was documented to have the highest population amongst others, around 2.8 x10⁶ CFU/g. This shows that yeast is present in the cherries as indigenous microbes in larger amounts rather than other microorganisms. The growth profile of yeast is relatively steady in the first 8 days of fermentation, approximately around 2.80-3.30x10⁶ CFU/g, then it...
steady climbs up to 9.65 x10^6 CFU/g on the 12 days of fermentation and surprisingly goes down on 16 days which later rises to 22.15 x10^6 CFU/g on 20 days.

To have a better understanding, a comparison to the growth profile of yeast during wine production is explained here. Apiculate yeast is mostly present in mature and ripe grapes. As the skin and grapes damages, the nutrients availability increases and promotes a greater population of different yeast. These growth produces different kinds of metabolites, which either toxic or supportive to other species. Then by mid-fermentation, these types of yeast decline and die off, replace by Saccharomyces cerevisiae, and continues the fermentation until its completion [19]. As fermentation in wine coffee started with the red cherries, this explanation is aligned with the growth profile of yeast in wine coffee fermentation in Figure 3, which is dominant in the early fermentation and after 20 days of fermentation.

As yeast generates alcohol which considers as disinfectant for its growth, it also promoted the growth of other species such as lactic acid bacteria (LAB). Then oxygen, alcohol and lactic acid will generate and support the growth of acetic acid bacteria (AAB). Fleet Graham [19] stated that the large amount of yeast biomass produced during fermentation will die and autolysed, releasing amino acids and vitamins that may encourage the growth of other species later in the process such as LAB. In Figure 2, LAB is hand-in-hand exist with yeast and acetic acid bacteria which develop in a similar environment. After 20 days of fermentation, yeast and LAB population was declined since most of the pulp was destroy and lysis their cell walls. It can be showed by the amount of water in plastic bags in 20 up to 30 days of fermentation.

Fleet and Graham [19] also mentions that in terms of wine production many factors affect its microbial ecology, but the most significant factor is the chemical composition of the grape juice used as raw material. As it might be the potential factor is wine coffee, the chemical composition of coffee cherries before fermentation and during fermentation should be examined in further research, as it is not included in this research. Moreover, as the fermentation time is longer up to 30 days, the interaction might be occurred and contribute to wine coffee microbial ecology. Longer time fermentation led to complex microbial ecosystems. The fermentation might be promoting the growth of different species and strains. As the fermentation process prolongs, the interaction between these microorganisms might occur between the yeast itself or yeast to yeast interaction and yeast interaction with acid bacteria [19]. Therefore, the intensive research should be done further to understanding the wine coffee fermentation ecology and its impact in wine coffee quality.

4. Conclusions
Taking everything into consideration, it might conclude that during the fermentation of wine coffee, yeast, lactic acid bacteria and acetic acid bacteria are present. The changing temperature and pH during fermentation indicate the microbial activity during the 30 days of wine coffee fermentation. Yeast provenly acts as dominant microorganisms in the beginning and after 20 days of fermentation. The yeast metabolites promote the growth of other microorganisms such as LAB and AAB. However, further intensive research on microbial identification should be done to understanding the fermentation ecology of wine coffee.

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