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RESEARCH ARTICLE

Quality of Tea Fermented Drink from Natural Fermentation of Banana (*Musa paradisiaca*) and Fuji Apple (*Malus domestica)*

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ABSTRACT

Bananas and apples are fruits that are easily found in Indonesia. Those fruits are sometimes wasted during peak seasons. This research aimed to increase the shelf life, the additional value of those fruits by natural fermentation, and used them as a starter for fermented tea drinks to provide probiotic beverage which contains beneficial lactic acid bacteria. Twenty percent of bananas and apples fruit cuts (1:1 ratio) were put in a jar and were immersed in a 5% sucrose solution at room temperature (27°C). The solution was stirred daily and harvested after 72 hours of fermentation. Results showed that the resulting starter contained 2.9 x 10^{11} CFU/mL lactic acid bacteria with 83 + 8.5% lactic acid content and a total of 1.7 x 10^{12} CFU/mL microbes. The pH of the starter was 3.7 + 0.1. Microscopic observations showed that most microorganisms in the starter were yeast and Gram-positive bacteria (rod and sphere). The starter was then diluted into tea drinks sweetened by honey 9% with four types of dilution ratios, namely a ratio of 1:5, 1:10, 1:15, and 1:20 (tea: starter). The organoleptic tests on 20 untrained panellists showed that the most preferred tea was the one with 15 times dilution in terms of taste, aroma, and colour. The number of lactic acid bacteria in fermented tea with a 1:15 ratio was 1.8 x 10^{10}, which still met the requirements of a probiotic drink.

KEYWORDS: *Apple, Banana, Fermentation, Lactic acid bacteria, Tea*

Introduction

Probiotics are additional food in the form of live microorganism cells that benefit the host body when consume in adequate amount (Koirala & Anal, 2021; Küçükgöz & Trząskowska, 2022), because they balance the presence of intestinal microflora (Wulansari et al., 2022; Gul and Durante-Mangoni, 2024). Probiotics can improve the immune system (Maldonado et al., 2019), help absorb nutrients (Yuan et al., 2024), reduce the risk of various cancers (Pino, 2020), and treat diarrhoea symptoms (Shafi et al., 2019; Bodke & Jogdand, 2022; Maftei et al., 2024). Some authors stated that fermented foods also beneficial in reducing the risk of cardiovascular disease and type 2 diabetes (Saryono et al., 2023). The probiotic bacteria contained lactic acid bacteria (LAB) (de Simone, 2019; Y. Wang et al., 2021; Zielińska et al., 2018).

Generally, probiotic drinks are made from milk because the ingredients can increase nutrition in the body (Koirala & Anal, 2021). Examples of products include acidophilus milk, yogurt, dahi, Bulgarian milk, and Yakult (Utami, 2018; Bintsis & Papademas, 2022). Unfortunately, 70% of the Asian population experiences lactose intolerance (Rahmawati et al., 2021; Akalın et al., 2024) and cholesterol sufferers are increasing (Rahmawati et al., 2021). Probiotic drinks made from milk are also relatively expensive, so plant-based

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probiotic drinks can be a more affordable alternative to healthy drinks (Rahmawati et al., 2021).

According to Pontonio et al. (2019), costumer preferences on ready to drink juice drink was increased in the last decade globally. They also prefer products that are free of chemical additive, but safe to consume (Plessas, 2021). Fermentation seems to be a method that offers a solution for consumers' demands and preferences. It is a low-cost and sustainable technology (Yuan et al., 2024), which also offer shelf-life extension of fruit products (Urbonaviciene et al., 2015; Paramithiotis et al., 2022). According to Kiczorowski et al. (2022), fermentation also results in increasing added value, nutrition, flavour, and product variety. It also degrades toxic compounds, create new active compounds, and retain nutrition values (Mojikon et al., 2022; Saud et al., 2024).

It is understandable that many authors stated that probiotic-based foods and beverages are considered as one of the future food products technologies (Yuan et al., 2024). The use of fruit or vegetables can increase antioxidant activity because vegetable products generally contain phenolic compounds, carotenoids, and glucosinolates, which have nutritional value (Pereira et al., 2017; Arias et al., 2022) and are also an essential source of antioxidants (Yahia, Celis and Svendsen, et al., 2017; Purkiewicz and Pietrzak-Fiećko, 2021; Arias, Feijoo and Moreira, 2022).Thus, plant-based probiotics have significant potential acceptance due to their low milk allergen content, economical, vegan friendly, low calories, and low cholesterol (Rahmawati et al., 2021; Domínguez-Murillo & Urías-Silvas, 2024).

Fermentation can be carried out by natural fermentation (Skowron et al., 2022), in which the process takes place with native bacteria, which lead to the growth of various lactic acid bacteria (Saud et al., 2024), yeast, and fungi (Rezac et al., 2018). According to Domínguez-Murillo & Urías-Silvas (2024), it is commonly accepted that at least 10^6 to10^7 CFU/mL of probiotics must be present in the final product at the time of consumption (Rasika et al., 2021).

Bananas are tropical fruits that are abundant in traditional Indonesian markets. Indeed, banana is the fruit that is in the first rank of Indonesian fruit productions (Badan Pusat Statistik, 2022) and Indonesia is the third largest banana productions in the world (Kurniati et al., 2022). However, bananas are prone to spoilage due to its high sugar and water content (Yuan et al., 2024) although they contain 1% inulin, a prebiotic in nature, which can promote the growth of probiotics (Arum Dalu et al., 2019),

One of the reasons for consumer's reluctancy to consume banana is due to its sweet astringent flavour (Damayanti et al., 2023). In this research, apples were added to mask the banana flavour. Both bananas and apples contain glucose, fructose, and sucrose which can be used as a carbon source for lactic acid bacteria (LAB) (Jibladze et al., 2024).

Tea is a very popular drink in Indonesia, and many people start to consume as part of their daily life due to people's awareness on health benefits of tea (Cita, 2023). The popularity and development of tea processing was marked by the tea houses, cafes, and shops that become trend among young people (Cita, 2023). Due to this reason, this study aims to investigate the possibility of banana and apple natural fermentation mixed with tea drink as a new probiotic product, which contains LAB which are known to be beneficial for human health. It is also hoped that the shelf life of bananas and apples can increase by fermentation (Yuan et al., 2024).

Materials and Method

Materials

The materials used in this research were: Cavendish bananas and Fuji apples, which were purchased from traditional markets in Salatiga, distilled water, honey (Madu TJ Brand), tea leaves (Tongtji Brand), NaOH (Merck, Germany) 0.1 N, mineral water, Man Rogosa Sharpe

Agar (MRSA) medium (Merck, Germany), Total Plate Count (TPC) medium (Merck), 0.1 M NaCl (Merck, Germany), phenolphthalein indicator (Merck, Germany), Gram stained (Merck, Germany).

Instrumentations

Instrumentations used in this research were: pH meter (Horiba, Japan), autoclave (Hirayama, Japan), oven (Memmert, Germany), titration equipment, scales (Sartorius, Germany), microscope (Olympus, Japan).

Preparation of Starter (Rahardjo & Sihombing, 2023)

Starter was made by selecting the banana and apple (ripe, fresh, and not wrinkled), then they were rinsed lightly to remove any dirt or insect. The fruits were then cut into small pieces. The size reduction is necessary to expand the fermentation surface area, which eventually will increase the release of food sources for the fermentation. Twenty percent of fruits cuts (bananas and apples (1:1 ratio)) were put in a jar and were immersed in a 5% sucrose solution at 27°C. The ratio of water to fruit was 2:1. The jar was then closed and left to ferment at room temperature (27°C). The solution was stirred daily and harvested after 72 hours fermentation.

Preparation of Fermented Tea

The ratio of starter and tea were 1:5, 1:10, 1:15, and 1:20, with the addition of honey at 9% of the total product volume, respectively. The Tea drink was made by brewing 3 grams tea leaves with 220 mL boiling water (90°C) for 5 minutes.

Measurement of Total LAB (Rahayu & Setiadi, 2023)

The LAB counted using the pour plate technique. One mL sample was added to 9 mL of sterilized water, mixed using vortex (dilution of 10^{-1}). Furthermore, serial dilutions were made up to 10^{-11} dilutions. One mL of the sample from 10^{-11} was put into a sterile petri dish (Duplo), then MRSA (cooled to 45°C) was added. The cultures were stored in incubator for 48 hours at 37°C.

Measurement of Total Microbes (Arifan et al., 2019)

One mL sample was added to 9 mL of sterilized water, and then the sample was mixed using vortex (dilution of 10^{-1}). Furthermore, serial dilutions were made up to 10^{-12} dilutions. One mL of the sample from 10^{-12} was put into a sterile petri dish (duplo), then TPC (cooled to 45°C) was added. The technique used for total microbes' counts was pour plate method. The cultures were stored in incubator for 48 hours at 37°C.

Gram Staining (Paray et al., 2023)

The staining technique was performed in four steps: (1) staining with crystal violet, (2) iodine mordant to bind the dye, (3) decolourization with 95% ethyl alcohol, (4) counter staining with Safranin. At first, the prepared smear was added with crystal violet for 1 minute (Paray et al., 2023), then washed by aquadest, and then Gram's iodine was added for 1 minute, and washed again by aquadest, and ethyl alcohol 95% as decolourizer was added for 10 seconds. The prepared sample was washed again. After that, the prepared smear was stained by counter staining for 1 minute, and was washed and finally allowed to dry. The sample was then observed on microscope using oil immersion lens with 1000x magnification. The Gram test was positive when the cells were purple and the Gram test was negative if the cells were red.

pH Measurement (Dari et al., 2021)

pH testing was performed using pH meter. At first, the pH meter was calibrated using buffer solution at pH 4 and pH 7. After calibration, the pH meter was dipped in sample until it appeared indicator number on the pH meter screen that showed the pH value of the sample. Measurement was performed in triplicate.

Lactic Acid Level (Touret et al., 2018)

Acid-base titration method was used to measure the total acid (titratable acidity) of the sample. Twenty mL sample was in an Erlenmeyer flask and dissolved with two times the volume of distilled water, then 2-3 drops of phenolphthalein indicator (1%) was added to the solution, followed by titration with 0.1 N NaOH until a pink colour is formed. Measurements were performed in three replicates. The formula for calculating total lactic acid was as follow:

$$total\ lactic\ acid = \frac{Volume\ (NaOH) x\ NaOH\ (N) x\ Acetic\ acid\ (MW)\ x\ Dilution\ Factor}{volume\ of\ sample} x\ 100\%$$

N: normality of NaOH, MW: molecular weight

Sensory Test (Nugerahani et al., 2024)

The sensory test was carried out by 20 untrained panellists using hedonic scale scoring methods. Fermented tea drink (about 15 mL) was served in a small plastic cup and encoded with three random numbers. Panellists were asked to evaluate of colour, taste, and aroma of the fermented tea drinks with 5 point hedonic scale, i.e. 1 = dislike very much, 2 = dislike moderately, 3 = neither like nor dislike, 4 = like moderately, 5 = like very much.

Results and Discussion

Microscopic observation on the starter showed that most microorganisms were yeast and Gram-positive bacteria. Only a few were Gram-negative bacteria. Most of the Gram-positive bacteria were in the shape of spheres and rods. It can be assumed that most of the bacteria are Lactobacillus, which is Gram-positive, non-spore-forming, and rod-shaped (Ibrahim, 2016). Gram-positive bacteria stain blue/purple when applied with the Gram stain due to thick cell walls. Figure 1 showed the result of Gram staining of the starter.

Based on chemical and biological tests, the starter from fermented bananas and apples had 83% of lactic acid. The chemical and biological characteristics of the starter can be seen in Table 1. Lactic acid is produced from the fermentation of LAB, which grows naturally using sucrose, fructose, and glucose in bananas and apples. According to Zheng et al. (2024), the use of sugar causes chemical changes in the fermentation, resulting in a decrease in pH, an increase in acidity levels, and the formation of aroma in the product.

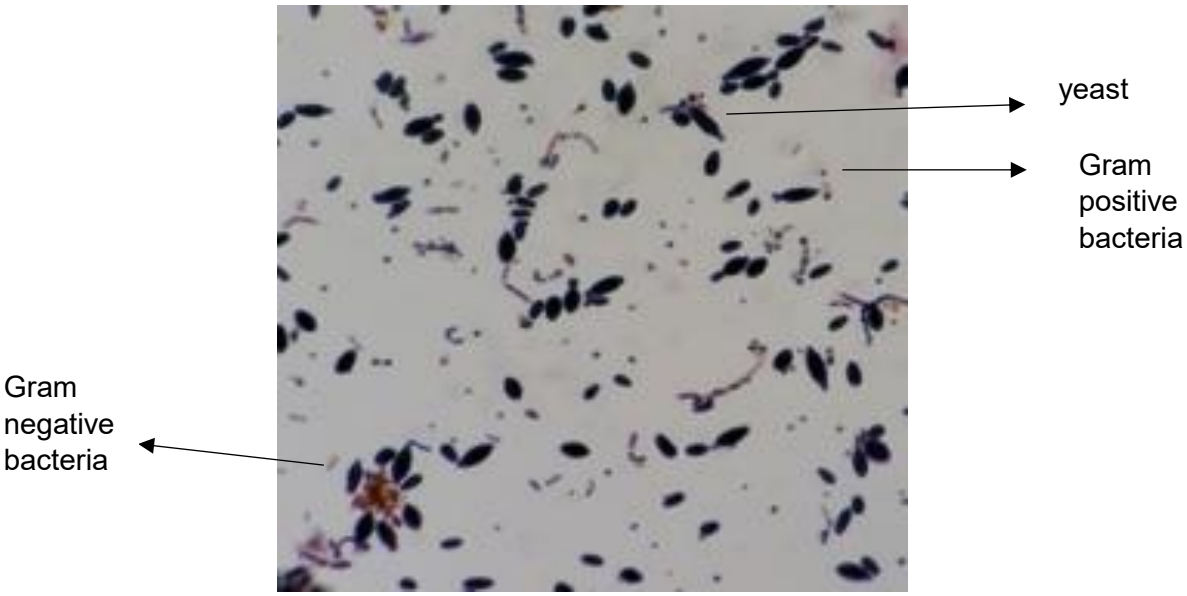

Figure 1. Microscopic display of Gram-stained starter with 1000 times magnifications.

The lactic acid levels obtained were relatively high compared to cheese whey probiotic drinks, ranging from 0.2-0.5%, with a maximum incubation of 36 hours (Nurhartadi et al., 2018). In this research, incubation was carried out for 72 hours so that the high levels of lactic acid obtained indicated that the bacteria were still in an active state carrying out the fermentation process. The level of lactic acid in a product is influenced by temperature and incubation time (Nurhartadi et al., 2018; Utami, 2018; Rahmawati, et al., 2021).The pH value in this study (pH 3.7) was also much lower than the cheese whey probiotic drink (Nurhartadi et al., 2018). A decrease in pH in line with an increase in lactic acid levels. It is caused by the accumulation of lactic acid, which is the main product of lactic acid bacteria (Li et al., 2022). The longer fermentation time will increase the presence of organic acids due to microbial metabolism (Puspaningrum, et al., 2022). Probiotic drinks with low pH are safe to consume, with an average shelf life of 1 month (Utami, 2018).

Table 1. Biological and chemical characteristics of the starter after 72 hours of fermentation at room temperature (27°C).

No.	Parameter	Value
1.	pH	3.7 + 0.06
2.	Lactic acid	83 ± 8.5%
3.	Total LAB	2.9×10^{11} CFU/mL
4.	Total Plate Count	1.7×10^{12} CFU/mL

Microbiological observation showed that the number of LAB in the starter was 2.9×10^{11} CFU/mL, and the total number of microbes was 1.7×10^{12} CFU/mL. Some studies stated that numerous bacteria and fungi are involved in spontaneous fermentation (Selvanathan & Masngut, 2023). This is in line with the results (Table 1) that shows the total microbial growth in the TPC medium are much more numerous than in LAB bacteria, i.e. 1.7×10^{12}CFU/mL.

Table 2. Number of lactic acid bacteria and pH value of fermented tea drink in several dilutions.

No.	Dilution	LAB counts (CFU/mL)	pH
1.	1:5	4.8×10^{10}	4.9 ± 0.03
2.	1:10	2.6×10^{10}	5.5 ± 0.02

3.	1:15	1.8 x 10^{10}	6.0 ± 0.02
4.	1:20	1.3 x 10^{10}	6.3 ± 0.03

The naturally obtained starter was then mixed with tea as a fermented drink. In this research, dilution of tea was mixed with a ratio of 1:5,1:10. 1:15 and 1:20. Table 2 showed the number of LAB in fermented tea drinks that underwent dilution (1:5), (1:10), (1:15), and (1:20). The amount of LAB obtained met international standards for probiotic drinks, namely at least 10^7 cells (Davidson et al., 2000). Spontaneous fermentation causes the growth of other microbes that are not LAB, which can grow in acidic conditions (Rezac et al., 2018). Studies showed that the first microorganism in natural bread fermentation was yeast (Yuan et al., 2024), *Lactobacillus* and *Acetobacter* was found in yoghurt and kimchi (Yuan et al., 2024). There is no consensus how many probiotic microorganisms that needed to be present in the probiotic drinks (Rasika et al., 2021). However, some researches indicated that at least 10^6 to 10^7 CFU/mL viable probiotic cells requisite in final product (Rasika et al., 2021). The present of mixed microorganisms also helps the high number of LAB counts. According to Wang et al. (2022), single fermentation results in lower number of viable bacteria compared to mixed fermentation condition in pear juice fermentation. Table 2 shows that LAB counts meet to the requirements, and the number of LAB become lesser due to dilutions. The results showed that Fuji apple juice was a good fermentation substrate for lactic acid bacteria.

Several studies state that probiotic drinks help the digestion of nutrients. Thus, tea drinks mixed with a natural starter of apples and bananas have the potential to be a food that has health benefits. Generally, LAB produces short-chain organic acids with anticarcinogenic properties (Rahmawati et al., 2021).

The pH value of fermented drink increases as the dilution increases. The pH value is an important factor for LAB growth. According Yuan et al. (2024), the most suitable condition for lactic acid fermentation is pH <4. This is in line with the results shown in Table 2, the lower the pH the higher the LAB counts. The low pH value is obtained from organic acids produced during fermentation (Wahyudi et al., 2023).

Sensory testing showed that panellists preferred sample formulation with a starter-to-tea ratio of 1:15. The sour taste resulting from the fermentation process limits the level of consumer preference. Table 3 showed that the highest score for sensory assessment of tea's color, taste, and aroma parameters was subjected to fermented tea drink with 1:15 ratio. The fermentation results produce a distinctive aroma from volatile organic acids produced during the fermentation process (Anggraeni et al., 2021; Dwiloka et al., 2024).

Table 3. Organolectic test of fermented tea drink at various dilutions.

Starter:tea	Color	Taste	Aroma	Score
Ratio 1:5	3.3 ± 0.6	3.1 ± 0.7	2.6 ± 0.8	4
Ratio 1:10	3.0 ± 0.9	3.1 ± 0.9	2.8 ± 0.7	3
Ratio 1:15	3.3 ± 0.7	3.3 ± 0.8	2.8 ± 1.0	1
Ratio 1:20	3.1 ± 0.7	2.9 ± 0.9	2.9 ± 0.8	2

Conclusion

Based on the research conducted, the results of organoleptic tests on fermented tea drinks from bananas and apples can be accepted as probiotic drinks by the panelists. The panelists preferred probiotic tea using a 1:15 dilution formulation. The fermented tea drinks met the amount of LAB required as a probiotic drink.

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Conflict of Interest

The authors have no conflict of interest to declare.

Author Contributions

Regina N. Budi, Rafaelle Jovanka, and Bintang Carouline carried out the experiment. Karina B. Lewerissa and Monika Rahardjo conducted the research method, wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

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