BIODIVERSITAS
Volume 24, Number 12, December 2023
Pages: 6440-6446

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d241205

Lactic acid bacteria viability and antibacterial activity of Etawa goat's synbiotic fermented milk

SAMSUL RIZAL[1,♥], SUHARYONO[1], ERDI SUROSO[1], ATRI MELATININGSIH[2], VIRDA AULIA SUYARTO[2]

[1]Department of Agricultural Product Technology, Faculty of Agriculture, Universitas Lampung. Jl. Sumantri Brojonegoro No. 1, Bandar Lampung 35145, Lampung, Indonesia. Tel./fax.: +62-813-68061924, ♥email: samsul.rizal@fp.unila.ac.id

[2]Graduated Program of Agricultural Product Technology, Faculty of Agriculture, Universitas Lampung. Jl. Sumantri Brojonegoro No. 1, Bandar Lampung 35145, Lampung, Indonesia

Manuscript received: 30 September 2023. Revision accepted: 13 December 2023.

Abstract. *Rizal S, Suharyono, Suroso E, Melatiningsih A, Suyarto VA. 2023. Lactic acid bacteria viability and antibacterial activity of Etawa goat's synbiotic fermented milk. Biodiversitas 24: 6440-6446.* The aim of this research was to determine the effect of kepok banana peel extract and white ginger extract (*Zingiber officinale* Var. *Amarum*) on the lactic acid bacteria viability and the antibacterial activity of Etawa goat's synbiotic fermented milk. This study used two treatments and three replications. The first treatment was the concentration of kepok banana peel extract (0, 1, 2, and 3% (v/v)) and the second treatment was the concentration of white ginger extract (0, 1, 2, and 3% (v/v)). All treatments were incubated at 37°C for 48 hours. The characteristics of Etawa goat's synbiotic fermented milk was evaluated by total lactic acid bacteria (LAB), total lactic acid, pH value, and antibacterial activity against pathogenic bacteria, *Salmonella typhimurium*. The data were analyzed using analysis of variance (ANOVA) and Least Significance Different (LSD) at 5%. The results showed that kepok banana peel extract had a significant effect on increasing total LAB, total lactic acid, pH value, and antibacterial activity. Meanwhile, white ginger extract had no significant effect on total LAB, total lactic acid, and pH but had a significant effect on the antibacterial activity. The addition of 3% kepok banana peel extract and 3% white ginger extract produced the best Etawa goat's synbiotic fermented milk. The best treatments produced Etawa goat's synbiotic fermented milk with a total LAB of 9.13 Log cfu/mL, a total lactic acid of 0.82%, 3.41 pH, and antibacterial activity against *Salmonella typhimurium* with a 16.33 mm diameter clear zone.

Keywords: Etawa goat's milk, kepok banana peels, prebiotic, symbiotic, white ginger

Abbreviations: LAB: Lactic Acid Bacteria, FOS: fructooligosaccharides

INTRODUCTION

Busy and fast-paced lifestyles encourage the development of beverage products that have multiple positive impacts on health. Among them are fermented functional drinks which contain nutritional and non-nutritional components that can provide benefits to the body. Functional beverages not only fulfill nutritional intake and maintain health, but also help physiological health and ensure the safety of daily consumption (Konuray and Erginkaya 2017; Markowiak and Slizewska 2017). They may contain probiotics that provide additional benefits. Probiotics are live microorganisms that have a beneficial effect on consumers by improving the gastrointestinal microflora (Konuray and Erginkaya 2017). As consumer demand for functional beverages that are safe, healthy, and highly nutritious increases, research on functional drinks with probiotics is also increasing (Konuray and Erginkaya 2017). Several functional beverages contain probiotics derived from various strains of lactic acid bacteria (LAB), especially *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* genera (Aleta et al. 2020).

In functional beverages, probiotics can be combined with prebiotics to improve their health properties. Prebiotics are non-digestible food ingredients that benefit the body by selectively stimulating the activity of colon-dwelling bacteria (Konuray and Erginkaya 2017). Prebiotics support probiotic growth by acting as a substrate in fermentation process, which will produce antimicrobial substance against pathogenic bacteria in the colon. Prebiotic compounds can be obtained from natural materials, such as oligosaccharide compounds found in banana peels. Based on research conducted by Kurtoğlu and Yildiz (2011), almost 33% of the sugar component in banana peel extract is fructooligosaccharides (FOS). The FOS content in kepok banana peels is high enough to be used as a source of prebiotics. At the same time, it can act as an effort to utilize kepok banana peel waste.

The combination of probiotics and prebiotics is called synbiotics. The probiotic agents, such as lactic acid bacteria, will still be alive and active when they reach the digestive tract (Konuray and Erginkaya 2017). Meanwhile, the prebiotics agent will not be digested in the upper digestive system but are able to function as fermentation substrates for probiotics in the colon. Examples of synbiotic products that have been developed are synbiotic beverages made from kepok bananas (Jouki et al. 2021) and green grass jelly leaves (Rizal et al. 2020b). Synbiotics containing kepok banana peel extract can be combined with other food ingredients that can increase their nutritional

value and efficacy. For example, the combination of kepok banana peel extract with the highly nutritious Etawa goat's milk and antioxidant-rich spices might be viable.

According to Larassati et al. (2023), Etawa goat's milk is included in livestock milk which has good nutritional content but is not widely used optimally in making processed products. Etawa goat's milk can be utilized as raw material for making synbiotic fermented milk due to its high digestibility, beneficial chemical composition, and low allergenicity (Sumarmono 2022). Fermented beverages from Etawa goat's milk, such as kefir and yogurt, are popular and widespread in Indonesia. However, the drawback of Etawa goat's milk is its unpleasant aroma. Therefore, it is necessary to add compounds that can reduce unpleasant aroma while improving the functional properties of the synbiotic fermented milk. The unpleasant smell of Etawa goat's milk may be reduced by adding a small amount of white ginger extract, which contains refreshing essential oils. Additionally, white ginger can inhibit the growth of pathogenic bacteria such as *E. coli*, *S. typhi*, and *S. aureus* (Handayani et al. 2018).

The research on utilizing banana peel FOS as a prebiotic in synbiotic beverages is emerging (Martharini and Indratiningsih 2017). FOS needs to be added in appropriate concentrations to produce the best functional characteristics of beverages (Kurtoğlu and Yildiz 2011). Meanwhile, research on adding ginger to fermented beverages has been carried out by Hakim et al. (2021), who added red ginger to goat's milk kefir in 1.5, 3, 4.5, and 6% concentrations. The concentration of ginger extract added to functional beverages will affect the synbiotics beverage characteristics (Rizal et al. 2020a). If the concentration of ginger extract is too high, clumping will occur in the resulting beverage. Therefore, it is necessary to study the effect of adding kepok banana peel extract and white ginger extract at various concentrations to obtain a synbiotic fermented goat's milk with the best characteristics.

MATERIALS AND METHODS

Study area

The main ingredients used in this research were Etawa goat's milk (*Capra aegagrus hircus*) obtained from an Etawa goat farm in Metro City (Lampung Province, Indonesia), kepok banana peel (*Musa paradisiaca* Linn) obtained from banana chips MSMEs in Metro City (Lampung Province, Indonesia), white ginger (*Zingiber officinale* var. *amarum*), sucrose (Gulaku), skim milk (Indoprima), *Lactobacillus casei* 4% (v/v) culture (Inter-University Center, Gadjah Mada University, Indonesia), citric acid, Himedia brand deMan-Rogosa Sharpe Agar (MRSA) and Nutrient Agar, Merck brand deMan-Rogosa Sharpe Broth (MRSB) and Nutrient Broth, distilled water, NaCl (0.85%), NaOH 0.1N, and phenolphthalein indicator (Merck). Pure culture of targeted bacteria (*Salmonella typhimurium*) was also obtained from the Inter University Center at Gadjah Mada University, Yogyakarta, Indonesia for antibacterial activity measurement.

The first step was starter culture preparation. It was prepared from the stock culture, then added into Etawa goat's milk to produce a synbiotic fermented milk. This study used two treatments and three replications. The first treatment was the concentration of kepok banana peel extract: 0% (P0), 1% (P1), 2% (P2), and 3% (P3) (v/v). The second treatment was the concentration of white ginger extract, namely 0% (J0), 1% (J1), 2% (J2), and 3% (J3) (v/v). All treatments were incubated at 37°C for 48 hours. The characteristics of Etawa goat's synbiotic fermented milk were then evaluated by total lactic acid bacteria (LAB), total lactic acid, pH, and antibacterial activity. The data were analyzed by ANOVA and Least Significance Different (LSD 5%).

Culture starter preparation

For culture preparation of *L. casei*, the procedure by Rizal et al. (2020b) was followed. The inoculum was rejuvenated on sterile MRSB in test tubes and incubated at 37° C for 48 hours. *L. casei* (4% v/v) was inoculated on 5% sterile skim milk medium and incubated at 37° C for 48 hours to produce stock culture. The 4% stock culture was inoculated onto intermediate media (50 mL peel extract added with 5% skim milk) and incubated at 37° C for 48 hours. The next step was to inoculate 4% of intermediate media on culture starter media (50 mL peel extract, 5% skim milk and 3% sucrose) and incubate it like the intermediate culture.

White ginger extraction

The white ginger extract was prepared based on the procedure given by Rizal et al. (2020a) with slight modifications. First, white ginger rhizomes were cleaned, peeled, and washed using clean running water. Then, 50 grams of white ginger were blended with warm water in 1:2 ratio for 2 minutes. Finally, the mixture was filtered to obtain white ginger extract.

Kepok banana peels extraction

For production of kepok banana peel extract, the procedure of Dante et al. (2016) was followed with modifications. First, 250 grams of kepok banana peels were sorted and washed using clean running water. After that, they were soaked in 0.5% citric acid for 15 minutes and rinsed. Then, the kepok banana peels were sterilized at 121° C for 15 minutes. They were then mashed with 300 mL of sterile distilled water at 3000 rpm for 2 minutes and filtered to obtain the kepok banana peel extract.

Production of Etawa goat's synbiotic fermented milk

The synbiotic beverage was prepared based on the procedure of Rizal et al. (2020b) with modifications. As much as 2% skim milk (w/v) and 2% sucrose (w/v) were added to 100 mL of Etawa goat's milk and pasteurized at 72° C for 15 minutes. Then, it was let cool until 37° C. Kepok banana peel extract and white ginger were then added according to the treatments and stirred. Subsequently, *Lactobacillus casei* (4% v/v) were incubated at 37° C for 48 hours.

Total lactic acid bacteria enumeration

Total LAB enumeration was carried out after the incubation of the synbiotic beverage at 37°C for 48 hours using the total plate count method (Yelnetty and Tamasoleng 2019). One gram of each sample was mixed with 45 mL of sterile NaCl 0.85%. An appropriate dilution (10^7-10^9) was made and inoculated on de Man Rogosa Sharpe Agar (Merck) and incubated at 37°C for 48 hours. The number of LAB colonies was expressed as log unit colony count (log cfu) per gram sample.

Titratable acidity determination (total lactic acid)

Analysis of total lactic acid followed the AOAC procedure (AOAC 2012).

Determination of pH

The measurement of the pH of the Etawa goat's synbiotic fermented milk used a pH meter (AOAC 2012). Before using the pH meter, its indicator cathode tip was washed with distilled water then dried. The cathode tip was then dipped in a sample of Etawa goat's synbiotic fermented milk, and the results were read on the pH meter.

Analysis of antibacterial activity against *Salmonella typhimurium*

The antibacterial activity of Etawa goat's synbiotic fermented milk was determined using the diffusion well method following the procedure by Murhadi (2002) against *S. typhimurium. Salmonella typhimurium* was used as test bacteria with the consideration that *S. typhimurium* are pathogenic bacteria that often cause digestive disorders besides *E. coli*. Pure cultures of *Salmonella typhimurium* from agar media were inoculated into test tubes with liquid media contents in the form of sterile Nutrient Broth, then incubated at 37° C for 24 hours and homogenized using a vortex. A total of 40µL of the culture was inoculated into an Erlenmeyer containing 60 mL of sterile Nutrient Agar (NA) media at 44-45° C, homogenized, poured onto four sterile petri dishes evenly, and allowed to freeze. Then, a hole (well) was made in each petri dish aseptically with a uniform diameter of 6 mm, and 60 mL of Etawa goat's synbiotic fermented milk was added. The test well was incubated at 37° C for 48 hours, and the inhibitory power of the Etawa goat's synbiotic fermented milk was calculated by measuring the diameter of the inhibition area around the well.

Determination of the best treatment

Determination of the best treatment was conducted using a weighting effectiveness test (De Garmo et al. 1984). Each parameter was weighted based on its level of importance in synbiotic beverages. The one resulting in the highest score was declared as the best treatment.

RESULTS AND DISCUSSION

LAB viability

The viability of LAB was indicated based on the amount of LAB contained in the Etawa goat's synbiotic fermented milk. The results of ANOVA showed that the addition of kepok banana peel extract had a significant effect on LAB viability of Etawa goat's synbiotic fermented milk, while the addition of white ginger extract did not affect LAB viability. The Etawa goat's synbiotic fermented milk with 3% kepok banana peel extract contained the highest total LAB with 9.130 Log cfu/mL. Meanwhile, the lowest total LAB was found in 0% kepok banana peel extract with 8.94 Log cfu/mL (Table 1).

Based on further analysis of 5% LSD as presented in Table 1, the addition of 1, 2, and 3% kepok banana peel extract resulted in the amount of LAB in Etawa goat synbiotic fermented milk which was not significantly different. In contrast to the addition of 1 and 2% kepok banana peel extract which produced the amount of LAB of Etawa goat synbiotic fermented milk which was not significantly different from the synbiotic drink without the addition of kepok banana peel extract, the 3% concentration of kepok banana peel extract produced the amount of LAB which was significantly different from the LAB in synbiotic drinks without the addition of kepok banana peel extract. Even though it was not statistically significantly different, the amount of LAB in Etawa goat synbiotic milk tended to increase along with increasing concentration of kepok banana peel extract. The increase in LAB is thought to be caused by the presence of FOS compounds in kepok banana peel extract, which acts as a selective substrate for the growth of *L. casei*. This finding is in line with existing research (Lopes et al. 2016) which states that FOS can increase LAB growth.

FOS is a prebiotic group that may be broken down by the fructosidase enzyme. However, the enzyme can only be produced by lactic acid bacteria if the substrate is present in the growth of LAB (Martharini and Indratiningsih 2017). FOS acts as a carbon source used in the metabolism of lactic acid bacteria. Increasing the amount of substrate causes the number of lactic acid bacteria to increase.

According to Setiarto et al. (2017), prebiotics in the form of FOS can function as a source of nutrition to increase the growth rate of lactic acid bacteria (*Lactobacillus* sp.*)*. The total LAB results of the Etawa goat's synbiotic fermented milk in this research are in accordance with the SNI standard of the minimum amount of LAB that lives in a product, which is 10^6 cfu/mL (BSN 2009).

Table 1. Effect of different kepok banana peel extract concentrations on the total lactic acid bacteria of Etawa goat's synbiotic fermented milk

Concentration of kepok banana peel extract	Total LAB ± SD (Log cfu/mL)
3 %	9.130 ± 0.000^{a}
2 %	9.078 ± 0.005^{ab}
1 %	8.980 ± 0.000^{ab}
0 %	8.940 ± 0.000^{b}

Note: The total LAB marked with the same letter means that it is not significantly different at 5% LSD (α = 0.152); SD = standard deviation

The addition of white ginger extract had no significant effect on the lactic acid bacteria number. It is because ginger is not the right substrate for the growth of lactic acid bacteria. White ginger contains low carbohydrates. On the other hand, white ginger contains secondary metabolites, namely flavonoids, phenols, oleoresins, essential oils, and tannins, that can act as antibacterials. In this research, the concentration of white ginger extract added were 0, 1, 2, and 3% (v/v) and no treatment resulted in any decrease nor any significant increases in total lactic acid bacteria. This is in accordance with research by Istiqomah (2018) where ginger extract at 1-3% did not reduce the total lactic acid bacteria on red guava probiotic with adding by red ginger. However, a decrease in the total lactic acid bacteria occurred when adding more than 3% of ginger extract.

Total lactic acid

The results of ANOVA and LSD analysis at 5% showed that the addition of kepok banana peel extract had a significant effect on the total lactic acid of the Etawa goat's synbiotic fermented milk, while the addition of white ginger extract had no significant effect on the total lactic acid. Table 2 shows that kepok banana peel extract, which acts as a source of nutrition, can affect the total lactic acid in the Etawa goat's synbiotic fermented milk. Lactic acid is the result of metabolism in lactic acid bacteria fermentation. The increase in total lactic acid occurred because LAB activity increased along with the addition of kepok banana peel extract. The FOS contained in banana peel extract supported LAB growth by providing a source of carbon during fermentation, thus increasing lactic acid as the main end-product of LAB metabolism (Abdel-Rahman et al. 2013). This is in line with research carried out by Rizal et al. (2020b); Mora-Villalobos et al. (2020), where the formation of lactic acid as a product of LAB metabolism increased as the number of LAB increased. The total lactic acid yields in this study met the SNI 7552:2009 standard of having around 0.2 to 0.9% of total lactic acid in fermented beverages.

The addition of kepok banana peel extract, which contains a substrate in the form of FOS, can be a source of energy for LAB in metabolic processes, thus affecting the final product. This aligns with research by İçier et al. (2015) and Fidina et al. (2018) on fermented soymilk beverage that states the increase in total lactic acid occurs because the kepok banana peel is used by *Lactobacillus casei* as a fermentation substrate. The FOS content as a prebiotic in green kepok banana peel extract is used as a supporting medium for LAB growth and activity, such as *Lactobacillus* and *Bifidobacterium* (Castro-Zavala et al. 2015; Brubaker 2019), in breaking down lactose and glucose into lactic acid. The lactic acids formed due to LAB metabolism accumulate, increasing the acidity of the Etawa goat's synbiotic fermented milk.

Meanwhile, the addition of white ginger extract had no significant effect on the total lactic acid. This occurred because white ginger contains not only low carbohydrates but also secondary metabolites that can function as antibacterial, resulting in low total LAB. Low total LAB causes lactic acid produced to also be low. However, the addition of the highest white ginger extract in this study, namely 3%, did not decrease the total lactic acid. This is in accordance with research by Wakhidah et al. (2017), who says the total lactic acid tends to decrease in the use of at least 7.5% white ginger extract.

pH value

The results of ANOVA and LSD analysis at 5% showed that the addition of kepok banana peel extract had a significant effect on the pH value of the Etawa goat's synbiotic fermented milk, while the addition of white ginger extract did not. Table 3 shows that kepok banana peel extract, which acts as a source of nutrition, can affect the degree of acidity in the Etawa goat's synbiotic fermented milk. The degrees of acidity (pH) of the Etawa goat's synbiotic fermented milk ranged from 3.41 to 3.75, all within the optimum pH range for LAB growth (Table 3). According to research by Afshari et al. (2021), *Lactobacillus casei* is a bacterium that is resistant to acidic pH and can still survive at pH as low as 2.5.

The decrease in pH value was caused increased total LAB in the Etawa goat's synbiotic fermented milk. The higher the kepok banana peel extract concentration, the higher the FOS content that will be broken down by LAB into metabolic products in the form of lactic acid, which can affect pH value.

Table 2. Effect of different kepok banana peel extract concentrations on the total lactic acid of Etawa goat's synbiotic fermented milk

Concentration of kepok banana peels extract	Total lactic acid ± SD (%)
3 %	0.812 ± 0.004^{a}
2 %	0.566 ± 0.004^{b}
1 %	0.366 ± 0.002^{c}
0 %	0.320 ± 0.002^{d}

Note: Total lactic acid marked with the same letter means that it is not significantly different at 5% LSD ($\alpha = 0.014$); SD= standard deviation

Table 3. Effect of different kepok banana peel extract concentrations on the pH value of Etawa goat's synbiotic fermented milk

Concentration of kepok banana peel extract	pH value±SD
0 %	3.750 ± 0.005^{a}
1 %	3.620 ± 0.006^{b}
2 %	3.545 ± 0.003^{c}
3 %	3.410 ± 0.003^{d}

Note: The pH value marked with the same letter means that it is not significantly different at 5% LSD (0.019); SD= standard deviation

The lactic acid bacteria used in this study were *Lactobacillus casei*, a type of LAB with a heterofermentative fermentation type (Huang et al. 2018). This type of LAB breaks down glucose and lactose into simpler structural compounds. Through glycolysis, these simple compounds are then converted into lactic acid as the main product. Additionally, *L. casei* can produce citric acid, malic acid, acetic acid, succinic acid, acetaldehyde, butyric acid, and propionic acid (Setiarto 2017) as well as small amounts of carbon dioxide. The acids produced accumulate in the synbiotic fermented milk, lowering the degree of acidity (Rizal et al. 2020b). These acids release protons H^+ that cause a decrease in pH value. Synbiotic fermented milk with a pH value of 4 makes the acid content dissociate easily, so more H^+ ions are produced. Assessing the degree of acidity with a pH meter will detect H^+ ions. The more H^+ ions are detected, the lower the pH value.

The results of pH value in this study were lower than the results of research by Rizal et al. (2020a) on a green grass jelly extract synbiotic beverage with a storage time of 0 hours with a pH value of 4.08. This is presumably due to the differences in the raw material and characteristics of the materials used. This study used Etawa goat's milk as raw material, which had a higher lactose content. Having more substrates—in this case, lactose—to break down led to more LAB metabolic products and lower pH.

Antibacterial activity

The antibacterial activity was determined by measuring the diameter of the clear zone formed around the diffusion wells of the test bacteria (Murhadi 2002; Sartika et al. 2019). Based on ANOVA and LSD at 5%, the addition of kepok banana peel extract, white ginger extract, and both had a significant effect on the antibacterial activity (Table 4). The increase in the antibacterial activity of the Etawa goat's synbiotic fermented milk was tested on the pathogenic bacteria *Salmonella* sp.

The higher the concentration of kepok banana peel extract added, the larger the diameter of the clear zone produced. This aligns with research by Sartika et al. (2019) where the antibacterial compound increased along with the amount of kepok banana peel extract, resulting in greater inhibition of pathogenic bacteria. Kepok banana peel extract contains secondary metabolites that can act as antibacterials and antioxidants, namely flavonoids, alkaloids, tannins, phenolic compounds, caretonoids, and saponin (Singh et al. 2016). Antioxidant compounds can influence the viability of pathogenic bacteria so they can function as antibacterials. In addition, the increasing antibacterial activity was also related to the increase in the total LAB caused by the increased concentration of the kepok banana peel extract added. Then, the total LAB produced lactic acid that lowered the pH value and could inhibit and prevent the growth of pathogenic bacteria (Gavriloka et al. 2019). *Salmonella typhimurium* grows at a minimum pH value of 4.1-9.0 with an optimum pH value of 6.5-7.5 (Pui et al. 2011). This pH mismatch inhibited the growth of *Salmonella* sp. as indicated by the size of the clear zone produced. These results went according to Kalkan (2016) and Rizal et al. (2020b) that stated how producing lactic acid could lower pH and inhibit the growth of pathogenic microorganisms.

The addition of white ginger extract also enlarged the diameter of the clear zone. White ginger extract contains secondary metabolites from the phenol group, flavonoids, essential oils, and terpenoids (Handayani 2018), which can inhibit the growth of *Salmonella* sp. These compounds inhibit the growth of pathogenic bacteria by forming complex compouds with proteins so that they can cause protein denaturation, which causes protein coagulation and disrupts the metaboliism and physiology of *Salmonella* sp. According to Wang et al. (2020), the essential oils in fresh ginger rhizome, composed of the compounds isocaryophyllene, cineol, dl-camphor, caryophyllene, camphene, α-pinene, β-pinene, α-farnesene, germacrene, and caryophyllene oxide, are antibacterial compounds. The diameters of the clear zones, which indicated the antibacterial activity of the Etawa goat's synbiotic fermented milk in this study, ranged from 12-16.3 mm and were well within the strong antibacterial activity standards of 10-20 mm. These results went according to several research where probiotic and synbiotic beverages might inhibit the growth of pathogenic bacteria such as *Salmonella* sp. (Carter et al. 2017). Additionally, green grass jelly synbiotic beverages effectively inhibited *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus* in research by Rizal et al. (2020b), and Zimbabwean commercial probiotic beverage inhibited the growth of *E. coli* in research by Chingwaru and Vidmar (2017).

Table 4. Interaction between different concentrations of kepok banana peel extract and white ginger extract on the antibacterial activity of Etawa goat's synbiotic fermented milk

Concentration of kepok banana peel extract (P) and white ginger extract (J)	Diameter of inhibition zone ± SD (mm)
P3J3 (3% : 3%)	16.267 ± 0.058[a]
P3J2 (3% : 2%)	15.667 ± 0.058[b]
P2J3 (2% : 3%)	15.067 ± 0.058[c]
P3J1 (3% : 1%)	14.833 ± 0.058[c]
P2J2 (2% : 2%)	14.533 ± 0.058[d]
P1J3 (1% : 3%)	14.433 ± 0.058[d]
P0J3 (0% : 3%)	14.367 ± 0.058[d]
P3J0 (3% : 0%)	14.100 ± 0.100[e]
P1J2 (1% : 2%)	13.967 ± 0.058[e]
P2J1 (2% : 1%)	13.967 ± 0.058[e]
P0J2 (0% : 2%)	13.767 ± 0.058[f]
P2J0 (2% : 0%)	13.667 ± 0.058[f]
P1J1 (1% : 1%)	13.233 ± 0.058[g]
P0J1 (0% : 1%)	13.067 ± 0.115[g]
P1J0 (1% : 0%)	12.767 ± 0.058[h]
P0J0 (0% : 0%)	12.000 ± 0.100i

Note: Inhibition zone diameter values marked with the same letter mean that they are not significantly different at 5% LSD (α = 0.329); SD = standard deviation

Table 5. Recapitulation of the weighting effectiveness index on the observation parameters of the Etawa goat's synbiotic fermented milk

Treatments	Total LAB (Log cfu/mL)	Total Lactic Acid (%)	Antibacterial Activity (d, mm)	pH	Weighted index value
P0J0	8.94	0.32	12.00	3.75	0.00
P0J1	8.94	0.32	13.06	3.75	0.10
P0J2	8.93	0.32	13.77	3.75	0.15
P0J3	8.94	0.32	14.37	3.76	0.21
P1J0	8.98	0.37	12.77	3.61	0.16
P1J1	8.98	0.37	13.23	3.62	0.21
P1J2	8.98	0.36	13.97	3.63	0.26
P1J3	8.99	0.37	14.43	3.62	0.31
P2J0	9.10	0.56	13.67	3.54	0.52
P2J1	9.10	0.57	13.97	3.54	0.56
P2J2	9.10	0.57	14.53	3.55	0.61
P2J3	9.10	0.56	15.07	3.54	0.65
P3J0	9.13	0.81	14.10	3.41	0.80
P3J1	9.13	0.81	14.83	3.41	0.86
P3J2	9.13	0.81	15.67	3.41	0.95
P3J3	9.13	0.82	16.27	3.41	1.00

Note: Kepok banana peel extract: P0: 0%, P1: 1%, P2: 2%, P3: 3%, white ginger extract: J0: 0%, J1: 1%, J2: 2%, and J3: 3%Result-2

Figure 1. Etawa goat's synbiotic fermented milk produced with 3% kepok banana peel extract and 3% white ginger extract (the best treatment)

The best treatment for the best Etawa goat's synbiotic fermented milk

Determining the best treatment for Etawa goat's synbiotic fermented milk was conducted by comparing the characteristics produced with the Indonesian national standards (SNI) as used Sutikno et al. (2013) and analyzing each parameter observed using the weighting method by De Garmo et al. (1984). The parameters for the observations were total lactic acid bacteria, total lactic acid, pH value, and antibacterial activity. The expected Etawa goat's synbiotic fermented milk was a beverage that had the highest functional properties and met the SNI requirements for flavored fermented milk (SNI 7552:2009). The weight values for determining the best treatment focused on total lactic acid bacteria, antibacterial activity, and total lactic acid with weight values of 1, 1, and 0.75, respectively. This was because these three parameters were important points in the overall functionality of a synbiotic product.

The results of the observation parameter weighting of synbiotic fermented milk are shown in Table 5. Based on the analysis, the best Etawa goat's synbiotic fermented milk was obtained by adding 3% kepok banana peel extract and 3% white ginger extract with the highest total weight value of 1. This treatment resulted in a beverage with a total acid bacteria of 1.35 x 10^9 cfu/mL or 9.13 log cfu/mL and total lactic acid of 0.82% with an inhibitory power of 16.27 mm and a pH value of 3.41. Etawa goat's synbiotic fermented milk produced from the best treatment is presented in Figure 1.

In conclusion, the viability of lactic acid bacteria and the antibacterial activity of the Etawa goat's synbiotic fermented milk were influenced by the concentration of banana peel extract added, but only the antibacterial activity was influenced by the concentration of white ginger extract added. The addition of 3% kepok banana peel extract and 3% white ginger extract produced the best Etawa goat's synbiotic fermented milk with a total lactic acid bacterium of 9.13 Log cfu/mL, a total lactic acid of 0.82%, pH value of 3.41, and antibacterial inhibition zone diameter of 16.3 mm.

ACKNOWLEDGEMENTS

The authors would like to thank the students and all parties involved in the research.

REFERENCES

Abdel-Rahman MA, Tashiro Y, Sonomoto K. 2013. Recent advances in lactic acid production by microbial fermentation processes. Biotechnol Adv 31 (6): 877-902. DOI: 10.1016/j.biotechadv.2013.04.002.

Afshari A, Hashemi M, Tavassoli M, Eraghi V, Noori SMA. 2021. Probiotic bacteria from 10 different traditional Iranian cheeses: Isolation, characterization, and investigation of probiotic potential. Food Sci Nutr 10 (6): 2009-2020. DOI: 10.1002/fsn3.2817.

Aleta A, Hrvat F, Dzuho A. 2020. Probiotics review and future aspects. Intl J Innov Sci Res Technol 5: 270-274.

Badan Standarisasi Nasional [BSN]. 2009. Minuman susu fermentasi berperisa. SNI 7552:2009. Badan Standarisasi Nasional. Jakarta. [Indonesia]

Brubaker M. 2019. Fermentation of prebiotics in whole food powders by pro biotic lactic-acid producing bacterial strains to identify synbiotic combinations. [Thesis]. Utah State University, Longan. [USA]. DOI: 10.26076/9161-2986.

Carter A, Adams M, Ragione RML, Woodward MJ. 2017. Colonisation of poultry by *Salmonella enteritidis* S1400 is reduced by combined administration of *Lactobacillus salivarius* 59 and *Enterococcus faecium* PXN-33. Vet Microbiol 199: 100-107. DOI: 10.1016/j.vetmic.2016.12.029.

Castro-Zavala A, Juarez-Flores BI, Pinos-Rodriguez JM, Delgado-Portales RE, Aguirre-Rivera JR, Alcocer-Gouyonnet F. 2015. Prebiotic effects of Agave salmiana fructans in *Lactobacillus acidophilus* and *Bifidobacterium lactis cultures*. Nat Prod Commun 10 (11): 1985-1988. DOI: 10.1177/1934578X1501001145.

Chingwaru W, Vidmar J. 2017. Potential of Zimbabwean commercial probiotic products and strains of *Lactobacillus plantarumas* prophylaxis and therapy against diarrhoea caused by *Escherichia coli* in children. Asian Pac J Trop Med 10 (1): 57-63. DOI: 10.1016/j.apjtm.2016.12.009.

Dante LJC, Suter IK, Darmayanti. 2016. Pengaruh konsentrasi sukrosa terhadap karakteristik yougurt dari susu kulit pisang kepok (*Musa paradisiaca* formatypica) dan kacang hijau (*Phaseolus radiatus L.*). Jurnal Ilmu dan Teknologi Pangan 5: 74-84.

De Garmo EP, Sullivan WG, Canada JR. 1984. Engineering Economy. McMillan Publishing Company, New York.

Fidina N, Sukarminah E, Sumanti DM. 2018. The effects of the addition of banana puree to the total number of total probiotic bacteria, pH value and organoleptic characteristics of the synbiotic yogurt made from goat milk and banana puree. J Ind Inf Technol Agric 2 (1): 12-21. DOI: 10.24198/jiita.v2i1.17649.

Gavriloka E, Anisimova E, Gabdelkhadieva A, Nikitina E, Vafina A, Yurullina D, Bogachev M, Kayumov A. 2019. Newly isolated lactic acid bacteria from silage targeting biofilms of foodborne pathogens during milk fermentation. BMC Microbiol 19 (248): 1-12. DOI: 10.1186/s12866-019-1618-0.

Hakim GL, Nefasa AN, Abdurrahman ZH. 2021. Effect of additional red ginger extract (*Zingiber officinale* Var. *Rubrum*) on organoleptic quality and pH of goat milk kefir. Trop Anim Sci 3 (1): 19-24. DOI: 10.36596/tas.v3i1.724. [Indonesian]

Handayani H, Achmad H, Suci AD, Firman M, Mappangara S, Ramadhany S, Pratiwi R, Wulansari DP. 2018. Analysis of antibacterial effectiveness of red ginger extract (*Zingiber Officinale Var Rubrum*) compared to white ginger extract (*Zingiber Officinale* Var. *Amarum*) in mouth cavity bacterial *Streptococcus Mutans* (In-Vitro). J Intl Dent Med Res 11: 676-681.

Huang C-H, Li S-W, Huang L, Watanabe K. 2018. Identification and classification for the *Lactobacillus casei* groub. Front Microbiol 9: 1-13. DOI: 10.3389/fmicb.2018.01974.

İçier F, Gündüz GT, Yılmaz B, Memeli Z. 2015. Changes on some quality characteristics of fermented soy milk beverage with added apple juice. LWT - Food Sci Technol 63 (1): 57-64. DOI: 10.1016/j.lwt.2015.03.102.

Istiqomah L. 2018. The influence of red ginger and lemongrass concentration on the characteristics of red guava (*Psidium guajava* L.) juice probiotic beverages using *Lactobacillus casei* bacteria. [Thesis]. Universitas Lampung, Bandar Lampung. [Indonesian]

Jouki M, Khazaei N, Rashidi-Alavijeh S, Ahmadi S. 2021. Encapsulation of *Lactobacillus casei* in quince seed gum-alginate beads to produce a functional synbiotic drink powder by agro-industrial by-products and freeze-drying. Food Hydrocoll 120: 106895. DOI: 10.1016/j.foodhyd.2021.106895.

Kalkan S. 2016. Probiyotik laktik asit bakterilerinin *Staphylococcus aureus* akarşı antimikrobiyel etkilerinin farklı matematiksel modeller ileanalizi. Sinop Univ J Nat Sci 1 (2): 150-159. [Turkey]

Konuray G, Erginkaya Z. 2017. Antimicrobial effect of probiotics, prebiotics and synbiotic. Novel bioknowledge and educational programs (*A. Méndez-Vilas, Ed.*). In book: Antimicrobial Research: Novel Bioknowledge and Educational Programs. Publisher: Formatex Research Center. Adana Turkey: 213-218.

Kurtoğlu G, Yildiz S. 2011. Extraction of fructooligosaccharide components from banana peels. Gazi Univ J Sci 24: 877-882.

Larassati DP, Susilawati, Nuariny F. 2023. The sensory properties on addition goat's milk and acetic acid coagulant of tofu milk. Jurnal Agroindustri Pangan 2 (2): 17-27. DOI: 10.47767/agroindustri.v2i2.538. [Indonesian]

Lopes SMS, Francisco MG, Higashi B, de Almeida RTR, Krausová G, Pilau EJ, Goncalves JE, Goncalves RAC, de Oliveira AJB. 2016. Chemical characterization and prebiotic activity of fructooligosaccharides from *Stevia rebaudiana* (*Bertoni*) roots and in vitro adventitious root cultures. Carbohydr Polym 152: 718-725. DOI: 10.1016/j.carbpol.2016.07.043.

Markowiak P, Slizewska K. 2017. Effects of probiotics, prebiotics, and synbiotics on human health. Nutriens 9 (9): 1021. DOI: 10.3390/nu9091021.

Martharini D, Indratiningsih I. 2017. Microbiological and chemical quality of goat milk kefir with the addition of *Lactobacillus acidophilus* FNCC 0051 and plantain peel flour (*Musa paradisiaca*). Agritech 37 (1): 22-29. DOI: 10.22146/agritech.17002.

Mora-Villalobos JA, Montero-Zamora J, Barboza N, Rojas-Garbanzo C, Usaga J, Redondo-Solano M, Schroedter L, Olszewska-Widdrat A, Lopez-Gómez JP. 2020. Multi-product lactic acid bacteria fermentations: A review. Fermentation 6 (1): 23. DOI: 10.3390/fermentation6010023.

Murhadi. 2002. Solasi dan karakteristik komponen anti bakteri dari biji atung. [Dissertation]. Program Pascasarjana, Institut Pertanian Bogor, Bogor. [Indonesian]

Pui CF, Wong WC, Chai LC, Tunung R, Jeyaletchumi P, Noor HMS, Ubong A, Farinazleen MG, Cheah YK, Son R. 2011. Review article *Salmonella*: A foodborne pathogen. Intl Food Res J 18: 465-473.

Rizal S, Suharyono S, Nurainy F, Merliyanisa M. 2020a. The influence of glucose and red ginger on the probiotic beverage characteristics of honey pineapple skin. Jurnal Teknologi Industri Hasil Pertanian 25 (2): 110-119. DOI: 10.23960/jtihp.v25i2.110-119. [Indonesian]

Rizal S, Suharyono AS, Nurainy F, Amelia JR. 2020b. The effects of low temperature storage on the viability of *Lactobacillus casei* and the stability of antibacterial activity in green grass jelly synbiotic drinks. Biodiversitas 21 (8): 3826-3831. DOI: 10.13057/biodiv/d210851.

Sartika D, Herdiana N, Kusuma SN. 2019. Antimicrobial activity of leather extract and muli banana flower (*Musa acuminata*) against *Escherichia coli* bacteria. Agritech 39 (4): 355-363. DOI: 10.22146/agritech.26625.

Setiarto RHB, Widhyastuti N, Rikmawati NA. 2017. Concentration optimization of fructooligosaccharides to increase growth of lactic acid bacteria yoghurt starter. Jurnal Veteriner 18: 428-440. DOI: 10.19087/jveteriner.2017.18.3.428. [Indonesian]

Sutikno, Rizal S, Marniza. 2013. Effects of sugar type and concentration on the characteristics of fermented turi (*Sesbania grandiflora* (L.) Poir) milk. Emir J Food Agric 25 (8): 576-584. DOI: 10.9755/ejfa.v25i8.15062.

Singh B, Singh JP, Kaur A, Singh N, 2016. Bioactive compounds in banana and their associated health benefits - A review. Food Chem 206: 1-11. DOI: 10.1016/j.foodchem.2016.03.033.

Sumarmono J. 2022. Current goat milk production, characteristics, and utilization in Indonesia. Intl Conf Environ Energy Earth Sci 1041: 1-8. DOI: 10.1088/1755-1315/1041/1/012082.

The Associate of Official Analytical Chemist (AOAC). 2012. Officinal Methods of Analysis. The Associate of Analytical Chemist (AOAC) Inc. Washington DC. 1728 pages.

Wakhidah N, Jati GM, Utami R. 2017. Fresh milk yoghurt with addition extracts of ginger pulp from distillation essential oil. Proc Biol Educ Conf 14: 278-284.

Wang X, Shen Y, Thakur K, Han J, Zhang J-G, Hu F, Wei Z-J. 2020. Antibacterial activity and mechanism of ginger essential oil against *Escherichia coli* and *Staphylococcus aureus*. Molecules 25 (17): 1-17. DOI: 10.3390/molecules25173955.

Yelnetty A, Tamasoleng M. 2019. The addition of yam tuber (*Dioscorea alata*) flour as a source of prebiotic on biomilk synbiotic characteristics. Intl Conf Anim Sci Technol: Earth Environ Sci 247: 6. DOI: 10.1088/1755-1315/247/1/ 012052.