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The effect of kweni mango and randu honey addition on the characteristics of synbiotic etawa goat milk

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Abstract. *Rizal S, Suharyono AS, Astuti S, Dewanti AS, Nugroho YB. 2024. The effect of kweni mango and randu honey addition on the characteristics of synbiotic etawa goat milk. Biodiversitas 25: 1580-1587.* The addition of kweni mango and randu honey as ingredients for producing synbiotic etawa goat milk is widely known to have a significant effect on the characteristics of the product. Therefore, this study aimed to identify the best formulation of kweni mango and randu honey in producing synbiotic etawa goat milk. The experiment was conducted using a completely randomized block design with 4 replications. A single factor with six levels of formulations of kweni mango extract and randu honey was used in producing synbiotic etawa goat milk. These formulations included F1 (0%:25%), F2 (5%:20%), F3 (10%:15%), F4 (15%:10%), F5 (20%:5%), and F6 (25%:0%). Subsequently, the microbiological, chemical, and sensory properties, as well as antimicrobial activity of the product were evaluated. The data obtained were statistically analyzed using ANOVA and the Least Significant Difference (LSD) test at a 5% significance level. The results showed that formulation F4 containing 15% kweni mango extract and 10% randu honey produced the best synbiotic Etawa goat milk. In addition, F4 formulation showed higher antibacterial activity against *Staphylococcus aureus* than *Escherichia coli*. The optimal beverage met the standards for lactic fermentation products from a chemical perspective and was favored by the panelists.

Keywords: Antibacterial activity, etawa goat milk, kweni mango, randu honey, synbiotic

INTRODUCTION

The advancement of food science and technology is a significant contributor to the increased awareness of healthy lifestyles in society. This increased awareness is characterized by individuals becoming more health-conscious and opting for food that provides beneficial effects to the body, leading to the development of functional foods. In addition, a category of functional food gaining popularity is synbiotic beverages containing both probiotics and prebiotics. The balance of microorganisms in the digestive tract has a significant impact on health. An increase in the number of pathogenic bacteria in the intestines disturbs its balance, making the human body less resistant to intestinal infections (Rizal et al. 2020). According to Oelschlaeger (2010), consuming beverages containing probiotics is known to suppress the growth of pathogenic bacteria. These beverages primarily affect the development of microbiota inhabiting organisms by ensuring the proper balance between pathogens and bacteria necessary for normal organism function.

Synbiotics refers to the combination of probiotics and prebiotics, which provide various benefits to the host. These include increasing survival and providing food supplements for the survival of microbes in the digestive tract (Scavuzzi et al. 2014; Biplab and Narayan 2014; Thilagavathi 2020; Markowiak and Śliżewska 2017). Several studies have also shown that synbiotics contain live and active probiotic bacteria, such as Lactic Acid Bacteria (LAB), which remain viable and active upon reaching the digestive tract. In addition, it also comprises prebiotics that are not digested in the upper digestive system but serve as substrates for probiotics (Boeni and Pourahmad 2012). Probiotic bacteria are known to play various essential roles, including improving intestinal health the immune system, as well as protecting the host from pathogenic infections (Chandra et al. 2022; Lokapirnasari et al. 2019). Recent reports showed that synbiotics improved the gut microbial environment and activated immune function (Biplab and Narayan 2014).

According to several reports, a popular synbiotic beverage in Indonesia is often made from milk of etawa goat with the addition of kepok banana peel extract and white ginger extract (Rizal et al. 2023). Rizal et al. (2023) combined kepok banana as a prebiotic with kweni mango, which had a strong aroma to cover the less favorable typical smell of goat. Ratya et al. (2017) have developed goat's milk as a substrate in the production of functional beverages because of its advantages, including high digestibility, nutritional composition that is important for health, and low allergenicity.

In this study, a synbiotic drink based on etawa goat milk was produced by adding randu honey as a prebiotic component and *Lactobacillus casei* as a probiotic component. Previous studies have reported the presence of several prebiotic components in randu honey, including 14.76% Fructo-Oligosaccharides (FOS) and 6.60% inulin. Randu honey also contains sugar, which can act as an energy source for LAB growth (Triwanto et al. 2021). In addition, the use of randu honey as a component in making synbiotic drink can be attributed to the presence of antimicrobial compounds, such as organic acids, essential oils, flavonoids, low pH, and hydrogen peroxide content (Abubakar et al. 2012).

Despite its potential, the drawback in the use of etawa goat milk as a synbiotic beverage is the presence of a distinctive "goaty" odor. However, the distinctive aroma can be reduced through the fermentation process and the addition of fruits with fresh aromas, such as mango, star fruit (blimbing), lime, and ginger (Yelnetty et al. 2014). A fruit that can be used to eliminate this "goaty" odor is kweni mango due to its beneficial constituents. These include volatile components, such as ethyl butanoate, α-pinene, camphene, myrcene, butyl butanoate, limonene, terpinolene, and linalool, forming a strong kweni aroma (Mustofa et al. 2022). Kweni mango has also been reported to contain compounds, such as flavonoids, tannins, saponins, mangiferin, terpenoids, and alkaloids, which are believed to inhibit bacterial growth (Afifa et al. 2014).

Although several studies have explored the development of etawa goat milk using various ingredients, there are limited studies on the use of randu honey and kweni mango. Therefore, this study aimed to determine the effect of adding randu honey and kweni mango on the sensory characteristics, growth of LAB, and antibacterial activity of synbiotic etawa goat milk. The results are expected to provide information on the formulation of ingredients with the best synbiotic characteristics of etawa goat milk according to SNI 7552:2018.

MATERIALS AND METHODS

Materials

The ingredients used in present study included etawa goat milk, fully ripe kweni mango, randu honey, mineral water, skim milk, pure cultures of *L. casei*, *S. aureus*, and *E. coli* obtained from Inter-University Center for Food and Nutrition, Universitas Gadjah Mada, Yogyakarta, Indonesia. MRSA, MRSB, distilled water (aquadest), $CaCO_3$, NaOH 0.1 N, phenolphthalein indicator, NaCl, 70% alcohol, sucrose, buffer solution pH 7.0, $CuSO_4.5H_2O$, boric acid, hexane, K_2SO_4, and Mueller Hinton Agar (MHA) medium.

Methods

The study was conducted using a Completely Randomized Block Design (CRBD) with a single factor comprising 6 levels and 4 replications. In addition, the single factor used as treatment was the formulation of kweni mango and randu honey, consisting of F1 (0%:25%), F2 (5%:20%), F3 (10%:15%), F4 (15%:10%), F5 (20%:5%), and F6 (25%:0%). Various characteristics of synbiotic Etawa goat milk were carefully assessed, covering microbiological aspects (LAB count), chemical attributes (pH, total lactic acid, and proximate analysis), sensory properties (evaluated through hedonic tests for taste, aroma, color, and overall acceptance), and antimicrobial activity against *S. aureus* and *E. coli*.

For determining the optimal treatment, a weighting method as outlined by De Garmo (1984) was used. Subsequently, proximate analysis was performed exclusively on the product identified as the best treatment. This meticulous approach ensured a robust statistical evaluation and allowed for the identification of the most effective treatment based on the specified criteria.

Preparation of kweni mango juice

The first stage of producing kweni mango juice was the preparation of 300 g of kweni mango with perfect ripeness. The fruits were washed with clean water and the skin was peeled, followed by separation of the flesh from the seeds and cutting into small pieces. The fruit flesh was then blended by adding water at a ratio of 3:1 (water: kweni mango) at a speed of 16,000 rpm for 45 seconds. Subsequently, the pulp obtained was filtered using a filter cloth until the juice and pulp were separated. Kweni mango juice was then pasteurized at 70°C for 15 minutes.

Preparation of working culture

The first step in preparing the starter culture was to obtain a pure culture of *L. casei*, with a volume of 1 oz. The pure culture was transferred to a tube containing sterilized liquid MRS Broth medium. Furthermore, the mixture was incubated at a temperature of 37°C for 48 hours, and a total of 4% (v/v) of *L. casei* culture was inoculated into 10 mL of sterilized 5% (w/v) skim milk. The mixture of pure culture and skim milk was mixed and then incubated at a temperature of 37°C for 48 hours. The culture obtained was referred to as the starter culture in this study. A total of 4% (v/v) of the starter culture was inoculated into 50 mL of sterilized kweni mango juice with the addition of 5% (w/v) sterilized skim milk, followed by incubation at a temperature of 37°C for 24 hours. The culture obtained was referred to as the intermediate culture. A total of 4% (v/v) of the intermediate culture was inoculated into 50 mL of sterilized kweni mango juice with the addition of 5% (w/v) sterilized skim milk and 3% (w/v) sterilized sucrose. The mixture was then incubated at a temperature of 37°C for 48 hours using an incubator. The culture obtained was referred to as the working culture. The process of providing a working culture began with making an intermediate culture using a mixed medium, containing kweni mango and randu honey. The aim was to facilitate the adaptation of the microbial inoculum before inoculating it into the actual substrate.

Production of synbiotic etawa goat milk

To prepare synbiotic etawa goat milk with the addition of kweni mango and randu honey, the process began with the preparation of 345 mL of etawa goat milk for pasteurization. The pasteurization process was carried out with the addition of 2% (w/v) skim milk and a combination of kweni mango juice and randu honey formulations in the ratio F1 (0%: 25%), F2 (5%: 20%), F3 (10 %: 15%), F4 (15%: 10%), F5 (20%: 5%), and F6 (25%: 0%) (v/v total) of 500 mL volume. Subsequently, the mixture was homogenized with a stir bar, and a total of 4% (v/v) working culture of *L. casei* from 500 mL total volume was added. The mixture of ingredients was then incubated at 37°C for 48 hours.

Enumeration of total LAB

Enumeration of total LAB was conducted using the Total Plate Count method (Rahayu and Nurwitri 2012). A total of 1 mL of sample was placed into a test tube containing 9 mL of sterile physiological saline solution. The samples and physiological solutions were then homogenized using a vortex mixer, with a 10^{-1} dilution. Subsequently, 1 mL of the 10^{-1} dilution solution was taken and placed into a test tube containing 9 mL of physiological salt solution to obtain the 10^{-2} dilution. The dilution was then repeated until a level of 10^{-8} was reached. A total of 1 mL of sample was taken from each dilution 10^{-6}, 10^{-7}, and 10^{-8} and placed into 3 different sterile petri dishes using a micropipette. In the cup, 15 mL of sterile MRS media was added and homogenized. The samples were then incubated at 37°C for 48 hours until colonies were formed and then counted.

Evaluation of total lactic acid

The total lactic acid of synbiotic etawa goat milk was analyzed using the titration method (AOAC 2019).

Acidity level (pH)

The pH of synbiotic drink was measured using a pH meter (AOAC 2019).

Sensory evaluation

Sensory evaluation was performed using the hedonic method with 30 untrained panelists. The sensory parameters included aroma, taste, color, and overall acceptance, with the rating scale comprising 1 = very dislike, 2 = dislike, 3 = somewhat like, 4 = like, and 5 = very like.

Antibacterial activity testing

Staphylococcus aureus and *E. coli* were selected for bacterial test, representing gram-positive and gram-negative bacterial populations commonly found in etawa goat milk. Testing of antibacterial activity of synbiotic etawa goat milk was carried out using the paper disc diffusion method. The bacterial test suspension was prepared by taking pure cultures of *S. aureus* and *E. coli*, each cultured on agar media in amounts of 2 oz. The cultures were then dissolved in 0.85% sterile NaCl solution aseptically and homogenized with a vortex. Subsequently, the turbidity level of the bacterial test suspension was measured through comparison with McFarland 0.5 standards. McFarland 0.5 was commonly used as a reference for comparing the turbidity of liquid media with a density equivalent to 1 x 10^{8} CFU/mL. A total of 1 mL of bacterial suspension was inoculated into a sterile petri dish, followed by the addition of 15 mL of Mueller Hinton Agar (MHA). The petri dish was shaken to ensure that the MHA media and bacterial suspension were homogenized, and then media was allowed to solidify. Filter paper discs (6 mm in diameter) that were dipped in each sample concentration were then placed on MHA agar surfaces mixed with bacterial suspension and then incubated at 37°C for 24 hours. After the incubation process was complete, the diameter of the inhibition zone formed around the filter paper disc was measured using a caliper. The enumeration of the inhibition zone followed the procedure used by Rizal et al. (2019), where measurement was performed based on the diameter (d, mm) of inhibition, which formed as a clear area around the paper disc.

Determination of the optimal formulation

Determination of the best formulation of kweni mango juice and randu honey in the production of synbiotic Etawa goat milk was based on the treatment that produced the best values for most parameters observed, including total LAB, total lactic acid, degree of acidity (pH), sensory properties (taste, aroma, color, and overall acceptability), and antibacterial activity against *S. aureus* and *E. coli.*

Analysis of nutritional components

The nutritional components of synbiotic etawa goat milk were tested using proximate analysis, which included protein, fat, ash, and carbohydrate content (AOAC 2019). The proximate analysis was carried out on synbiotic etawa goat milk, which was made with the best formulation.

Data analysis

All the collected data were subjected to Bartlett's test to evaluate the homogeneity of variances, and Tukey's test was used for pairwise comparisons. In addition, the statistical analysis comprised the use of Analysis of Variance (ANOVA). To identify the differences among treatments, the Least Significant Difference (LSD) test was performed at a 5% significance level.

RESULTS AND DISCUSSION

Effect of kweni mango and randu honey addition on characteristics of synbiotic etawa goat milk

Based on the analysis of variance results, addition of kweni mango and randu honey influenced all the characteristics of synbiotic etawa goat milk tested in this study. The results showed that the addition of mango kweni juice and randu honey significantly influenced the amount of LAB, total lactic acid, and pH value**,** as shown in Table 1.

Total LAB of synbiotic etawa goat milk

Total LAB values in synbiotic etawa goat milk with the addition of mango kweni juice and randu honey ranged from 8.61 log CFU/mL to 9.00 log CFU/mL (Table 1) or equivalent to 4.02 x 10^{8} CFU/mL to 1.00 x 10^{9} CFU/mL. All treatments produced synbiotic milk that met the quality requirements (1.00 x 10^{6} CFU/mL) for total LAB based on SNI 7552:2018. The post hoc test (LSD 5%) for total LAB in synbiotic etawa goat milk with the addition of mango kweni juice and randu honey showed that treatment F4 was significantly different from other treatments.

Based on Table 1, F4 was the best formulation of kweni mango juice and randu honey, which produced the highest total LAB compared to other formulations. The composition of these 2 ingredients was thought to be able to provide balanced nutrition between the sugar content as an energy source for LAB growth and kweni mango, which had antimicrobial compounds. According to Triwanto et al. (2021), kapok honey contained FOS (%), which could provide an energy source for LAB growth. Harun et al. (2013) stated that LAB could grow more under optimum conditions with sufficient energy sources, while a decrease was likely to occur when the growth medium contained excess energy sources. Excessive energy sources typically reduced the optimization of multiplication and growth of LAB cells due to the formation of hypertonic environmental conditions.

During the fermentation process, LAB often went through the lag phase (adjustment phase) more quickly because the working culture had adapted to the substrate environment. This showed that total LAB value increased with the addition of kweni mango juice and randu honey formulations that met the nutritional needs. In addition, LAB reached the logarithmic phase, characterized by the formation of a minimum density of cells of around $1x10^7$ CFU/mL. This caused an increase in the total LAB value due to cell multiplication.

Total lactic acid of synbiotic etawa goat milk

Total lactic acid values in synbiotic etawa goat milk with the addition of mango kweni juice and randu honey ranged from 0.82 to 0.89% (Table 1). All treatments led to synbiotic etawa goat milk that met the quality standards (0.2-0.9%) for total lactic acid based on Indonesian standards (SNI 7552:2018).

Further testing using the LSD at a 5% significance level for the total lactic acid in synbiotic etawa goat milk with the addition of mango kweni juice and randu honey showed that treatment F4 was significantly different from others. In addition, treatment F3 was not significantly different from F5 but significantly different from others. The results also showed that F2 was not significantly different from F5 and F6 but significantly different from others. F1 was not significantly different from F6 but significantly different from others.

In this study the total lactic acid values in etawa goat milk synbiotic with mango kweni juice and randu honey were consistent with the total LAB produced. Based on the data in Table 1, the increase in total LAB value led to an increase in the total lactic acid value produced. This was in line with Pranayanti and Sutrisno (2015) that sufficient nutrition from prebiotic ingredients could trigger the growth of LAB, leading to an increase in LAB activity, along with the increase in the total lactic acid value as a product of fermentation. LAB broke down sucrose and fructose found in randu honey and mango kweni juice into simple sugar monomers using sucrase enzymes, forming lactic acid (Rachman et al. 2018).

Acidity (pH) of synbiotic etawa goat milk

The pH values of synbiotic etawa goat milk with the addition of mango kweni juice and randu honey ranged from 3.71 to 3.91 (Table 1). The optimal pH range for the growth of LAB in fermented beverages was approximately 3-6 (Rasbawati et al. 2019). The further test (LSD 5%) for acidity (pH) in synbiotic etawa goat milk with the addition of mango kweni juice and randu honey showed that treatment F4 was significantly different from others. In addition, F5 produced synbiotic Etawa goat milk that was not significantly different from F2 but significantly different from others. The results also showed that F6 was not significantly different from treatments F2 and F1 but significantly different from others. Synbiotic etawa goat milk obtained from P0 was not significantly different from F1 but significantly different from other treatments.

A strong relationship between total LAB, total lactic acid, and pH in synbiotic etawa goat milk (Table 1). When total LAB and total lactic acid increased, the pH of the product with the addition of mango kweni juice and randu honey tended to decrease. The results showed that the proper formulation of mango kweni juice and randu honey served as a substrate. LAB metabolized and produced secondary metabolites, such as lactic acid, which could lower the pH value of the product. This was consistent with a study by Martharini and Indratiningsih (2017), where the increasing lactic acid content reduced the pH of fermented goat milk-based beverages. Purnomo and Muslimin (2012) also stated that the increasing content of lactic acid decreased the pH of fermented beverages based on goat milk.

Table 1. Results of 5% LSD on total LAB, total lactic acid, and pH value of synbiotic Etawa goat milk with the addition of mango kweni juice and randu honey

Mango kweni and randu honey formulations	Total LAB (Log CFU/mL)	Total lactic acid (%)	pH value
F4 (15% mango kweni juice: 10% randu honey)	9.00^{a}	0.89^{a}	3.71^{a}
F3 (10% mango kweni juice: 15% randu honey)	8.85^{b}	0.86^{b}	3.74^{b}
F5 (20% mango kweni juice: 5% randu honey)	8.82^{c}	0.85^{bc}	3.75^{c}
F2 (5% mango kweni juice: 20% randu honey)	8.77^{d}	0.84^{cd}	3.79^{cd}
F6 (25% mango kweni juice: 0% randu honey)	8.66^{e}	0.83^{de}	3.81^{de}
F1 (0% mango kweni juice: 25% randu honey)	8.61^{f}	0.82^{e}	3.91^{ef}
LSD 5%	0.04	0.02	0.20

Note: Values given the same letter notation have no significantly different meaning in the 5% LSD test

Randu honey contained Fructooligosaccharides (FOS) and inulin as a source of prebiotics as well as sugar (fructose, glucose, and sucrose), which could increase the growth activity of LAB. LAB typically produced lactic acid during the fermentation process. A high total BAL value caused metabolic products in the form of lactic acid to increase and reduce the pH of synbiotic etawa goat milk drink. The acid accumulation could lower the pH and inhibit the growth of pathogenic bacteria (Nurainy et al. 2017), leading to an increase in antibacterial activity of the product.

Sensory properties of synbiotic etawa goat milk

The color scores in synbiotic Etawa goat milk with the addition of mango kweni juice and randu honey ranged from 3.33 to 4.15. The analysis of variance showed that the formulation of mango kweni juice and randu honey significantly influenced the color of the product. According to the post hoc test (LSD 5%), color score of treatment F4 (15% kweni mango : 10% randu honey) was significantly different from others (Table 2). The results showed that F4 had the highest color-liking score of 4.15 (like). In addition, mango kweni contained carotenoid pigments that ranged from yellow to orange, and randu honey comprised carotenoid and flavonoid pigments, which were golden yellow (Sumarlin et al. 2014), influencing the color of the product (Putri et al. 2017).

The aroma scores in synbiotic etawa goat milk with the addition of mango kweni juice and randu honey ranged from 2.90 to 4.38. The analysis of variance showed that the formulation of mango kweni juice and randu honey significantly influenced the aroma of the product. Based on the post hoc test (LSD 5%), F5 (20% kweni mango : 5% randu honey) was significantly different from other treatments. The results showed that F5 obtained the highest (4.38) score for aroma liking (Table 2).

The addition of mango kweni juice and randu honey with the right formulation masked the distinctive "prengus" aroma in synbiotic Etawa goat milk. According to Mustofa et al. (2022), mango kweni contained volatile components, such as ethyl butanoate, camphene, α-pinene, butyl butanoate, terpinolene, limonene, and linalool, which contributed to the strong characteristic aroma of kweni. Harjiyanti et al. (2013) also showed that it has volatile compounds that underwent oxidation during fermentation, enhancing its distinctive aroma. Randu honey contained sucrose, which could suppress the number of unwanted carbonyl compounds, allowing the volatile compounds present in mango kweni to be released optimally. According to Apandi et al. (2016), consumer assessment of food products was influenced by their aroma. A distinct aroma could be perceived by the sense of smell due to the presence of volatile components.

The taste scores of synbiotic etawa goat milk with the addition of mango kweni juice and randu honey ranged from 2.00 to 4.01. The analysis of variance showed that the formulation of mango kweni juice and randu honey significantly influenced the taste of the product. Based on the LSD 5% tes, treatment F4 (15% kweni mango : 10% randu honey,) was significantly different from other treatments (Table 2). In addition, treatment F4 obtained the highest (4.01) score for taste liking. According to Apandi et al. (2016), taste is the sensation perceived by the taste buds in response to the combination of compositions used in a product.

The right formulation of kweni mango and randu honey created a distinctive lactic fermentation sour taste in synbiotic Etawa goat milk. The addition of randu honey served as a prebiotic and a source of sugar that could be used as an energy source for the growth of LAB, leading to higher lactic acid production and the emergence of the typical sour taste of lactic fermentation. In addition, the results showed that treatment F6 (25% kweni mango: 0% randu honey) had the lowest (2.00) score. According to Harun et al. (2013), total LAB decreased when the growth medium contained an excess of energy sources, causing a decrease in lactic acid production, and synbiotic drinks tended to be less acidic.

The overall acceptance scores for synbiotic etawa goat milk with the addition of mango kweni juice and randu honey ranged from 2.34 to 4.08. The analysis of variance showed that the formulation of mango kweni and randu honey significantly influenced the overall acceptance of synbiotic etawa goat milk. Based on the post hoc test (LSD 5%) in Table 2, F4 was significantly different from other treatments. F4 (15% kweni mango: 10% randu honey) had the highest overall acceptance score of 4.08 (like). Factors influencing the increase in overall acceptance scores by the panelists for synbiotic Etawa goat milk were taste, color, and aroma. The appearance of synbiotic milk products from the Etawa goat is presented in Figure 1.

Table 2. Results of 5% LSD on the color of synbiotic etawa goat milk with the addition of kweni mango and randu honey

Mango kweni and randu honey formulations	Color scores	Aroma scores	Taste scores	Overall acceptance
F4	4.15[a]	4.19[b]	4.01[a]	4.08[a]
F5	3.86[b]	4.38[a]	2.62[d]	2.98[c]
F6	3.67[c]	2.91[e]	2.00[e]	2.34[d]
F3	3.57[cd]	3.44[c]	3.37[b]	3.33[b]
F2	3.44[de]	3.20[d]	3.11[bc]	3.23[b]
F1	3.33[e]	3.08[de]	2.87[cd]	3.19[b]
LSD 5%	0.17	0.18	0.30	0.20

Note: Numbers followed by the same letter have no significantly different meaning in the 5% LSD test; F1: 0% mango kweni juice: 25% randu honey, F2: 5% mango kweni juice: 20% randu honey, F3: 10% mango kweni juice: 15% randu honey, F4: 15% mango kweni juice: 10% randu honey, F5: 20% mango kweni juice: 5% randu honey, F6: 25% mango kweni juice: 0% randu honey

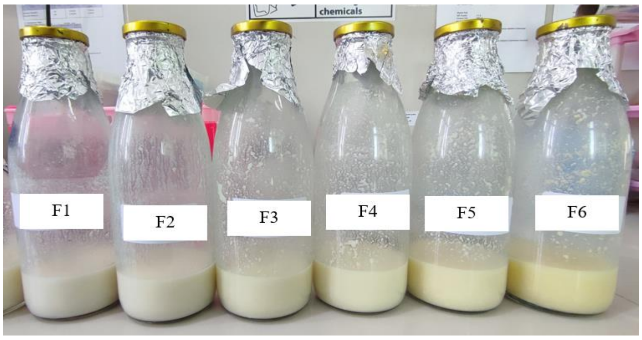

Figure 1. The appearance of synbiotic milk of Etawa goat milk with the addition of various combinations of kweni mango and randu honey (F1: 0%: 25%; F2: 5%: 20%; F3: 10%: 15%; F4: 15%: 10%; F5: 20%: 5%; F6: 25%: 0%)

Antibacterial activity of synbiotic etawa goat milk

The analysis of variance showed that the inclusion of mango kweni juice and randu honey had a significant impact on antibacterial activity in synbiotic etawa goat milk. The antibacterial activity was shown by the formation of a clear zone. Results of the antibacterial activity test showed that formulation of mango kweni juice and randu honey formed clear zones ranging from 3.28 mm to 9.27 mm against *S. aureus*, while in *E. coli*, it ranged from 3.07 mm to 4.47 mm (Table 3). The post hoc test (LSD 5%) results of antibacterial activity against *S. aureus* and *E. coli* showed that treatment F4 was significantly different from other treatments.

Antibacterial activity of synbiotic Etawa goat milk against *S. aureus* produced larger clear zone diameters compared to *E. coli*. The difference in the ability to inhibit the growth of *S. aureus* and *E. coli* is caused by the differences in cell wall structure (lipid content, peptidoglycan, enzyme activity) in Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*E. coli*), leading to variations in antibacterial activity against these bacteria (Eleazu et al. 2013)

S. aureus had a relatively simple cell wall structure, facilitating the entry of antibacterial compounds into the cell. Furthermore, it consisted of a thick peptidoglycan layer (40% - 50%) and relatively little fat. Several studies have shown that *E. coli* had a more complex cell wall structure, consisting of several layers, including an outer membrane protecting the peptidoglycan, an inner layer (phospholipid), and an outer layer (lipopolysaccharide). The difference in cell wall structure between *S. aureus* and *E. coli* led to varying activities in inhibiting bacterial growth. *E. coli* was more difficult to penetrate compared to *S. aureus* (Combarros-Fuertes et al. 2020).

In line with previous reports, randu honey contained Fructo-Oligosaccharides (FOS) and inulin as prebiotic sources, along with sugars (fructose, glucose, and sucrose) that could enhance the growth activity of LAB. This enhancement led to an increase in total LAB, which produced lactic acid during the fermentation process. The elevated values of total LAB contributed to an increase in the metabolic product (lactic acid), thereby reducing the pH value of the product. The accumulation of lactic acid, which caused a decrease in pH, played an essential role in inhibiting the growth of pathogenic bacteria, as suggested by Nurainy et al. (2017). This mechanism led to an increase in antibacterial activity of synbiotic Etawa goat milk.

The best formulation of kweni mango juice and randu honey

Results showed a recapitulation of the assessment results in determining the best treatment, which had the highest value based on the parameters. In Table 4, the treatment with the highest value was F4 (15% kweni mango juice: 10% randu honey). Therefore, the best synbiotic fermented etawa goat milk was obtained by adding 15% kweni mango juice and 10% randu honey. This treatment produced a total of 9.00 log CFU/mL LAB or the equivalent of 1 x 10^9 CFU/mL, total lactic acid of 0.89%, acidity degree (pH) of 3.71, sensory properties using the hedonic method, a taste score of 4.01 (like), aroma score of 4.19 (like), color score of 4.15 (like), and overall acceptability score of 4.08 (like). The results also showed that F4 had antibacterial activity with a clear zone diameter of 9.27 mm for *S. aureus* bacteria and 4.47 mm for *E. coli* bacteria.

Nutritional components of synbiotic Etawa goat milk

Proximate analysis was conducted on the best treatment in line with SNI 7552:2018. Based on the proximate analysis results presented in Table 5, the amount of ash, protein, and fat content in the product met the requirements outlined in SNI 7552:2018 for flavored fermented milk beverages.

Table 3. Results of 5% LSD on antibacterial activity of synbiotic Etawa goat milk with the addition of mango kweni juice and randu honey

Mango kweni and randu honey formulations	Clear zone diameter (mm)	
	S. aureus	***E. coli***
F4 (15% mango kweni juice: 10% randu honey)	9.27^a	4.47^a
F3 (10% mango kweni juice: 15% randu honey)	6.38^b	3.86^b
F5 (20% mango kweni juice: 5% randu honey)	6.17^c	3.69^c
F2 (5% mango kweni juice: 20% randu honey)	4.99^d	3.64^d
F6 (25% mango kweni juice: 0% randu honey)	3.67^e	3.13^e
F1 (0% mango kweni juice: 25% randu honey)	3.28^f	3.07^f
LSD 5%	0.05	0.03

Note: Values given the same letter notation have no significantly different meaning in the 5% LSD test

Table 4. Determination of the best formulation of kweni mango juice and randu honey in the production of synbiotic Etawa goat milk

Observation parameters	Formulation of kweni mango and randu honey					
	F1	F2	F3	F4	F5	F6
Total LAB (Log CFU/mL)	8.61	8.77	8.85	9.00	8.82	8.66
Total lactic acid (%)	0.82	0.84	0.86	0.89	0.85	0.83
Acidity (pH)	3.91	3.79	3.74	3.71	3.75	3.81
Diameter of the inhibition zone in *S. aureus* (mm)	3.28	4.99	6.38	9.27	6.17	3.67
Diameter of the inhibition zone in *E. coli* (mm)	3.07	3.64	3.86	4.47	3.69	3.13
Taste scores	2.87	3.11	3.37	4.01	2.62	2.00
Aroma scores	3.08	3.20	3.44	4.19	4.38	2.91
Color scores	3.33	3.44	3.57	4.15	3.86	3.67
Overall acceptance	3.19	3.23	3.33	4.08	2.98	2.34

Note: F1: 0% mango kweni juice: 25% randu honey, F2: 5% mango kweni juice: 20% randu honey, F3: 10% mango kweni juice: 15% randu honey, F4: 15% mango kweni juice: 10% randu honey, F5: 20% mango kweni juice: 5% randu honey, F6: 25% mango kweni juice: 0% randu honey

Table 5. Proximate analysis of synbiotic Etawa goat milk with the addition of kweni mango and randu honey

Nutritional components	Value	Indonesian Standard (SNI 7552:2018) for fermented drink
Water content (%)	82.56	-
Ash content (%)	0.89	≤ 1.00
Protein content (%)	1.72	≥ 1.00
Fat content (%)	4.78	≥ 0.6
Carbohydrate content (%)	10.05	-

The results of proximate analysis of synbiotic etawa goat milk showed that the nutritional composition of the product met the quality requirements for fermented drinks based on SNI 7552:2018. The water content obtained was 82.56%, which was considered good despite not being included in the requirements. The ash content in synbiotic Etawa goat fermented milk with the addition of kweni mango and randu honey was 0.89%. According to Desnilasari and Lestari (2014), the ash content in fermented products was influenced by the minerals from raw materials, such as etawa goat milk and other additional ingredients, including kweni mango, kapok honey, and skim milk. Based on the results, the protein content and fat content obtained were 1.72 and 4.78%, respectively, with goat milk being a major contributor. In addition, the carbohydrate content was 10.05%, and the product was reported to contain glucose, fructose, and sucrose. The carbohydrate content in synbiotic etawa goat milk was largely contributed by the addition of randu honey.

Based on the Indonesian standard SNI 7552:2018 for flavored fermented milk drinks, the best synbiotic Etawa goat milk was obtained with F4 formulation (15% kweni mango juice: 10% randu honey). Synbiotic Etawa goat milk with this formulation was preferred by the panelists in terms of taste, aroma, color, and overall acceptability. In addition, it showed antibacterial activity against *S. aureus* and *E. coli*, where the antibacterial activity against *S. aureus* was higher than *E. coli*.

REFERENCES

Association of Official Analytical (AOAC). 2019. Official Methods Of Analisis. Chemist, Washington DC, USA.

Abubakar MB, Abdullah WZ, Sulaiman SA, Suen AB. 2012. A review of molecular mechanisms of the anti-leukemic effects of phenolic compounds in honey. Intl J Mol Sci 13 (11): 15054-15073. DOI: 10.3390/ijms131115054.

Afifa K, Kamruzzaman M, Mahfuza I, Afzal H, Arzina H, Roksana H. A comparison with antioxidant and functional properties among five mango (*Mangifera indica* L.). Intl Food Res J 21 (4): 1501.

Apandi I, Fajar R, Yusmarini. 2016. Consumers preference mapping analysis for the sensory attribute of soyghurt product within the student Agricultural Faculty of University of Riau. Jurnal JOM Faperta 3 (1): 1-16. [Indonesia]

Badan Standarisasi Nasional. 2018. SNI-7552:2018 Flavoured fermented milk drinks. Badan Standarisasi Nasional Indonesia, Jakarta. [Indonesian]

Biplab B, Narayan C. 2014. Probiotics, prebiotics and synbiotics - In health improvement by modulating gut microbiota: The concept revisited. Intl J Curr Microbiol App Sci 3 (3): 410-420.

Boeni S, Pourahma R. 2012. Use of inulin and probiotic *Lactobacilli* in synbiotic yogurt production. Ann Biol Res 3 (7): 3486-3491.

Chandra EH, Lokapirnasari WP, Hidanah S, Al-Arif MA, Yuniarti WM, Luqman EM. 2022. Probiotic potential of on feed efficiency, weight, and carcass percentage in ducks. Jurnal Medik Veteriner 5 (1): 69-73. DOI: 10.20473/jmv.vol5.iss1.2022.69-73. [Indonesian]

De Garmo EP, Sullivan WG, Canada JR. 1984. *Engineering economy 7th Edition*. Mc Millan Publishing Company, London.

Desnilasari D, Lestari N. 2014. Formulation of synbiotic beverage based on banana puree (*Musa paradisiaca* var *sapientum*) using *Lactobacillus casei*. Jurnal Agritech 34 (3): 257-265. DOI: 10.22146/agritech.9453. [Indonesian]

Eleazu CO, Eleazu KC, Chukwuma S, Essien UN. 2013. Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. J Diabetes Metab Disord 12 (1): 60. DOI: 10.1186/2251-6581-12-60.

Harjiyanti MD, Pramono YB, Mulyani S. 2013. Total asam, viskositas dan kesukaan pada yogurt drink dengan sari buah mangga (*Mangifera indica*) sebagai Perisa Alami. Jurnal Aplikasi Teknologi Pangan Universitas Diponegoro 2 (2): 104-107. [Indoneisan]

Harun N, Rahmayuni, Sitepu Y. 2013. Addition of coconut sugar and time of fermentation on quality of red beans milk fermented (*Phaesolus vulgaris* L.). Jurnal Sagu 12 (2): 9-16. [Indonesian]

Lokapirnasari WP, Pribadi TB, Al Arif A, Soeharsono S, Hidanah S, Harijani N, Najwan R, Huda K, Wardhani HC, Rahman NF, Yulianto AB. 2019. Potency of probiotics *Bifidobacterium spp*. and *Lactobacillus casei* to improve growth performance and business analysis in organic laying hens. Vet World 12 (6): 860. DOI: 10.14202/vetworld.2019.860-867.

Martharini D, Indratiningsih. 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. [Indonesian]

Mostafa S, Wang Y, Zeng W, Jin B. 2022. Floral scents and fruit aromas: Fuctions, compositions, biosynthesis, and regulation. Front Plant Sci 13: 860157. DOI: 10.3389/fpls.2022.860157.

Nurainy F, Rizal S, Suharyono S. 2017. Antibacterial activity and characteristics of green grass jelly extract synbiotic drink with added pineapple and guava juice during cold storage. Prosiding Seminar Nasional BKS PTN Wilayah Barat Bidang Pertanian: 1186-1195. [Indonesian]

Oelschlaeger TA. 2010. Mechanisms of probiotic actions-A mini-review. Intl J Med Microbiol 300 (1): 57-62. DOI: 10.1016/j.ijmm.2009.08.005.

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

Combarros-Fuertes P, M. Estevinho L, Teixeira-Santos R, G. Rodrigues A, Pina-Vaz C, Fresno JM, Tornadijo ME. 2020. Antibacterial action mechanisms of honey: Physiological effects of avocado, chestnut, and polyfloral honey upon *Staphylococcus aureus* and *Escherichia coli*. Molecules 25 (5): 1252. DOI: 10.3390/molecules25051252.

Pranayanti I, Sutrisno A. 2015. The making of coconut water (*Cocos nucifera* L.) probiotic drink with starter *Lactobacillus casei shirota* strain. Jurnal Pangan dan Agroindustri 3 (2): 763-772. [Indonesian]

Purnomo H, Muslimin LD. 2012. Chemical characteristics of pasteurised goat milk and goat milk kefir prepared using different amount of indonesian kefir grains and incubation times. Intl Food Res J 19 (2): 791-794.

Putri K, Herawati K, Hamzah F. 2017. The usage of kuini fruit to make the product fruit leather with add on red dragon fruit. Jurnal Online Mahasiswa Fakultas Pertanian Universitas Riau 4 (2): 1-15. [Indonesian]

Rachman A, Taufik E, Arief II. 2018. Characteristics of roselle probiotic yoghurt made from goat's milk and cow's milk during storage at room temperature. Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan 6 (2): 73-80. [Indonesian]

Rahayu P, Nurwitri C. 2012. Mikrobiologi Pangan. IPB Press, Bogor. [Indonesia]

Rasbawati R, Irmayani I, Novieta ID, Nurmiati N. 2019. Organoleptic characteristics and ph value of yogurt with addition of noni fruit extract (*Morinda citrifolia* L). Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan 7 (1): 41-46. DOI: 10.29244/jipthp.7.1.41-46.

Ratya N, Taufik E, Arief II. 2017. Chemical, physical, and microbiological characteristics of etawa crossbred goat milk in Bogor. Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan 5 (1): 1-4. DOI: 10.29244/jipthp.5.1.1-4. [Indonesian]

Rizal S, Suharyono, Amelia JR. 2019. The effect of addition of sucrose solution on the antibacterial activities of green grass jelly extract sinbiotic beverages during storage in cold temperature. AGRIC Jurnal Ilmu Pertanian 31 (1): 53-66. DOI: 10.24246/agric.2019.v31.i1.p53-66

Rizal S, Suharyono S, Nurainy F, Amelia JR. 2020. 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.

Rizal S, Suharyono S, Suroso E, Melatiningsih A, Suyarto VA. 2023. Lactic acid bacteria viability and antibacterial activity of etawa goat's synbiotic milk. Biodiversitas 24 (12): 6440-6446. DOI: 10.13057/biodiv/d241205.

Scavuzzi BM, Henrique FC, Miglioranza LH, Simão AN, Dichi I. 2014. Impact of prebiotics, probiotics and synbiotics on components of the metabolic syndrome. Ann Nutr Disord Ther 1 (2): 1009.

Sumarlin L, Muawanah A, Wardhani P et al. 2014. Anticancer and antioxidant activity of honey in the market local Indonesia. Jurnal Ilmu Pertanian Indonesia 19 (3):136-144.

Thilagavathi T. 2020. Probiotics, prebiotics, synbiotics and its health benefits. Intl J Curr Microbiol App Sci 9 (11): 497-511. DOI: 10.20546/ijcmas.2020.911.061.

Triwanto J, Herlinda K, Muttaqin T. 2021. Physicochemical quality in honey from nectar of randu flower (*Ceiba pentandra*) and kaliandra (*Calliandra callothyrsus*). J For Sci Avicennia 4 (2): 102-113. DOI: 10.22219/avicennia.v4i2.19750. [Indonesian]

Yelnetty A, Hadju R, Tamasoleng M, Sakul S. 2014. Characteristic of local banana starch as prebiotic and effect addition of starch on physico chemical properties of goat milk yoghurt. Proc 16th AAAP Anim Sci Congr 2: 339.