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Physicochemical, Microbiological, and Sensory Properties of Probiotic Drink Made from Wild Horse Milk with Modified Banana Flour Addition

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ABSTRACT: Dairy products from wild horses in Sumbawa Island, Indonesia, are promising to be developed as a probiotic source due to naturally occurring lactic acid bacteria. However, it is less favored due to its pronounced horse flavor. The development of products can be accomplished by converting them into a probiotic drink and blending them with other ingredients to create a product with enhanced nutritional and sensory characteristics. This research aims to formulate probiotic drinks by blending wild horse milk and modified banana flour (MBF), evaluating their physicochemical and microbiological attributes and sensory quality. A one-factor, Completely Randomized Design (CRD) experimental design was employed in this research. The treatment involved the addition of MBF at three specific concentrations: 0%, 5%, and 10%. The results revealed that the addition of MBF led to significant differences ($p<0.05$) in the physicochemical properties (pH, viscosity, total dissolved solids, and syneresis). All formulations exceeded the minimum Lactic Acid Bacteria (LAB) requirements set by the Indonesian National Standard (SNI) 2981:2009, ranging from $1.57x10^9$ to $2.35x10^9$ CFU.mL^{-1}. According to the sensory evaluation, probiotic drinks containing 5% MBF exhibited the highest sensory quality, stimulating appetite with dominant attributes such as a brownish-white color, sour taste, minimal skunky flavor, and a slightly sandy/coarse fiber texture. In conclusion, adding MBF enhanced the probiotic drinks' physicochemical properties, total LAB content, and sensory quality.

Keywords*:* Banana; Development product; Probiotic drinks; Wild horse milk

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INTRODUCTION

Horse milk serves as an alternative source of animal protein in addition to cow and goat milk. It comprises 10.20% total solid content, 6.40% lactose content, and 2.10% protein content, with a casein-to-whey protein ratio of 1.10 and 1.20% fat content (Faccia et al., 2020). One of the advantages of horse milk is its higher whey protein content compared to cow milk. The whey protein found in horse milk has the potential to act as an antimicrobial compound against pathogens like *Salmonella* and *Staphylococcus aureus* (Sanam, 2022). Whey protein in horse milk comprises α-lactalbumin, β-lactalbumin, immunoglobulin, serum albumin, lactoferrin, and lysozyme (Malacarne et al., 2002). Furthermore, horse milk naturally contains lactic acid bacteria (LAB). The total LAB count in fresh horse milk from Polish Coldblood horse breeds is approximately $3.69x10^3$ $CFU.mL^{-1}$ (Czyżak-Runowska, 2018).

Sumbawa Island is home to 80% of the total horse population in West Nusa Tenggara province, with approximately 38,173 horses (BPS 2020). Unlike horses in other regions primarily utilized for transportation, the horses on Sumbawa Island are predominantly raised for milk production and are referred to as wild horses. The term "wild" pertains to the extensive method of livestock management, wherein horses are allowed to roam freely on pastures owned by residents and fend for their food. Subsequently, farmers locate these horses when it is time for milking. The government of West Nusa Tenggara has officially recognized wild horses as an indication of origin and a cultural heritage of local customs (Prastyowati, 2021). Local inhabitants have been consuming wild horse milk for generations, attributing it to numerous health benefits (Laili, Setyowati, and Iravati, 2014). Fresh wild horse milk possesses a white hue, a liquid consistency, and a mildly sweet flavor. Each horse can yield 1-2 liters of milk daily during lactation. Most of the local residents earn their livelihoods as farmers and sell wild horse milk as a supplementary source of income. Wild horse milk is typically distributed through intermediaries in West Nusa Tenggara and Java (Prastyowati, 2021). Therefore, it has the potential to be developed as a means of food diversification to enhance the economic well-being of the local community.

Wild horse milk from Sumbawa Island is among the local foods that harbor probiotics. It is a natural source of probiotics due to the presence of Lactic Acid Bacteria (LAB) (Sujaya et al., 2008). *Lactobacillus sp.* is one of the LAB strains identified in wild horse milk (Antara, Dibia, and Aryanta, 2009). Widiada (2021) indicated that the LAB species identified in wild horse milk exhibit a homofermentative fermentation pattern, producing lactic acid as the final product. Lactic acid leads to a decrease in the milk's pH and inhibits the growth of pathogenic bacteria. Functional foods based on probiotics are reported to have the potential to modulate gut microbiota, enhancing the immune system's ability to reduce and control inflammation triggered by pathogens (Di Cerbo et al., 2017). LAB derived from wild horse milk also demonstrates the potential to serve as an antioxidant and antidiabetic component (Fidien et al., 2021).

During the storage period, wild horse milk undergoes spontaneous fermentation by LAB, which helps prevent coagulation and spoilage (Hakim, Suada, and Sampurna, 2013). However, the spontaneous fermentation process leads to variable outcomes, including the inability to control the final pH of the product. The likelihood of contamination during the unsanitary milk collection process and the absence of pasteurization before packaging further diminish the quality of the fermented milk produced. Additionally, fermented horse milk tends to develop a pronounced skunky flavor, making it less appealing to consumers (Ardiansyah et al., 2021; Sofiyatin and Widiada, 2018). Skunky is an unpleasant taste and aroma commonly

associated with animals. Hence, products derived from wild horse milk can be enhanced by converting it into a probiotic drink and incorporating it with other ingredients to create a product with improved nutritional and sensory attributes, such as blending it with banana.

Banana is a horticultural product with the highest production among all fruits in Indonesia, totaling 9.24 million tons annually (BPS, 2022). Because of its dietary fiber and resistant starch content, bananas possess the potential to serve as a source of prebiotics (Jaiturong et al., 2020; Powthong et al., 2020). Unripe bananas inherently possess a low resistant starch (RS) type 2 content and exhibit instability during processing. Converting RS type 2 into RS type 3 enhances starch stability. The retrogradation process produces RS type 3, necessitating starch heating and cooling (Bojarczuk et al., 2022). Resistant starch produced by modified banana flour (MBF) contains a higher amylose content and a lower glycemic index than unmodified banana flour (Jenie, Putra, and Kusnandar, 2012).

The incorporation of bananas into dairy products is a common practice. The prebiotics in bananas enhance LAB's viability and improve yogurt's sensory quality (Falah et al., 2021). Additionally, RS type 3 contributes to an increase in total solids and enhances the final viscosity of yogurt (He et al., 2019; Mwizerwa et al., 2017). The substitution of modified Uli banana flour (MUBF) in yogurt significantly enhanced the texture, color, and overall preference for the yogurt (Jenie, Saputra, & Widaningrum, 2013). The objective of this research was to create a probiotic drink using wild horse milk with the addition of MBF, assess its physicochemical and microbiological characteristics, and evaluate the sensory quality of the probiotic drink.

MATERIALS AND METHODS

Materials

Fresh wild horse milk was sourced from Penyaring Village, Sumbawa Island, Indonesia, while Kepok bananas were procured from a traditional market in Mataram, West Nusa Tenggara, Indonesia. Furthermore, starter cultures (*Lactobacillus bulgaricus* FNCC 0472, *Streptococcus thermophilus* FNCC 0040, and *Lactobacillus rhamnosus* FNCC 0052) were acquired from the Food and Nutrition Center Studies at Gadjah Mada University (UGM), Indonesia. Additional ingredients included skimmed milk (Indoprima, Indonesia) and liquid stevia sweetener (Drip Sweet CV. Oil M3, Indonesia).

Table 1. The formula of probiotic drinks made from wild horse milk with MBF

Ingredients	Unit	0% MBF	5% MBF	10% MBF
Wild horse milk	mL	1000	1000	1000
Modified banana flour (MBF)	g	0	50	100
Culture starter	%	3	3	3
Skimmed milk	g	50	50	50
Stevia sweetener	drop	4	4	4

This experimental research employs a Completely Randomized Design (CRD) with a single factor. The treatment in this research involves the addition of modified banana flour (MBF) at different concentrations, specifically 0%, 5%, and 10%. Table 1 illustrates the formulation of probiotic drinks.

Production of modified banana flour (MBF)

The production of MBF was adapted from (Sukasih et al., 2021). In this research, half-ripe Kepok bananas were utilized. The Kepok bananas were peeled and sliced to a thickness of approximately 2 mm using a fruit slicer. The banana segments were

immersed at room temperature for 24 hours in a 3:4 ratio of distilled water to encourage natural fermentation. Subsequently, the banana segments were drained and subjected to high-pressure heating in an autoclave at 121°C for 15 minutes. The subsequent step involved a cooling period of 36 hours to reduce the temperature to below 10°C. In the subsequent step, the bananas underwent an 8-hour drying process at 60°C inside a cabinet dryer. Subsequently, they were pulverized using a blender and sifted through a sieve with a 60-mesh opening.

Production of probiotic drinks using MBF addition

The production of probiotic drinks was adapted from Raut et al. (2015), originally intended for the production of yogurt drinks. The process involved several sequential stages. In the initial stage, 1000 mL of fresh wild horse milk was mixed with 5% skim milk and MBF, depending on the treatments (0%, 5%, and 10%). The blend was homogenized at 4000 revolutions per minute (rpm) for 10 minutes. Subsequently, the mixture underwent pasteurization at a temperature of 65°C for 20 minutes and was cooled to 37°C. Following the cooling step, starter cultures were introduced, consisting of 3% of a mixture comprising *L. bulgaricus, S. thermophilus, and L. rhamnosus* in a 1:1:1 ratio, all at a temperature of 37°C. The inoculation process lasted for 24 hours. Following fermentation, liquid stevia sweetener was incorporated and agitated until achieving homogeneity. Finally, probiotic drinks were stored at temperatures below 10°C.

Analysis of physicochemical properties and resistant starch content

The physicochemical analysis of probiotic drinks, encompassing parameters such as pH, viscosity, total dissolved solids, total titratable acidity, and syneresis, was conducted following the methods outlined by the Association of Official Agricultural Chemists (AOAC, 2005). The pH analysis was performed utilizing a pH meter, wherein the pH electrode was immersed into the sample solution and allowed to equilibrate until the pH meter displayed a stable pH reading. Viscosity analysis used a viscometer with spindle number 1, operating at 60 rpm. The results can be read directly on a viscometer in units of mPa.s. Total dissolved solids analysis employs a refractometer, where the sample is applied onto the prism of the refractometer, and the results can be directly observed in units of °Brix.

The analysis of total titratable acidity (TAT) employs the titration method. Three drops of phenolphthalein (PP) indicator are added to the sample and titrated using NaOH 0.1 N. The titration process is halted when the solution changes to a pink color. The result is calculated using the following formula.

$$\text{TAT (\%)} = \frac{\text{volume of NaOH (mL)x 10 (Normality of NaOH)x 64(dilution factor)x 100}}{\text{0,1 x sample weight (g)}}$$

Syneresis analysis utilizes a centrifuge. The sample is centrifugated at 1500 rpm for 20 minutes to obtain the supernatant. The result is calculated using the following formula.

$$\text{Syneresis (\%)} = \frac{\text{initial weight of the sample} - \text{final weight of sample (the precipitate)}}{\text{final weight of sample}} \text{ x } 100$$

The analysis of resistant starch in MBF is based on the method described by Goñi et al. (1996). The determination of resistant starch content involves four primary stages. Initially, protein removal is carried out using pepsin enzyme at 40°C for 60 minutes. Next, non-resistant starch is removed using α-amylase enzyme at 37°C for 16 hours. Subsequently, the resistant starch is dissolved and hydrolyzed using amyloglucosidase (AMG) at 60°C for 45 minutes, and the determination of glucose

resulting from the hydrolysis of resistant starch is performed using the GOPOD reagent.

Determination of Total LAB (Lactic Acid Bacteria)

The total LAB analysis of probiotic drinks follows the procedure outlined in Pelczar and Chan (2007). This method employs a stratified dilution approach, wherein a 1 mL sample is mixed with 9 mL of phosphate buffer, forming what is referred to as dilution-1. The outcome of dilution-1 (1 mL) is mixed with 9 mL phosphate buffer to produce dilution-2. This dilution process is repeated until reaching dilution-9. The results from the final three dilutions are selected for inoculation onto De Man Rogosa Sharp Agar (MRSA) media through the pour plate method, followed by incubation at 37°C for 48 hours. The results are subsequently calculated using the standard plate count method.

Analysis of sensory quality of probiotic drinks

In this research, we performed sensory analysis utilizing Quantitative Descriptive Analysis (QDA). QDA was employed to assess the intensity of sensory attributes in probiotic drinks, employing an intensity rating scale ranging from 0 to 10 (<6 = categorized as poor; 6-7 = rated as fair; 8-10 = considered high) (Chapman, Lawless, and Boor, 2001). The analysis involved eight trained panelists who had previously undergone selection and training in the analysis of sensory attributes. All panelists signed an informed consent form before the sensory evaluation.

Data Analysis / Statistics

The collected data is initially processed in several stages: coding, entering, cleaning, and editing. Microsoft Excel 2019 was employed for data processing, and subsequently, IBM Statistical Program for Social Science (SPSS) version 23 was used for data analysis. The data of sensory quality is presented in a spider web using Microsoft Excel 2019. The data about physicochemical properties and total LAB were subjected to Analysis of Variance (ANOVA). In cases where significant differences in the results were observed, further testing was conducted utilizing Duncan's Multiple Range Test, with a significance level (p-value) less than 0.05 ($p<0.05$).

RESULTS AND DISCUSSION

Resistant starch content in MBF

Table 2 displays the resistant starch (RS) content identified in MBF (Modified Banana Flour). The transformation of RS type 2 into RS type 3 was accomplished through fermentation and the autoclaving-cooling processes. Bananas inherently have a low RS type 2 content. The process of modification increases the RS content in banana flour. RS type 2 can be converted into RS type 3 via retrogradation by using autoclaving and cooling processes (Ashwar et al., 2016). Fermentation is conducted before retrogradation to facilitate the cleavage of -1,6-glycosidic bonds, particularly those in branching bonds within amylopectin molecules, shortening amylose chains (Setiarto et al., 2015). The fundamental principle underlying the retrogradation process involves the heating and cooling of starch. During the heating phase, amylopectin molecules break down into amylose. These amylose chains form robust and increasingly stable double-helix bonds as the cooling phase advances, resulting in the formation of RS type 3 (Wang et al., 2015).

Table 2. Resistant starch content in MBF

Sample	Banana type	Resistant starch (mg/100g)
Modified banana flour (MBF)	Kepok	11.35±0.16

RS type 2 in conventional banana flour tends to be unstable during processing. During the heating process, it undergoes gelatinization, which increases digestibility

in the gastrointestinal (GI) tract and reduces RS levels. The conversion of RS type 2 to RS type 3 makes RS more resistant to changes during the heating process, consequently leading to a higher RS content (Han et al., 2023; Nurhayati et al., 2014). According to Jenie et al. (2012), the RS content in conventional banana flour ranged from 6.39% and increased to 12.99% after modification.

Digestive enzymes in the human gastrointestinal (GI) tract do not readily digest RS type 3. RS type 3 is beneficial as it supports the growth of gut microbiota since it undergoes fermentation to produce short-chain fatty acids (SCFA) (Chang et al., 2021). Gut bacteria generate three primary SCFAs: acetic acid, butyric acid, and propionic acid. SCFAs function as anti-inflammatories, enhancing the body's immune system (Xiong et al., 2022).

Incorporating RS into yogurt can also influence the sensory acceptability of the products. RS augments viscosity, thereby influencing the ultimate texture of yogurt. Mwizerwa et al. (2017) demonstrated that adding RS can enhance the texture of yogurt compared to yogurt without RS. The inclusion of RS can also enhance the flavor of yogurt. RS enhances the fermentation activity of LAB, producing lactic acid, which imparts an acidic aroma and taste to the yogurt. Furthermore, the additional flavor is influenced by the type of starch used in yogurt (Saleh et al., 2020).

Physicochemical characteristics of probiotic drinks

Table 3 displays the physicochemical characteristics of probiotic drinks. A significant difference ($p<0.05$) was observed among probiotic drinks' pH, viscosity, syneresis, and total dissolved solids. Nevertheless, this difference did not impact the overall total titratable acidity. The incorporation of MBF resulted in a decrease in pH, with the lowest pH observed in the 10% MBF (3.58±0.13) formulation, followed by the 5% MBF (3.95±0.07) and 0% MBF (4.27±0.09) formulations. The viscosity of probiotic drinks was highest in the 10% MBF formula (79.35±0.91 mPa.s), followed by the 5% MBF (70.00±4.24 mPa.s) and 0% MBF (61.50±2.11 mPa.s) formulations, while syneresis values for the 0%, 5%, and 10% of MBF are 87.38±0.73%, 80.16±0.51%, and 61.75±0.35%, respectively. Total dissolved solids increased with the addition of MBF, with values of 11.60±0.14°Brix for the 0% MBF, 12.60±0.42°Brix for the 5% MBF, and 13.35±0.07°Brix for the 10% MBF formulations. The total titratable acidity of probiotic drinks did not show significant differences among the treatments. However, the values for 10% MBF (0.85±0.08%), 5% MBF (0.63±0.13%), and 0% MBF (0.56±0.56%) increased with the addition of MBF.

Adding MBF to probiotic drinks results in a decrease in pH during fermentation and an increase in the total titratable acidity of the probiotic drinks. A similar discovery was reported by Handayani and Nuraini (2022), who indicated that higher additions of banana flour would lower the pH of yogurt. Nonetheless, it led to an overall increase in the generated acidity. MBF contributed more resistant starch than conventional flour, promoting increased acid production during fermentation and lowering the pH of probiotic drinks (Jenie et al., 2013; Nurhayati et al., 2014). All the formulations met the titratable acidity requirements for yogurt as specified in SNI 2981:2009, ranging from 0.5% to 2.0% (BSN, 2009).

The presence of lactose decreased during the fermentation of probiotic drinks with the addition of MBF. Lactose is one of the primary carbohydrate sources in milk. MBF enhanced the fermentation activity of LAB, leading to an increased breakdown of lactose into lactic acid and other components (Huppertz, 2017). Lactic acid impacts the flavor of probiotic drinks by imparting the characteristic acidic taste. Additionally, some aroma compounds produced by LAB, such as acetaldehyde, ethanol, and diacetyl, contribute to the overall flavor profile of yogurt (Chen et al., 2017).

On the other hand, the presence of viscosity and total dissolved solids increased during fermentation. RS type 3 derived from MBF finds wide application in food manufacturing. RS can be employed as a thickening agent to enhance the sensory quality of the product (Han et al., 2023). The addition of RS type 3 increases the viscosity of yogurt (He et al., 2019). Mwizerwa et al. (2017) also demonstrated that adding modified cassava starch raised the total solids content of yogurt from 17.08±0.17% to 19.26±0.09%.

Table 3. Physicochemical characteristics of probiotic drinks with MBF

Characteristics	0% MBF	5% MBF	10% MBF
pH	4.27±0.09[a]	3.95±0.07[a]	3.58±0.13[b]
Total titratable acidity (%)	0.56±0.57[a]	0.63±0.13[a]	0.85±0.08[a]
Lactose content (%)	11.53±0.31[a]	9.89±0.12[b]	8.57±0.52[c]
Viscosity (mPa.s)	61.50±2.12[b]	70.00±4.24[a]	79.35±0.91[a]
Total dissolved solids (°Brix)	11.60±0.14[b]	12.60±0.42[a]	13.35±0.07[a]
Syneresis (%)	87.38±0.73[a]	80.16±0.51[b]	61.75±0.39[c]

Notes: The average values in the same row with different letters (a, b, c) indicate a significant difference ($p<0.05$). MBF= modified banana flour.

The occurrence of liquid separation from the gel is associated with a process known as syneresis (Mizrahi, 2010). Because of the instability of the gel (curd), the gel formed from acidified milk will experience syneresis, which is the separation of liquid (whey) from the gel (curd) (Walstra, 1993). The inclusion of MBF reduces syneresis in probiotic drinks. The greater the amount of MBF added, the less pronounced the syneresis in probiotic drinks becomes. A similar observation was reported by Saleh et al. (2020), who noted that adding starch could diminish the syneresis of yogurt. Mwizerwa et al. (2017) also demonstrated that yogurt containing modified cassava starch exhibited the lowest syneresis during the 0-21-day period compared to yogurt without modified cassava starch. Incorporating RS into probiotic drinks leads to a firmer gel structure, reducing the extent of whey separation (He et al., 2019).

The total of lactic acid bacteria (LAB) in probiotic drinks

The total number of LAB in fresh wild horse milk was $3.05x10^8$±0.21 CFU.mL^{-1}. The total LAB count increased when wild horse milk was transformed into probiotic drinks. Following the conversion into probiotic drinks, fermentation raised the total LAB count in wild horse milk from 10^8 CFU.mL^{-1} to 10^9 CFU.mL^{-1}. The starter culture comprised *L. bulgaricus, S. thermophilus, and L. rhamnosus.* Figure 1 illustrates the total LAB content.

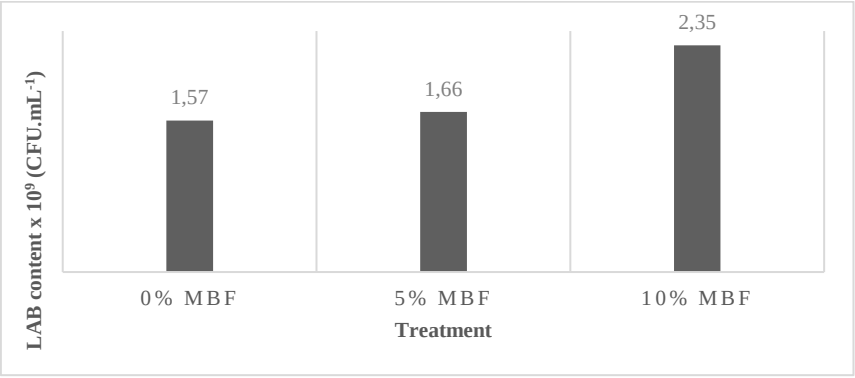

Figure 1. The total LAB in probiotic drinks with MBF addition

Adding MBF to probiotic drinks has a notable impact ($p<0.05$) on the total LAB count. The incorporation of MBF significantly influenced the total LAB count, with values of 0% MBF ($1.57x10^9±0.04$ CFU.mL^{-1}), 5% MBF ($1.66x10^9±0.04$ CFU.mL^{-1}), and 10% MBF ($2.35x10^9±0.23$ CFU.mL^{-1}). Resistant carbohydrates present in MBF display potential prebiotic activity. LAB are capable of fermenting these resistant carbohydrates due to the inability of human gastrointestinal tract enzymes to metabolize them (Bojarczuk et al., 2022).

The 10% MBF formula resulted in the highest total LAB count compared to the other formulations. All treatments exceeded the minimum LAB content requirement for yogurt specified in SNI 2981:2009, 10^7 CFU.g^{-1} (BSN, 2009). Similar outcomes were reported by Jenie et al. (2013), who indicated that yogurt containing modified Uli banana flour yielded a total LAB count of up to 10^9 CFU.mL^{-1}. Furthermore, in yogurt containing unmodified Ambon banana flour at 0-5% concentrations, the total LAB count reached only 10^8 CFU.mL^{-1} (Handayani and Nuraini, 2021). Incorporating RS type 3 into yogurt helps maintain the viability of probiotic bacteria at levels exceeding 10^8 CFU.g^{-1} throughout a storage period of 0-21 days, surpassing yogurt formulations containing RS type 2 or without RS (He et al., 2019).

The sensory quality of probiotic drinks

Quantitative Descriptive Analysis (QDA) was conducted for all three formulations.

Figure 2 illustrates the intensity of sensory attributes in probiotic drinks. According to the QDA, the sensory attributes encompassed appearance (color, roughness, and shine), aroma (sour, banana, skunky), texture (thickness and sandy/coarse fibers), taste (sour, banana, skunky, sweet, and bitter), mouthfeel (sandy/coarse fibers and skunky), and aftertaste (sandy/coarse fibers and sweet).

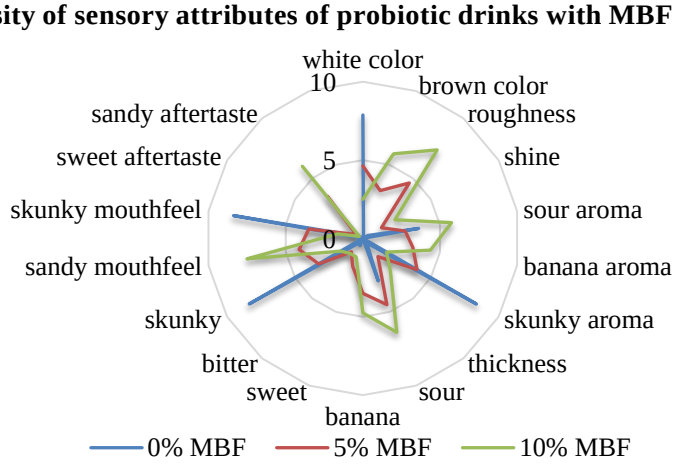

Figure 2. The intensity of sensory attributes in probiotic drinks with the addition of MBF

Probiotic drinks without MBF exhibited a white color, probiotic drinks with 5% MBF tended to be somewhat brownish-white, and those with 10% MBF had a brownish color. Probiotic drinks lacking MBF tended to exhibit more pronounced skunky characteristics, which were dominant (with a score>8) in terms of aroma, taste, and mouthfeel. Skunky is a characteristic animal-like flavor that consumers tend to dislike, which could lower the acceptability of probiotic drinks. However, the banana flavor from MBF and the acidic flavor resulting from the fermentation process reduced the skunky taste in probiotic drinks. Consequently, the intensity of the skunky flavor decreased from a score of 8 (high intensity) to 3-4 (low intensity). Probiotic drinks with 5% MBF primarily exhibit a sour taste with a slightly

sandy/coarse fiber texture. Adding MBF enhanced the sandy/coarse fibers texture, mouthfeel, and aftertaste in probiotic drinks. Probiotic drinks containing 10% MBF have a high intensity (with a score of 7.5) for coarse fibers, while those with 5% MBF exhibit a lower intensity (score of 3-4), and probiotic drinks without MBF do not possess the coarse fibers attribute. A more significant proportion of MBF will intensify the coarse fiber texture in probiotic drinks. Similar outcomes were also observed by Tsyganov et al. (2022), who reported that including starch in dairy products can evoke an unpleasant sensation of coarse fibers upon consumption. Generally, the acceptance rate of probiotic drinks with 5% MBF is the highest compared to other formulations. Probiotic drinks with 5% MBF exhibited a low intensity of skunky flavor (aroma, taste, and mouthfeel), a mild sandy/coarse fiber texture, and a sour taste that panelists found acceptable.

CONCLUSION

Probiotic drinks using wild horse milk as a base can be formulated using modified banana flour (MBF). The addition of MBF influences the physicochemical properties (pH, viscosity, total dissolved solids, and syneresis) and microbiological characteristics (total LAB content) of the probiotic drinks. All formulations of probiotic drinks exceeded the minimum total LAB requirements for yogurt, with counts ranging from $1.57x10^9$ to $2.35x10^9$ $CFU.mL^{-1}$ as regulated in SNI 2981:2009. Based on the sensory evaluation, probiotic drinks containing 5% MBF exhibit the highest sensory quality, likely stimulating the appetite by reducing the skunky flavor typically associated with wild horse milk. A brownish-white color, a sour taste, and a slight sandy/coarse fiber texture characterize these drinks. Additional research is needed to explore incorporating alternative flavors, such as fruit or herbal extracts, to mitigate the horse-like flavor in probiotic drinks formulated using wild horse milk.

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