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The Effectiveness of Kombucha as a Starter in the Production of Fermented Milk Beverages

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ABSTRACT. A symbiotic culture of kombucha (SCOBY) has the potential to act as a probiotic source of lactic acid bacteria, which can be combined with fermented beverage starters using fresh or reconstituted milk as a medium. This study aims to evaluate the effectiveness of kombucha in producing fermented milk products with microbiological characteristics. The experimental design used was a completely randomized factorial design with two treatment levels: skim milk concentration and kombucha concentration. The parameters observed included total lactic acid bacteria, pH, and total lactic acid. Data were analyzed by ANOVA using IBM SPSS Statistics program 29, and if significant effects were found, Duncan's post-hoc test was conducted. The results showed that using kombucha as a starter had a very significant effect ($P<0.01$) on all three variables observed. The amount of lactic acid bacteria (LAB) in the fermented milk met the SNI standard, with a concentration of 7 log CFU/ml. It can be concluded that kombucha can be used as a starter in fermented milk production. Although the percentage of lactic acid levels and pH values are not yet listed in the Indonesian National Standard, both pH value and lactic acid levels showed a very significant effect. A higher percentage of kombucha starter led to higher lactic acid content, which is a result of bacterial metabolism. Additionally, a higher percentage of kombucha starter resulted in a lower pH value in the kombucha-fermented milk.

Keywords: fermentation, fermented milk, kombucha, lactic acid bacteria

INTRODUCTION

In general, consumers aim to live longer lives with better quality. This goal often leads to the adoption of alternative lifestyles, including the consumption of more "functional" foods that help achieve desired health qualities and address growing health concerns. In line with increasing health issues in Indonesia and around the world, interest in functional products is on the rise, and consequently, the market share for these products has also increased. In addition to their nutritional benefits, functional foods provide active compounds that offer indirect health benefits (Kusumayanti et al., 2018). These foods can promote better health, have physiological effects, and help manage specific disorders and diseases (Çakiroğlu and Uçar, 2018; Tahergorabi et al., 2015). In recent years, public awareness of the importance of foods and beverages with functional health benefits has led to the rapid development of fermented products, particularly probiotic products that contain lactic acid bacteria.

Symbiotic cultures in the form of kombu mushrooms or SCOBY (Symbiotic Culture of Bacteria and Yeast) have shown potential as probiotics (Khaerah and Akbar, 2023). SCOBY, also known as tea mushroom, has a pancake-like shape and a white (pale) color, with a chewy texture similar to rubber and a gel-like consistency (Jayabalan et al., 2014). Kombucha is widely recognized for its benefits, including antibacterial activity and its role in producing anti-carcinogenic compounds, antioxidants, anti-hypertensive effects, and anti-inflammatory properties (Elkhtab et al., 2017; Malbasa et al., 2009; Zubaidah et al., 2014). The fermentation process in kombucha involves both bacteria and yeast, which are crucial for producing the tea drink. The bacteria involved in kombucha fermentation include lactic acid bacteria (LAB) and acetic acid bacteria (AAB) (De Roos and De Vuyst, 2018; Villarreal-Soto et al., 2018). Lactic acid bacteria are probiotic bacteria that can be used as a starter culture for fermented beverages made with fresh or reconstituted milk (Villarreal-Soto et al., 2018).

Milk fermentation, particularly using probiotic bacteria, holds great potential for innovation. This encourages new possibilities, such as using SCOBY and kombucha as starters in making probiotic drinks. The fermentation process not only extends the shelf life of milk but also improves its flavor, making fermented milk more popular among consumers. Probiotic drinks provide digestive health benefits by improving the balance of intestinal microflora and can survive stomach acidity if consumed in amounts of at least 1×10^6 CFU/ml (Rizal et al., 2016). Given these

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potential benefits, it is important to research the effectiveness of using kombucha bacteria as a starter for milk fermentation, with the aim of improving product quality and developing probiotic beverage products that contribute to health maintenance.

MATERIALS AND METHODS

Material

This research was conducted at the Integrated Laboratory of the Faculty of Animal Husbandry and Fisheries, West Sulawesi University. The materials used included skim milk, distilled water, SCOBY, kombucha products, methylated spirits, MRS agar (MRSA), MRS Broth (MRSB), Buffered Pepton Water (BPW), 70% alcohol, distilled water, 0.1 N NaOH, NaCl, tissue, cotton, and gloves.

Method

The experimental design used was a completely randomized design (CRD) with a factorial pattern, consisting of 2 treatment factors: milk concentration and kombucha concentration. Kombucha concentration levels were KK1 (10%) and KK2 (15%), while skim milk concentration consisted of 4 levels: SK1 (15% skim milk), SK2 (20% skim milk), SK3 (25% skim milk), and SK4 (30% skim milk). The percentage of skim milk (b/v) was calculated based on the volume of raw materials used. The total measurement data obtained were then analyzed by ANOVA (analysis of variance) using IBM SPSS Statistics program 29. If there was a significant effect between treatments, Duncan's further test was conducted.

Kombucha Starter Preparation

Starter preparation follows the method described by Malbaša et al. (2014). It begins with preparing the SCOBY and fermented tea to produce kombucha. Then, 10% (v/v) and 15% (v/v) of the kombucha supernatant were taken and inoculated into each concentration of skim milk.

Preparation of Fermented Milk

The preparation of fermented milk follows a modified method based on Malbaša et al. (2014) and Kruk et al. (2021). The initial stage involves pasteurizing the milk at 90°C for 5 minutes, then cooling it to 42°C. After that, the kombucha starter is inoculated into the pasteurized skim milk powder according to each treatment and homogenized. The mixture is then incubated for 24 hours at 42°C.

Variables Observed

Total Lactic Acid Bacteria

Total LAB was determined by counting the lactic acid bacteria colonies that grew on MRS (Man Rogosa Sharpe) agar medium. The sample was first diluted to prevent overcrowded colonies and ensure more accurate results. A total of 1 ml of fermented milk sample was diluted into 9 ml of 0.1% BPW solution. Dilution was carried out up to 10^{-7} (10^{-5}, 10^{-6}, 10^{-7}). From each dilution series, 1 ml of the solution was transferred to a sterile petri dish and then poured with 10-15 ml of deMan Rogosa Sharpe Agar (MRSA) medium, followed by homogenization. The mixture was left to solidify and then incubated at 37°C for 24-48 hours in an inverted position. Plates showing between 25 and 250 colonies were counted, and the result was expressed as colony-forming units per milliliter (CFU/ml) of the sample inoculated.

pH Value

pH measurement was carried out using a pH meter. The pH meter was first turned on and then standardized with pH 4 and pH 7 buffer solutions. A total of 30 ml of the fermented milk sample was placed in a 50 ml beaker. The pH meter electrode was then immersed into the sample and allowed to stabilize until the pH reading remained constant (Retnowati and Kusnadi, 2014).

Total Acid Test

The total acidity was determined using the Titration Method. Acidity was analyzed by titrating with 0.1 N NaOH, using 1% phenolphthalein as an indicator (Analysis, 2023).

RESULTS AND DISCUSSION

Total Acid Bacteria

Table 1 shows that using kombucha as a starter for making fermented milk significantly affects ($P<0.01$) the number of lactic acid bacteria (LAB). The total LAB in kombucha-fermented milk ranged from 5.1 to 9.3 CFU/ml, with an average of 9.9 CFU/ml. Table 3 further reveals that the highest number of LAB (9.3) was obtained at a 25% milk concentration with a 15% kombucha starter, while the lowest (5.1) was found at a 15% milk concentration using a 15% kombucha starter. The total LAB count meets the minimum SNI standard for fermented milk, which sets the threshold at 7 log CFU/ml. This increase in LAB indicates that the bacteria are actively growing and metabolizing in milk fermented with kombucha.

Bacteria utilize lactose from skim milk as a nutrient source for metabolism. The analysis of variance showed a very significant interaction (P<0.01) between milk concentration and kombucha concentration on the LAB value of fermented milk. This interaction occurred between 10% and 15% kombucha concentrations with a skim milk percentage close to 20% (Figure 1).

Table 1. Mean number of lactic acid bacteria with different milk concentration and kombucha concentration treatments (log transformation)

Skim Milk Concentration	Kombucha Concentration		Mean
	KK1	KK2	
KS1	6.6±2[b]	5.1±3[b]	5.8±6,9[b]
KS2	6.4±9,7[b]	7.1±1[b]	6.8±7,5[b]
KS3	7.6±1,5[a]	9.3±5.5[b]	8.5±1.3[a]
KS4	5.8±7,5[b]	8.2±2[b]	7±8.8[b]
Mean	7±1.5[b]	9.9±6.3[a]	

Note: [a,b] different superscripts indicate significant differences (P<0.01)

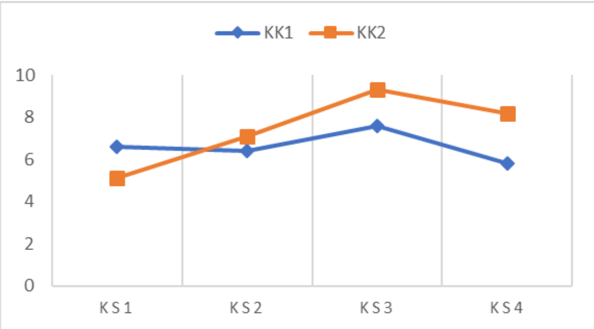

Figure 1. Interaction Between Milk and Kombucha Concentration on Total LAB

LAB play a crucial role in kombucha fermentation. According to Marsh et al. (2014), several types of LAB found in kombucha include bacteria from the Lactobacillus, Lactococcus, Bifidobacterium, and Leuconostoc groups. In addition, Suciati et al. (2019) found that the LAB count in whey fermented with kombucha ranged from 7.36 to 7.67 log CFU/ml. The LAB in kombucha can support the growth of acetic acid bacteria (AAB), which, in turn, increases cellulose production and stimulates the growth of Gluconoacetobacter (Marsh et al., 2014).

As shown in Figure 2, the higher the milk concentration, the greater the LAB count in the fermented milk. This occurs because microorganisms in the milk break down lactose into glucose and galactose with the aid of the enzyme lactase. These monosaccharides are processed through glycolysis into pyruvate, which is then reduced by lactic acid bacteria (Mandang et al., 2016). With more available nutrients, the number of LAB also increases. Currently, the number of microorganisms in kombucha-fermented milk drinks has met the SNI requirements. This indicates that dairy products as a medium can yield excellent microbiological quality in kombucha-fermented milk. Hrnjez et al. (2014) found, using kombucha as an inoculum for fermenting milk showed a significant increase in the LAB population and demonstrated bacterial inhibition activity.

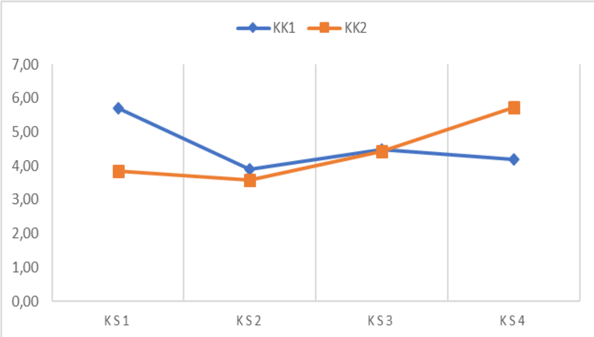

Figure 2. Interaction between Milk and Kombucha Concentration on Total Lactic Acid

pH Value

Based on Table 2, the pH of kombucha-fermented milk ranges from 5.22 to 5.63, with an average of 5.04. This indicates that the pH of all fermented milk treatments decreased compared to the pH of normal milk. The decrease in pH is attributed to the accumulation of acids produced by lactic acid bacteria (LAB) during the fermentation of lactose. Lactose in skim milk is readily fermented by LAB, which causes the milk to become more acidic (Mandang et al., 2016). However, the pH values of kombucha-fermented milk do not meet the SNI milk standard, which is set between 4.0 and 4.5. This discrepancy may be due to the 24-hour fermentation period, which may not have allowed the bacteria to work optimally in producing acids that lower the milk's pH. Kombucha fermentation typically lasts between 7 to 60 days, with biological activity increasing as fermentation progresses; the best results are usually observed after about 15 days (Villarreal-Soto et al., 2018). In general, the pH of fermented milk varies depending on the fermentation time, typically ranging from 4.5 to 4.7 (Kruk et al., 2019).

Kruk et al. (2021) also found that the pH of fermented milk using SCOBY culture was between 4.5 and 5.5. However, there is no definitive information about the ideal pH of kombucha starter culture for milk fermentation. Another factor influencing pH is the origin of the SCOBY, as different studies use various sources of SCOBY and kombucha with different microbiological compositions.

Table 2. Mean number of pH value with different milk concentration and kombucha concentration treatments

Skim Milk Concentration	Kombucha Concentration KK1	Kombucha Concentration KK2	Mean
KS1	5.45±0.17	5.22±0.15	5.33±0.16
KS2	5.53±0.08	5.37±0.15	5.45±0.12
KS3	5.52±0.06	5.30±0.18	5.41±0.15
KS4	5.63±0.18	5.25±0.17	5.44±0.27
Mean	5.53±0.08[a]	5.28±0.07[b]	

Note: [a,b] different superscripts indicate significant differences (P<0.01)

The analysis of variance showed a very significant difference (P<0.01) based on the kombucha concentration. However, there was no interaction between the two factors regarding the pH value of kombucha-fermented milk. The pH of commercially available fermented milk typically ranges between 3.9 and 4.7, depending on the type of product, fermentation duration, and other influencing factors.

Total Acid Test

Based on Table 3, the lactic acid content of kombucha-fermented milk ranges from 3.5% to 5.7%, with an average of 4.6%. The results of the analysis of variance indicated a very significant difference (P<0.01) in lactic acid content based on milk concentration. During fermentation, lactic acid bacteria (LAB) perform metabolic processes that produce lactic acid, which is measured as the total acid content. Therefore, as the sucrose content in skim milk increases, LAB can utilize these nutrients for their metabolic processes. This finding aligns with the opinion of Jonathan et al. (2022), who stated that the fermentation activity carried out by LAB during the production of fermented milk leads to the accumulation of lactic acid, thus increasing the total acid content. The growth of microbes is directly related to the amount of lactic acid produced. LAB break down sucrose into lactic acid, contributing to this increase.

However, the level of total lactic acid in kombucha-fermented milk does not meet the SNI standards, which could be attributed to the relatively short fermentation time. The fermentation period may not have been long enough for the microbial growth to reach the stationary and optimum phases, thus preventing the lactic acid production from complying with the Indonesian National Standards. The longer the fermentation time, the greater the conversion of lactose into lactic acid by LAB, which would increase the total lactic acid content. This is in line with findings by Suharto et al. (2021) and Maitimu et al. (2013), who observed changes in lactic acid levels during storage, which correspond with microbial population changes.

Table 3. Mean number of total lactic acid with different milk concentration and kombucha concentration treatments

Skim milk Concentration	Kombucha Concentration KK1	Kombucha Concentration KK2	Mean
KS1	5.7±0.80[a]	3.85±0.57[b]	4.78±1.31[a]
KS2	3.90±0.11[b]	3.59±0.02[b]	3.75±0.22[b]
KS3	4.48±0.43[b]	4.42±0.74[b]	4.45±0.04[a]
KS4	4.20±0.16[b]	5.74±0.62[a]	4.97±1.08[a]
Mean	4.57±0.79	4.40±0.96	

Note: [a,b] different superscripts indicate significant differences (P<0.01)

Additionally, another factor that could influence the results is the predominance of acetic acid bacteria (BAA) in SCOBY and kombucha cultures. Research by Marsh et al. (2014) indicated that BAA make up 90% of the total microorganisms in kombucha and SCOBY, with yeast and LAB contributing a smaller percentage. While BAA dominate, Marsh et al. (2014) also noted that the microbial composition in milk changes during fermentation. The percentage of BAA decreases, while the proportion of LAB and yeast increases as the total microbial count rises. Consequently, bacterial activity continues to degrade lactose, which still impacts the total lactic acid content.

The results of the analysis of variance revealed a very significant interaction (P<0.01) between milk concentration and kombucha concentration regarding total lactic acid in fermented milk. The interaction was most pronounced at the 25% skim milk concentration with both 10% and 15% kombucha starter concentrations (Figure 2). As the concentration of milk and kombucha increases, and fermentation time lengthens, more lactic acid bacteria remodel nutrients, particularly sugars (glucose and fructose), into lactic acid and other organic acids. As a result, the total acid content produced increases.

CONCLUSION

This study demonstrates that using kombucha as a starter for fermented milk is highly effective in influencing the number of lactic acid bacteria (LAB), lactic acid content, and pH value.

The use of kombucha starter increases the fermentation products in skim milk with varying concentrations. Additionally, the lactic acid content is inversely proportional to the pH conditions. Based on the number of LAB, a 25% skim milk concentration is recommended. However, the lactic acid content and pH value observed in this study do not meet the Indonesian National Standard (SNI). This discrepancy is likely due to the short fermentation time and the predominant presence of acetic acid bacteria (BAA) in the fermentation process.

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