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Saccharomyces cerevisiae's POTENTIAL APPLICATION IN IMPROVING QUALITY OF YOGURT PRODUCED IN SUBOPTIMAL FERMENTATION CONDITIONS

POTENSI PENGGUNAAN *Saccharomyces cerevisiae* DALAM MENINGKATKAN KUALITAS YOGURT YANG DIPRODUKSI DALAM KONDISI FERMENTASI KURANG OPTIMAL

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Abstract

The quality of a yogurt highly depends on the milk's quality, the culture, and the incubation temperature. However, many home yogurt makers do not have access to fresh milk and incubator which may lead to subpar-quality yogurt. This research explored the potential of using *Saccharomyces cerevisiae* to improve yogurt quality when fermentation conditions are suboptimal. The experiment was conducted by inoculating ultra-high temperature (UHT)--sterilized milk with a 10% yogurt starter containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, also various *S. cerevisiae* concentrations (0; 0.156; 0.625; 2.5; and 10%). The inoculated milk was fermented at 30 °C for 24 hours. Their sensory qualities were examined by the panelists. Their acidity, pH, and microorganism count were examined before and after incubation. *S. cerevisiae* addition at 2.5% displayed better taste and texture without discernable unpleasant aroma. These improvements might be due to the ethanol production by *S. cerevisiae*. *S. cerevisiae* addition was also found to slightly inhibit the growth of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*. However, the combined number of these probiotic microorganisms (2.7×10^8 CFU/mL) was still by the national standard. *S. cerevisiae* addition potentially improves yogurt quality made from UHT milk incubated at lower temperatures.

Keywords: Fermentation; Improvement; Quality; *Saccharomyces cerevisiae*; Yogurt

Abstrak

Kualitas yogurt sangat dipengaruhi oleh kualitas susu, kultur bakteri, dan suhu inkubasi. Meskipun demikian, banyak pembuat yogurt rumahan kesulitan untuk memperoleh susu segar dan inkubator, sehingga menghasilkan yogurt dengan kualitas yang kurang optimal. Penelitian ini bertujuan untuk mengeksplorasi potensi penggunaan Saccharomyces cerevisiae untuk meningkatkan kualitas yogurt ketika kondisi fermentasi kurang optimal. Percobaan dilakukan dengan menginokulasi ultra-high temperature (UHT)-sterilized milk *dengan 10% bibit yogurt yang mengandung Lactobacillus delbrueckii subsp. bulgaricus dan Streptococcus thermophilus serta berbagai konsentrasi S. cerevisiae (0%; 0,156%; 0,625%; 2,5%; dan 10%). Susu yang diinokulasi kemudian difermentasikan pada suhu 30 ℃ selama 24 jam. Karakteristik yogurt yang dihasilkan dinilai kualitasnya oleh panelis. Keasaman, pH, dan jumlah mikroorganisme diamati sebelum dan sesudah inkubasi. Hasil yang diperoleh menunjukkan penambahan S. cerevisiae pada konsentrasi 2,5% menghasilkan rasa dan tekstur yang lebih baik serta tidak ditemukan bau yang kurang enak. Peningkatan ini diduga disebabkan oleh produksi etanol S. cerevisiae. Penambahan S. cerevisiae juga diketahui sedikit menghambat pertumbuhan L. delbrueckii subsp. bulgaricus dan S. thermophilus. Namun, jumlah gabungan mikroorganisme probiotik tersebut ($2,7 \times 10^8$ CFU/mL) masih sesuai dengan standar nasional. Penambahan S. cerevisiae berpotensi meningkatkan kualitas yogurt yang dibuat dari susu UHT dan diinkubasi pada suhu yang lebih rendah.*

***Kata Kunci**: Fermentasi; Kualitas; Peningkatan; Saccharomyces cerevisiae; Yogurt*

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INTRODUCTION

Milk is not traditionally a big part of Indonesian people's diet. However, the trend of milk and milk-based product consumption is increasing every year. One of the products that gains popularity is yogurt due to its health benefits (Peramiarti, 2021). Yogurt, a popular fermented dairy product, mainly relies on the ability of thermophilic *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* to ferment milk (Zhao et al., 2023). This fermentation is crucial in achieving a good quality of yogurt including its flavor, texture, and aroma. Traditionally, the optimum fermentation is conducted at 42 °C using fresh (raw or pasteurized) milk because it is considered the standard for making high-quality yogurt (Krasaekoopt et al., 2004). Maintaining the high incubation temperature is fundamental to accommodate the optimum growth of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus.* If the optimum fermentation conditions are met, the milk will be transformed into yogurt with a soft-thick texture and a tangy flavor due to the production of lactic acid (Sfakianakis & Tzia, 2014). Therefore, a proper incubator and fresh milk become a necessity to produce a good-quality yogurt.

Other than optimum temperature, the quality of the milk as the main ingredient also holds the key to the yogurt's quality. Raw or pasteurized milk is considered the standard for making high-quality yogurt (Krasaekoopt et al., 2004). The problem arises when people want to make their yogurt at home. Many households in Indonesia are not equipped with a proper heating apparatus that is important for incubation, also high-quality fresh milk is not widely available and is not easily obtained. The widely available milk is ultra-high temperature (UHT)-sterilized milk. The yogurt produced using UHT-sterilized milk usually has its texture altered and its flavor compromised. This reduced quality was attributed to the change in milk protein structure during the sterilization at such a high temperature (Krasaekoopt et al., 2003). Unfortunately, deviations from these ideal conditions can occur due to various factors, potentially compromising the final product.

This study explored the potential of *Saccharomyces cerevisiae*, a well-known yeast strain known for its health benefits, fermentative metabolism, and ethanol production capabilities, to enhance yogurt quality when fermentation conditions are suboptimal for the primary yogurt cultures (Ballet et al., 2023; Lahue et al., 2020; Parapouli et al., 2020). Previously, it has been shown that *S. cerevisiae* could grow in milk and produce small amounts of ethanol, glycerol, and lactic acid which may improve the overall quality of fermented milk (Roostita & Fleet, 1996). While milk itself is not a suitable growth medium for *S. cerevisiae* due to limitations like the inability to ferment milk's lactose, it was hypothesized that its strategic introduction during the yogurt-making process could offer specific advantages.

By introducing *S. cerevisiae* in milk with both *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*, it was expected that their metabolic abilities would complement each other. While *S. cerevisiae* is natively unable to use lactose due to its inability to assimilate this sugar, *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* can utilize lactose (Liu et al., 2016a; Sfakianakis & Tzia, 2014). However, *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* cannot naturally use galactose, a sugar that is derived from lactose breakdown. In contrast, *S. cerevisiae* can metabolize galactose through its Leloir pathway producing UDP-glucose (Zhao et al., 2023).

Some studies have been conducted to examine the characteristics of yogurt supplemented with other probiotics and to assess the quality of yogurt from different types of milk (Niamah, 2017; Setyawardani et al., 2024; Stephen & Verwiyeh, 2016). However, the potential of using prevalent yeast *S. cerevisiae* in the yogurt-making process has not been fully elucidated. This research specifically investigates whether *S. cerevisiae* can contribute to improved yogurt quality under suboptimal fermentation conditions, potentially paving the way for a more robust and adaptable yogurt production process.

MATERIALS AND METHODS

Microorganisms

Saccharomyces cerevisiae, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus* were purchased from the Laboratory of Microbiology, Satya Wacana Christian

University. *S. cerevisiae* culture was transferred and grown on Malt Extract Agar (MEA) slants at 30 °C for 2 days. *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* cultures were regrown on MRS agar slants at 42 °C for 2 days and on M17 agar at 37 °C for 2 days, respectively. These cultures were then stored at 4 °C and regularly transferred onto new respective media slants every 2 weeks.

The culture was transferred and grown on Nutrient Agar (NA) slants at 30°C for 2 days. These cultures were then stored at 4°C and regularly transferred onto new NA slants every 2 weeks.

Yogurt Starter Preparation

L. delbrueckii subsp. *bulgaricus* and *S. thermophilus* subculture were then transferred into MRS broth and M17 broth media, respectively. Both *L. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* were grown overnight with constant 250 rpm shaking (New Brunswick Innova 2300) at 42 °C and 37 °C, respectively. The cells of each culture were harvested with centrifugation at 5,000 rpm (Hettich Universal 320 R) for 10 minutes, followed by washing in physiological saline (0.9% NaCl) twice. The optical density (OD) of both washed cultures was measured at 600 nm wavelength (Shimadzu UV-Vis Spectrophotometer UV-1280). Yogurt inoculum was prepared by growing *L. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* each at 10^7 cells/mL density in 250 mL ultra-high temperature (UHT) sterilized milk (Ultra Milk). The inoculated milk was then incubated at 40 °C for 8 hours until the density of both cultures reached 10^8 cells/mL and was used as the yogurt starter.

***Saccharomyces cerevisiae* Preparation**

Previously grown *S. cerevisiae* on MEA was further sub-cultured in 500 mL Malt Extract Broth medium. The culture was grown at 30 °C with 250 rpm constant shaking overnight. The actively growing culture was then harvested with centrifugation at 5,000 rpm for 10 minutes. The cells were then washed in physiological saline twice and the OD_{600} was set to 1.0. The washed cells were then transferred into sterile UHT-sterilized milk and were used as *S. cerevisiae* inoculum.

Experimental Design

The experimental groups were designed by inoculating 180 mL of sterile UHT milk with 20 mL of yogurt starter (10% of the final volume) and different amounts of *S. cerevisiae* inoculum. The control groups were designed similarly to those of the experimental group but contained only *S. cerevisiae* inoculum without the yogurt starter. The amount of *S. cerevisiae* was set to 0; 0.156%; 0.625%; 2.5%, as well as 10% by adding 0; 0.312; 1.25; 5; and 20 mL of *S. cerevisiae* inoculum. Later, they were labeled as Sc-0; Sc-1; Sc-2; Sc-3; and Sc-4, respectively. After inoculation, the milk was incubated at 30 °C for 24 hours. The incubation temperature was set to 30 °C to represent the suboptimal temperature for both *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* and to provide the optimal temperature for *S. cerevisiae*. The experiment and the parameter measurements were conducted in triplicates. The measurements were taken before and after incubation.

Hedonic Sensory Evaluation

The hedonic qualities were evaluated by 5 panelists who have a predilection towards yogurt. The evaluation was conducted blindly to examine the taste, texture, hint of ethanol's presence, aroma, and color of the milk after incubation (Table 1). The grading was conducted using a 5-point hedonic scale.

Table 1. Five-point hedonic quality rating description

Rating	Hedonic quality				
	Taste	Texture	Ethanol	Aroma	Color
1	Neither like nor dislike	Very poor	Imperceptible	Very unpleasant	Too light
2	Like slightly	Poor	Perceptible	Unpleasant	Slightly light
3	Like moderately	Fair	Slightly distinctive	Neutral	Just right
4	Like very much	Good	Distinctive	Pleasant	Slightly dark
5	Like extremely	Very good	Very distinctive	Very pleasant	Too dark

pH and Total Titratable Acid Measurements

The pH of the inoculated milk was measured using a pH meter (Lutron PH-201). Total titratable acid was measured using the titration method (Guevarra, 2016). Briefly, as much as 10 mL milk sample was titrated using 0.1 M NaOH with the presence of 0.5% phenolphthalein indicator to an endpoint of faint pink color. The amount of required NaOH to neutralize the milk sample was converted into a percentage of acids (calculated as lactic acids) using the equation given below. % lactic acid= (volume of 0.1 N NaOH (mL) × 0.9)/weight of samples (g).

Microbial Enumeration

The number of *S. cerevisiae*, *L. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* were quantified using the Total Plate Count method (Tavakoli et al., 2019). One milliliter of milk sample was serially diluted using sterile physiological saline and plated onto MEA, MRSA, and M17 agar for the examination of *S. cerevisiae*, *L. delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus*, respectively. To inhibit the growth of bacteria on MEA media for *S. cerevisiae* quantification, penicillin G (MEIJI) was added to the media at 100 µg/mL final concentration. The cultures were then incubated at the respective temperature as previously stated for 48 hours before enumeration.

Data Analysis

The obtained data were analyzed statistically using IBM SPSS Statistic 22 software. Means of variance were examined using One-Way ANOVA at a 99% confidence level (0.01 significance level). Each presented value was the mean from triplicate data ± their standard deviation. The superscript alphabet following each value indicated their significance statistically determined using Tukey's test.

RESULTS

Sensory Properties of the Fermented Milk

The incorporation of *S. cerevisiae* culture in the production of yogurt using *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* were found to change the characteristics of yogurt (Table 2). The panelists perceived an improvement in the yogurt's taste and texture when *S. cerevisiae* was added. Yogurt made with *S. cerevisiae* tended to taste better. Treatment Sc-3 (2.5% *S. cerevisiae*) produced the best-tasting yogurt according to the panelists. The yogurt made without *S. cerevisiae* (treatment Sc-0) had a relatively fair texture. *S. cerevisiae* addition enhanced the texture by making the yogurt firmer. Treatment Sc-4 with the highest *S. cerevisiae* displayed a yogurt with a very good texture. It was significantly better than the Sc-0 treatment.

A hint of ethanol was detected by the panelists in the yogurt that was inoculated with a relatively high amount of *S. cerevisiae* at treatment Sc-3 and Sc-4 (2.5% and 10% *S. cerevisiae*, respectively). At lower *S. cerevisiae* concentration (Sc-1 and Sc-2), the panelists did not detect ethanol. *S. cerevisiae* addition at concentrations used in treatments Sc-1, Sc-2, and Sc-3 did not significantly affect the yogurt's aroma. However, at the treatment with the highest number of *S. cerevisiae* (Sc-4), an unpleasant rancid aroma was perceived by the panelists. Therefore, all panelists gave this yogurt the lowest aroma rating. All treatments produced yogurt with a similar creamy white color. There were no significant differences in color observed among treatments.

Table 2. Perceptible hedonic quality of the experimental group

Treatment	Hedonic quality				
	Taste	Texture	Ethanol	Aroma	Color
Sc-0	2.20 ± 0.84^{a}	2.60 ± 0.55^{a}	1.00 ± 0.00^{a}	3.80 ± 1.09^{a}	2.80 ± 0.45^{a}
Sc-1	3.00 ± 1.00^{ab}	2.60 ± 0.55^{ab}	1.00 ± 0.00^{a}	2.80 ± 0.84^{ab}	2.80 ± 0.45^{a}
Sc-2	3.80 ± 0.45^{ab}	3.00 ± 0.71^{abc}	1.40 ± 0.55^{a}	2.80 ± 0.84^{ab}	2.60 ± 0.55^{a}
Sc-3	4.00 ± 0.71^{b}	3.60 ± 0.55^{bc}	3.20 ± 0.84^{b}	3.40 ± 0.89^{a}	3.20 ± 0.45^{a}
Sc-4	3.60 ± 0.55^{ab}	5.00 ± 0.00^{c}	4.60 ± 0.55^{c}	1.00 ± 0.00^{b}	3.20 ± 0.84^{a}

The milk in the control group that was inoculated with only *S. cerevisiae* did not display any coagulation and acid production normally seen in yogurt. There was no significant difference among all treatments in the control groups (Table 3). The incubated milk containing *S. cerevisiae* (Sc-1–Sc-4) tasted just like uninoculated milk (Sc-0). Their texture was like that of regular milk and stayed liquid due to the lack of coagulation. The same goes for the color where no significant differences were found. All treatments exhibited a similar creamy white color. However, a small amount of ethanol was perceived by some panelists at the high *S. cerevisiae* treatment (Sc-3 and Sc-4) although there were no differences statistically. Similarly, panelists also perceived a hint of unpleasant aroma in treatments Sc-3 and Sc-4.

Table 3. Perceptible hedonic quality of the control group

Treatment	Hedonic quality				
	Taste	Texture	Ethanol	Aroma	Color
Sc-0	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	3.00 ± 0.00 [a]	3.00 ± 0.71 [a]
Sc-1	1.00 ± 0.00 [a]	1.20 ± 0.45 [a]	1.00 ± 0.00 [a]	3.00 ± 0.00 [a]	3.20 ± 0.45 [a]
Sc-2	1.20 ± 0.45 [a]	1.20 ± 0.45 [a]	1.00 ± 0.00 [a]	3.00 ± 0.00 [a]	2.60 ± 0.55 [a]
Sc-3	1.40 ± 0.54 [a]	1.00 ± 0.00 [a]	1.40 ± 0.55 [a]	2.60 ± 0.55 [a]	3.40 ± 0.55 [a]
Sc-4	1.00 ± 0.00 [a]	1.20 ± 0.45 [a]	1.60 ± 0.55 [a]	2.40 ± 0.55 [a]	3.00 ± 0.71 [a]

Acidity of the Fermented Milks

Acidity as one of the key characteristics of yogurt was depicted as total titratable acid (TTA) and pH (Figure 1 & 2). There were observable differences in their TTA between the experimental and control groups of this experiment. The experimental group saw a substantial increase in their TTA after incubation compared to that of the control group (Figure 1). Similar to the TTA, the pH of yogurt under experimental groups decreased significantly compared to the control groups after incubation (Figure 2).

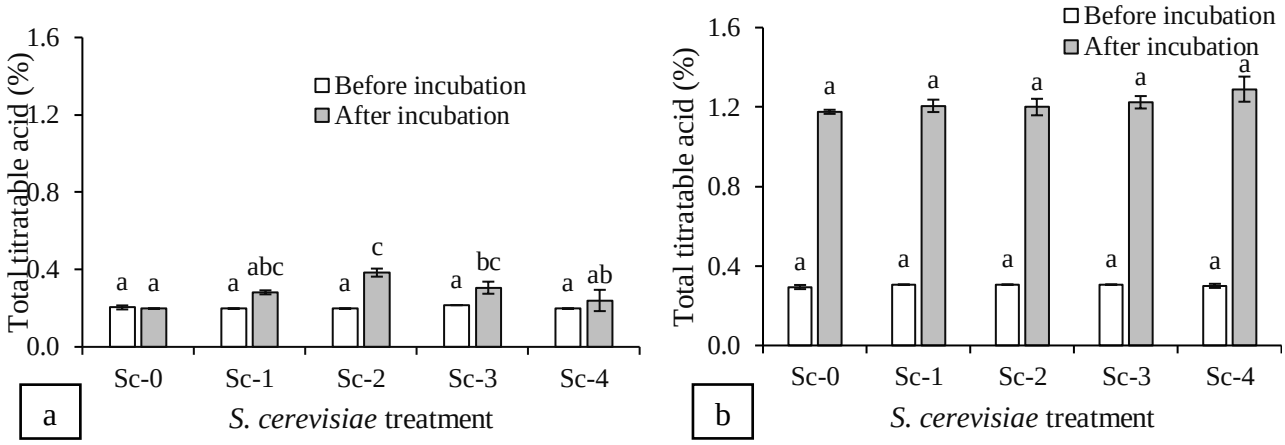

Figure 1. Total titratable acid (TTA) of the inoculated milk, TTA of the experimental group with yogurt culture addition (a), and TTA of the control group without yogurt culture addition (b). The error bars indicate standard deviations and the letters indicate the significant differences at the level of 1% of the Tukey's test

The initial TTA of the experimental group was higher than that of the control group at 0.3% compared to 0.2%, respectively. The TTA of the experimental group averaged 1.2% after 24 hours of incubation, four times compared to that before incubation (Figure 1a). The TTA of the experimental groups did not significantly differ among all five treatments, both before and after incubation. As predicted, the control groups only saw a small increase in TTA, averaging at around 0.28% (Figure 1b).

The pH of experimental groups decreased from the average of 6.43 to 4.66 after the incubation period and there was a significant difference at the final pH between Sc-0 and Sc-3 also Sc-4 (Figure 2a). Whereas, the control groups only experienced a small decrease from the average of 6.71 to 6.43 (Figure 2b). By the TTA described in Figure 1b, the treatment with *S. cerevisiae* addition (Figure 2b, Sc-1–Sc-4) showed a significantly lower pH than the milk without any culture (Figure 2b, Sc-0). In

both experimental and control groups, increasing the concentration of *S. cerevisiae* resulted in a lower pH after incubation.

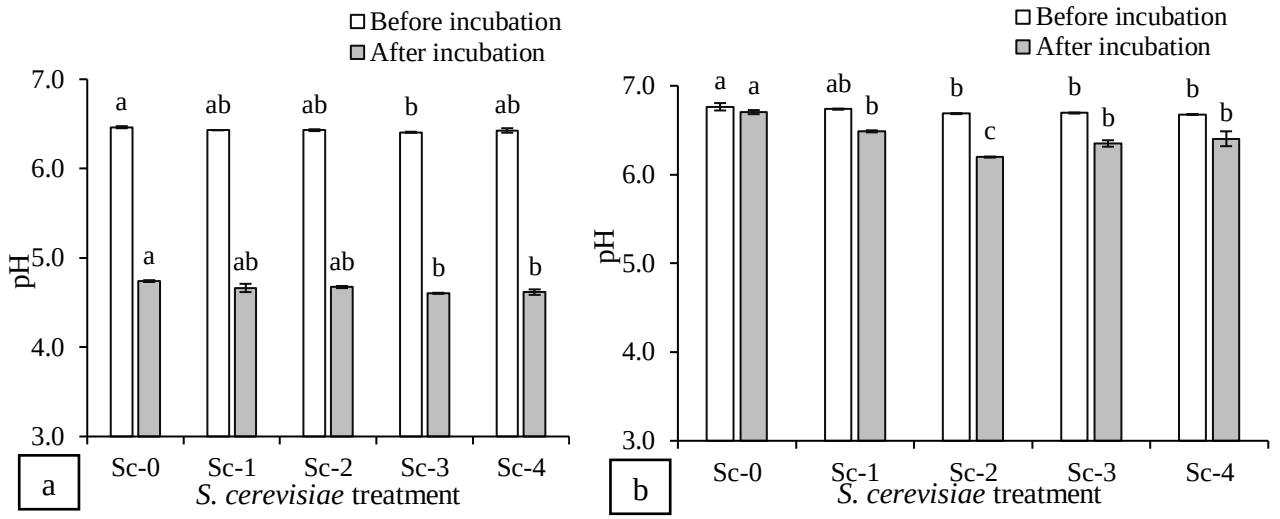

Figure 2. pH of the inoculated milk, pH of the experimental group with yogurt culture addition (a), and pH of the control group without yogurt culture addition (b). The error bars indicate standard deviations and the letters indicate the significant differences at the level of 1% of the Tukey's test

Microbial Dynamics During Fermentation

Dynamic changes in microbial numbers were observed during milk fermentation, reflecting interactions among the microorganisms (Figure 3 & 4). Initial S. cerevisiae counts before incubation were similar in the experimental and control groups (Figure 3a & 3b, white bars). After incubation, the number of *S. cerevisiae* in the experimental group was higher than that of the control group (Figure 3a & 3b, grey bars). The numbers of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* in the inoculated milk before incubation were similar across all treatments (Figure 4a & 4b, white bars).

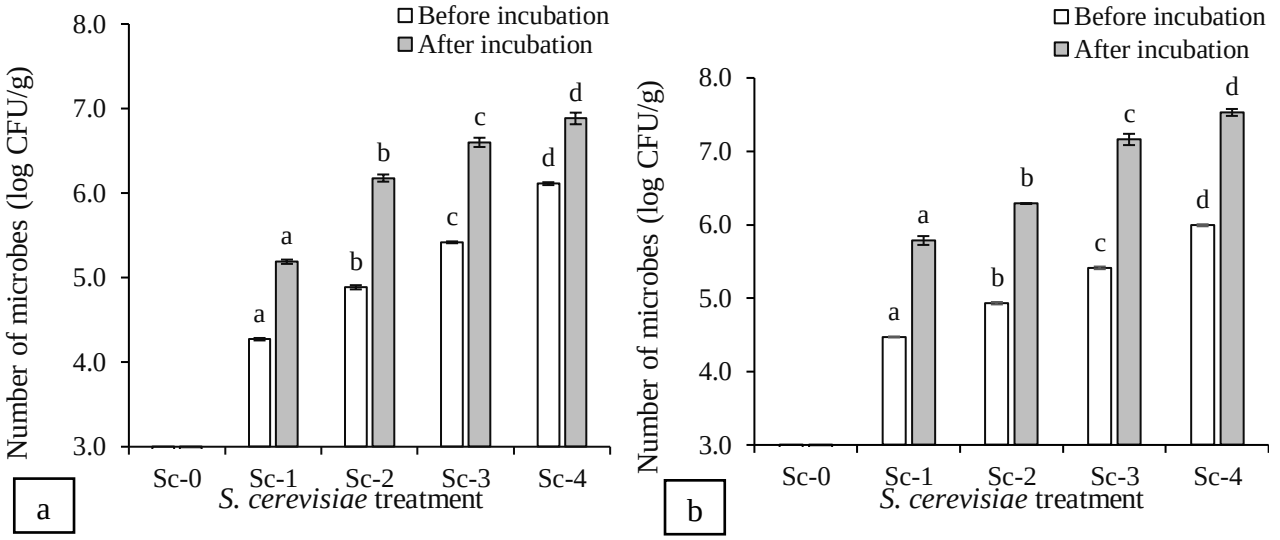

Figure 3. The number of *S. cerevisiae* of the inoculated milk. A = The number of *S. cerevisiae* of the experimental group with yogurt culture addition, B = The number of *S. cerevisiae* of the control group without yogurt culture addition. The error bars indicate standard deviations and the letters indicate the significant differences at the level of 1% of the Tukey's test

The number of both *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* displayed a similar pattern when grown with the presence of *S. cerevisiae*. The higher the number of S. cerevisiae being inoculated, the lower the number of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* would be after incubation (Figure 4a & 4b, grey bars). Treatment Sc-2, Sc-3, and Sc-4 showed a significant

difference in *L. delbrueckii* subsp. *bulgaricus* number compared to treatment Sc-0 which had no *S. cerevisiae*. Similar to that of *L. delbrueckii* subsp. *bulgaricus, S. thermophilus* in treatment Sc-1, Sc-2, Sc-3, and Sc-4 exhibited a decrease in their growth compared to the treatment Sc-0. A correlation test was conducted between the presence of *S. cerevisiae* and the number of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* showed a negative value, indicating that *S. cerevisiae* could inhibit the growth of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* to some degree.

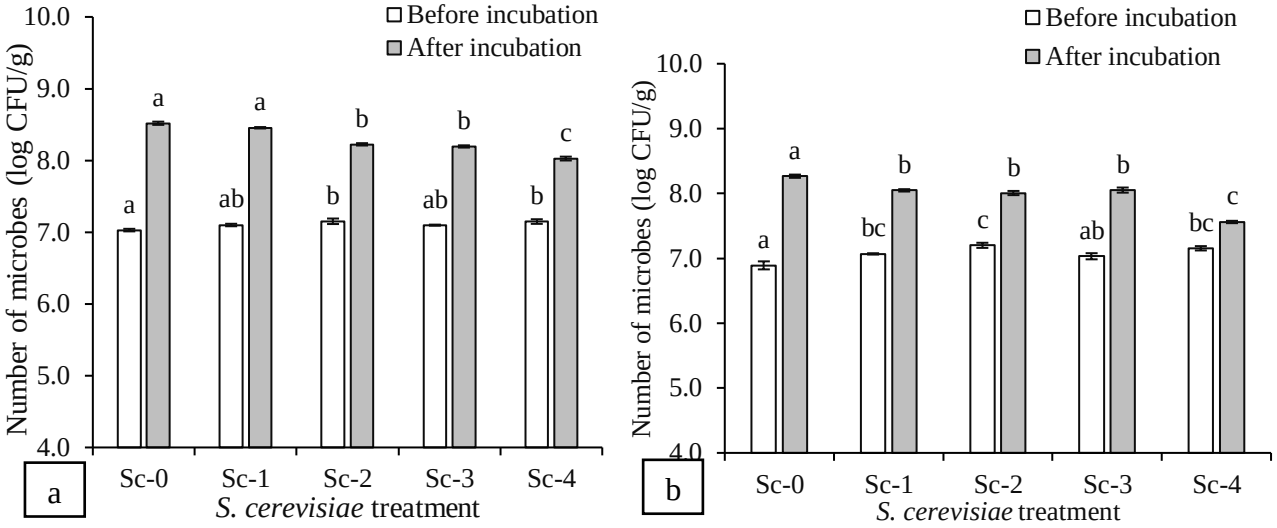

Figure 4. The number of lactic acid bacteria of the inoculated milk in the experimental group with the yogurt culture addition. A = The number of *L. delbrueckii* subsp. *bulgaricus*, B = The number of *S. thermophilus*. The error bars indicate standard deviations and the letters indicate the significant differences at the level of 1% of the Tukey's test.

The number of *L. delbrueckii* subs. *bulgaricus* and *S. thermophilus* were comparable among each treatment at day 0. After 24 hours of incubation, both bacteria grew significantly. However, the counts of both bacteria decreased as the concentration of *S. cerevisiae* increased (Figure 4).

DISCUSSION

Sensory Properties of the Fermented Milk

The role of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* were proven to be indispensable in producing acid and coagulate milk (Table 2). The control group which did not have these two microorganisms failed to become yogurt as they remained liquid and exhibited similar characteristics to uninoculated milk (Table 3). The experimental group which contained yogurt starter, although incubated at only 30 °C, successfully fermented milk to produce yogurt. This finding confirmed that *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* were still active and retained their ability to form yogurt at this temperature. However, the yogurt produced was of subpar quality (Table 2, Sc-0).

The suboptimal temperature may have hindered the optimal growth of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* (Glušac et al., 2015). Therefore, their lactic acid production might not be sufficient to coagulate milk rendering the overall yogurt texture as not firm as in normal yogurt production. The lack of lactic acid production might also be responsible for the subpar yogurt taste found in this experiment. The connection between lactic acid and yogurt's flavor trend was observed previously as lactic acid was a major contributor in determining desirable yogurt flavor (Chen et al., 2017).

S. cerevisiae addition during fermentation was shown to significantly affect some of yogurt's characteristics, especially its taste and texture. These improvements might be due to the presence of ethanol in the fermented milk produced by *S. cerevisiae* (Roostita & Fleet, 1996). Ethanol might improve yogurt's taste by introducing a fresh flavor not usually found in typical yogurt. Ethanol may have compensated for the lack of tanginess in this suboptimal yogurt. *S. cerevisiae* did have the ability to produce small amounts of acid (Figure 1b). A similar approach using carbon dioxide addition has

been explored to create a novel yogurt variant (Burton et al., 2014). The probable presence of ethanol at Sc-3 and Sc-4 treatments might have improved the texture compared to Sc-0 due to its ability to coagulate milk casein. Similarly, the role of ethanol in assisting milk curdling has been described through ethanol-induced milk coagulation (Alhaj et al., 2022).

Unlike the taste and texture which tend to be better when *S. cerevisiae* was present, the aroma of yogurt made with the highest amount of *S. cerevisiae* culture (Sc-4 treatment) was identified as very unpleasant (Table 2, Aroma). Panelists described its aroma as rancid, like oxidized oil. This rancid aroma might arise due to the high number of total microorganisms present in the milk (Chen et al., 2017). These microorganisms possess the ability to metabolize lipids and protein. The usage of full cream milk which is rich in fat and protein magnified the risk of lipid oxidation and protein degradation which resulted in a potent rancid aroma production (Folkenberg & Martens, 2003; García-Gómez et al., 2019).

Acidity of the Fermented Milks

The addition of yogurt culture was shown to successfully acidify the milk and therefore decrease the pH (Figure 1a Sc-0 & Figure 2a Sc-0). This finding confirmed that the yogurt culture being used in this study was viable and active. Meanwhile, the milk which was inoculated with *S. cerevisiae* alone only produced small amounts of acid and slightly decreased the pH (Figure 1b, Sc-1–Sc-4 & Figure 2b, Sc-1–Sc-4). This result is expected due to the ability of *S. cerevisiae* to produce lactic acid when grown in milk was limited (Roostita & Fleet, 1996). Yogurt culture together with *S. cerevisiae* was found to successfully acidify the milk although the TTA of the produced yogurt displayed no significant difference among different concentrations of *S. cerevisiae* treatments (Figure 1b, Sc-1–Sc-4). This indicated that the acid being produced by *S. cerevisiae* might not be comparable to that of the *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* from the original yogurt culture. Therefore, the increase in *S. cerevisiae* cell number among treatments in the experimental group did not significantly affect the total titratable acid and only displayed a slight decrease in their pH value (Figure 1a & 2a). Also, it can be assumed that the perceptible improvement of the yogurt's taste according to the panelists from the experimental group was not mainly related to *S. cerevisiae*'s acid production. Other than lactic acid, diacetyl, acetaldehyde, acetone, as well as 2-butanone were identified as the chemicals that contribute to yogurt's flavor profile (Cheng, 2010).

Microbial Dynamics During Fermentation

In this study, it was confirmed that *S. cerevisiae* could grow in UHT-sterilized milk with or without the presence of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* (Figure 3a & 3b). Due to *S. cerevisiae*'s limited proteolytic capacity and its ability to utilize galactose, *S. cerevisiae* grew better in milk when *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* were present (Figure 3a). Conversely, the addition of *S. cerevisiae* was also found to affect the growth of both *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* statistically (Figure 4a & 4b). *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* support each other through protocooperation where metabolite exchange occurs between them while exhibiting a competitive co-evolution (Liu et al., 2016b). The introduction of *S. cerevisiae* as the third microorganism changed the dynamics of these microorganisms. This inhibition was unlikely due to the possible ethanol production by *S. cerevisiae* because both *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* have ethanol tolerance (Mena & Aryana, 2012). *S. cerevisiae* might have inhibited *L. delbrueckii* subsp. *bulgaricus* and *S. thermophiles* growth by competing for nutrients such as free amino acids present in the media. This competition together with relatively low incubation temperature led to some inhibition on *L. delbrueckii* subsp. *bulgaricus* and *S. thermophiles* growth.

Although experiencing some inhibitions, the level of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* were still by the standard instituted by the Indonesian government. The national standard (yogurt quality requirements SNI 2981-2009) establishes that the minimum number of probiotic microorganisms in yogurt is 10^7 CFU/g. Whereas the number of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* in the Sc-3 treatment was around 2.7×10^8 CFU/g (8.4 in log CFU/g).

CONCLUSION

Yogurt production at suboptimal fermentation conditions resulted in a subpar product. *S. cerevisiae* addition at the beginning of the fermentation process together with *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* resulted in higher-quality yogurt. From the conducted examinations, it was determined that the best quality of the yogurt was found in treatment Sc-3 which was inoculated with 2.5% *S. cerevisiae* inoculum. The yogurt from this treatment displayed an improved taste and texture possibly due to the ethanol which assisted in creating a fresh flavor and firm curd. Also, the rancid aroma found in the Sc-4 treatment which contained four times the number of *S. cerevisiae* being inoculated was not observed in Sc-3 yogurt. This yogurt contained 2.7×10^8 CFU/g of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* combined which met the Indonesian national standard. Further research needs to be conducted to determine the exact chemical profile of the yogurt produced with *S. cerevisiae* addition and subsequently examine the exact role of ethanol in improving yogurt's quality.

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