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Optimization of Fermentation and Sensory Quality of Mango Yogurt Using Various Sucrose Concentrations Fermented by *Lacticaseibacillus paracasei* Strain Shirota

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Abstract

This research explores the optimization of mango yogurt production, leveraging mango as the primary fermentation ingredient and Lacticaseibacillus paracasei strain Shirota (LcS) as the probiotic inoculum. Traditional yogurt, typically derived from animal milk, faces competition from plant-based alternatives. The research objective is to investigate the impact of inoculum ratios and sucrose concentrations on LcS density and sensory attributes, with a focus on the Substrate to Inoculum Ratio (S/I) as a critical parameter influencing microbial metabolism and population dynamics. Various inoculum ratios (1:1, 1:2, 1:4, 1:8 v/v) and sucrose concentrations (0%, 2.5%, 5%, 7.5%) were applied in mango yogurt production. Results from ANOVA analysis indicated significant differences in LcS density among inoculum ratios ($F_{3,16} = 37.01$, $P = 0.000$) and sucrose concentrations ($F_{3,16} = 19.07$, $P = 0.000$). The 1:2 inoculum ratio and 7.5% sucrose concentration emerged as optimal, as evidenced by stable pH conditions and lactic acid profiles within recommended standards. Sensory analysis, employing a 5-point hedonic scale for color, odor, taste, and texture evaluation, revealed moderate acceptance. In conclusion, this study introduces an innovative approach to mango yogurt production, highlighting the potential of specific inoculum ratios and sucrose concentrations for optimal results. The findings contribute to the broader landscape of plant-based yogurt alternatives, underscoring the need for ongoing refinement to meet commercial standards and ensure consumer satisfaction

Keywords: *Lactic acid, Lacticaseibacillus paracasei strain Shirota, Mango, pH, Yakult*

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INTRODUCTION

Yogurt, renowned for its thick to semi-solid texture and distinct sour taste, is traditionally crafted using animal milk (Weerathilake et al. 2014). However, recent advancements in food technology have spurred the introduction of plant-based milk as a viable alternative, providing nutritional benefits comparable to conventional animal milk yogurt (Sridhar et al., 2023). Commonly utilized plant-based ingredients for yogurt production boast elevated levels of carbohydrates and reducing sugars (Montemurro et al., 2021). The fermentation process leads to the creation of organic acids, contributing to the characteristic flavor of yogurt. Consumption of yogurt is associated with digestive benefits, attributed to the enhancement of gut microflora (Adolfsson et al., 2004). Mango, due to its rich content of carbohydrates and reducing sugars, exhibits great potential for incorporation into yogurt production, fostering the growth of lactic acid bacteria (LAB) (Reddy et al., 2015).

Yakult, a fermented milk drink containing a LAB strain, particularly *Lacticaseibacillus paracasei* strain Shirota (LcS), is known for its ability to resist gastric conditions and colonize the small intestine. This colonization results in the production of antimicrobial substances, stimulating macrophage activity and proliferation (Cook et al., 2023; Naito et al., 2023). Renowned for its health benefits, Yakult is accessible in 15 countries, including various Southeast Asian countries and Indonesia. Mango-flavored yogurt, in particular, holds distinct appeal due to its unique taste and potential health benefits (Saeed et al., 2021). The commercial probiotics, LcS, adds an extra layer of functionality to the mango yogurt formulations. The optimization of fermentation conditions and the careful consideration of sensory attributes are critical aspects in ensuring the quality and acceptance of mango yogurt (Ahmad et al., 2022).

Sucrose concentration in the fermentation process plays a pivotal role in influencing both the fermentation kinetics and the sensory characteristics of the final product (de Souza et al., 2021; Nurhartadi et al., 2017). Understanding the interplay between sucrose concentrations and the fermentation process, particularly with LcS, is crucial for tailoring the product to meet consumer expectations. Despite the growing interest in flavored yogurts, comprehensive studies on the optimization of mango yogurt production, specifically focusing on varied sucrose concentrations and the associated sensory quality, remain limited. This study seeks to address this gap by investigating the fermentation outputs and sensory attributes of mango yogurt produced using different sucrose concentrations by LcS. This study is dedicated to create simple yet innovative probiotic beverages.

METHOD

The main ingredients in yogurt production are commercially available pasteurized milk, branded as Yakult®, serving as the inoculum source for *Lacticaseibacillus paracasei* strain Shirota (LcS) with a minimum concentration of 6.5×10^9 viable cells per 65 ml bottle (Sutula et al., 2013). Mangoes were purchased from a traditional market in Pekanbaru, Riau, Indonesia. Fresh mangoes were peeled, and the pulp was extracted and pressed to obtain mango juice. Inoculum loads with varying ratios of 1:1, 1:2, 1:4, and 1:8 (v/v) were prepared by mixing Yakult® with mango juice in a total volume of 100 mL and incubated at 37°C for 24 hours. The treatment with the highest LcS population density was used to produce mango yogurt with sucrose concentrations of 0% (control), 2.5%, 5.0%, and 7.5%, incubated at 37°C for 24 hours. All experiments were conducted in five replicates. Hedonic testing was conducted on all mango yogurt samples resulting from the treatments. The prepared samples were assessed using a 5-point hedonic scale (1–5), where sensory attributes such as color, odor, taste, and texture were scored from "1 = extremely disliked" to "5 = extremely liked." The sensory evaluation of the mango yogurts involved 15 semi-trained evaluators who were selected and trained following a standardized method, with respondents from the University of Riau campus in Pekanbaru, Indonesia.

LcS population density was determined using the standard plate count (SPC) method on de Man, Rogosa, and Sharpe (MRS) agar, expressed in colony-forming units (CFU)/g (Agustine et al., 2018). Lactic acid concentration was determined using the total titrated acid method, and pH was recorded using a digital pH-meter (Mega et al., 2020). Graphical images were generated using GraphPad Prism ver. 8.0, followed by statistical tests such as an analysis-of-variance (ANOVA) test and Tukey's post hoc test to identify mean differences among treatments. A radar plot showing sensory attributes from hedonic test was generated using MS. Excel ver. 2016.

RESULTS AND DISCUSSION

Yogurt production using mango as the main ingredient for fermentation and *Lacticaseibacillus paracasei* strain Shirota (LcS) inoculum from a commercial source has been optimized with various inoculum ratios as presented in Figure 1. ANOVA results indicate a significant difference among the mean density values obtained from the inoculum load variations ($F_{3,16} = 37.01$, $P = 0.000$). The inoculum ratio that resulted in the highest lactic acid bacterium (LAB) density was obtained from the 1:2 treatment with a log value of 8.12±0.26 CFU/g, while the lowest was from the 1:8 treatment with a log value of 6.40±0.12 CFU/g. The Substrate to Inoculum Ratio (S/I) is a crucial parameter optimized during the fermentation process, involving the interaction between microbes and metabolic pathways (Kong et al., 2016).

In the production of mango yogurt, the inoculum load of the fermenting agent, specifically LcS, is evaluated based on its population density in the final product. The LcS strain, a lactic acid bacterium, functions optimally in anaerobic to microaerophilic conditions, leading to the conversion of organic macromolecules into acetate, ethanol, lactic acid, methane, and volatile fatty acids (Lin et al., 2018). Theoretically, a low concentration of inoculum is introduced, slowing down fermentation but resulting in increased lactic acid production as cells gradually convert sugar to lactic acid after multiplication (Abdullah & Amalia, 2023). Conversely, a high inoculum concentration diminishes cell viability and hinders lactic acid generation (Othman et al., 2017). This explains that there is an optimum moderate inoculum load level in maintaining the stability of the population density of this strain.

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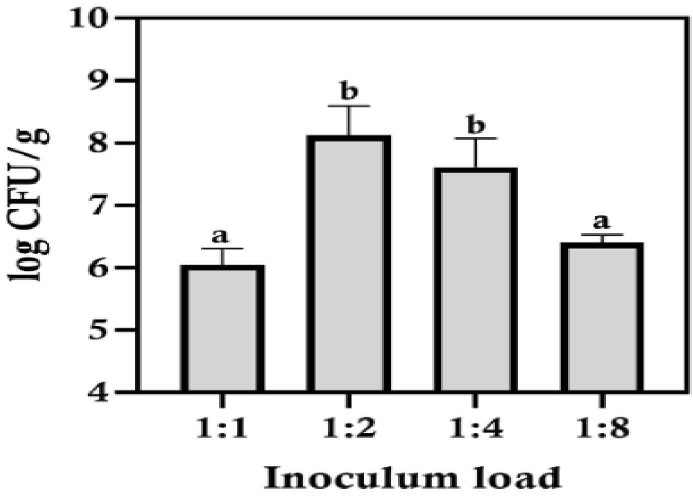

Figure 1. Population dynamics (log CFU/g) of lactic acid bacteria in mango yogurt with different inoculum loads or ratios

The effect of sucrose on LAB density in mango yogurt fermentation indicates a significant difference in the mean results ($F_{3,16}$ = 19.07, P = 0.000) as presented in Figure 2. The highest density value is shown by the 7.5% sucrose treatment, with a final LAB density reaching log 8.88±0.17 CFU/g, while the lowest density is observed in the 0% sucrose treatment (control) with log 8.06±0.26 CFU/g. This suggests that the addition of sucrose above 5% no longer significantly increases the LcS cell population in yogurt. Lactic acid bacteria have the ability to metabolize various types of sugars into lactic acid (Wang et al., 2021a). The accumulation of lactic acid in food materials or during fermentation creates a niche that is more supportive for the growth of a specific functional group of microorganisms, including LAB. This phenomenon also eliminates other competitors that are not resistant to drastic pH dynamics or high levels of organic acids. Consequently, the mechanism of competitive exclusion becomes advantageous for LAB (Blasche et al., 2021).

Sugars present in milk and fruit (mango juice) can stimulate growth, enhancing LAB activity in yogurt. Sucrose serves as a crucial nutrient for LAB growth, influencing cell viability, population density, and the final metabolites produced during fermentation in the fermented product (Wang et al., 2021b). A study suggests that fermented dairy or food products should contain a minimum of log 8 (10^8) CFU of probiotic strains, approximately meeting the recommended daily intake of viable probiotics in fermented milk (Shah, 2007). Therefore, based on this research, it appears that the treatment with 5% sucrose is considered adequate for producing mango yogurt. Other researchers reported a sucrose concentration of 12% as the optimum parameter for creating yogurt supplemented with butterfly pea (*Clitoria ternatea* L.) (Suharman et al., 2021).

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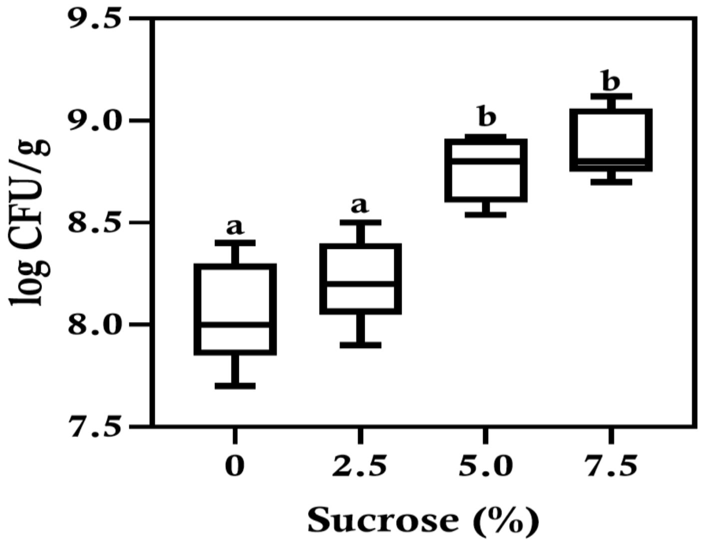

Figure 2. Population dynamics (log CFU/g) of lactic acid bacteria in mango yogurt with different sucrose concentrations (%)

Acid–base titration, employed to estimate the total acid concentration, is utilized for determining the overall acid content in fermented products (Fetzer & Jones, 1952). The pH and lactic acid profiles monitored in each mango yogurt treatment based on sucrose variations are presented in Figure 3. The highest lactic acid content was obtained from the treatment with the highest sucrose concentration (7.5%) at 0.79±0.19%, while the lowest was from the control treatment (0%) at 0.49±0.07%. The pH conditions remained stable, ranging from 4.61 (7.5% sucrose) to 4.83 (control). ANOVA results showed a significant difference in the mean values of lactic acid content ($F_{3,16}$ = 9.48, P = 0.001), but no significant difference in pH values ($F_{3,16}$ = 0.86, P = 0.482). The fermentation conditions in this study were controlled for only 24 hours, so further monitoring is needed regarding the stability of the product in both room and cold storage conditions to determine the quality of mango yogurt. According to the Indonesian National Standard (SNI 2981:2009), the lactic acid level in yogurt should be within 0.5-2.0%, which was achieved in our study. The pH decrease resulted from the metabolism of lactose and sucrose into lactic acid by LcS during fermentation (Pato et al., 2020). The highest sucrose concentration (12%) also increased the lactic acid content to 2% in butterfly pea yogurt (Suharman et al., 2021). Moreover, additional investigation can be undertaken to evaluate the antioxidant potential. It is recognized that lactic acid has the capability to act as a chelating agent, thereby inhibiting free radicals as a metabolic by-product (Łopusiewicz et al., 2021).

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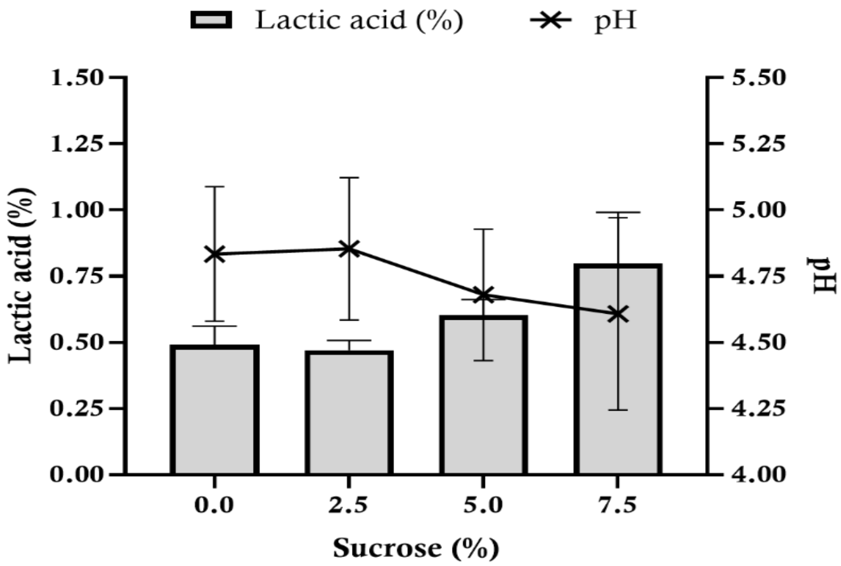

Figure 3. Lactic acid and pH profiles of mango yogurt fermented by *Lacticaseibacillus paracasei* strain Shirota (LcS) under different sucrose concentrations

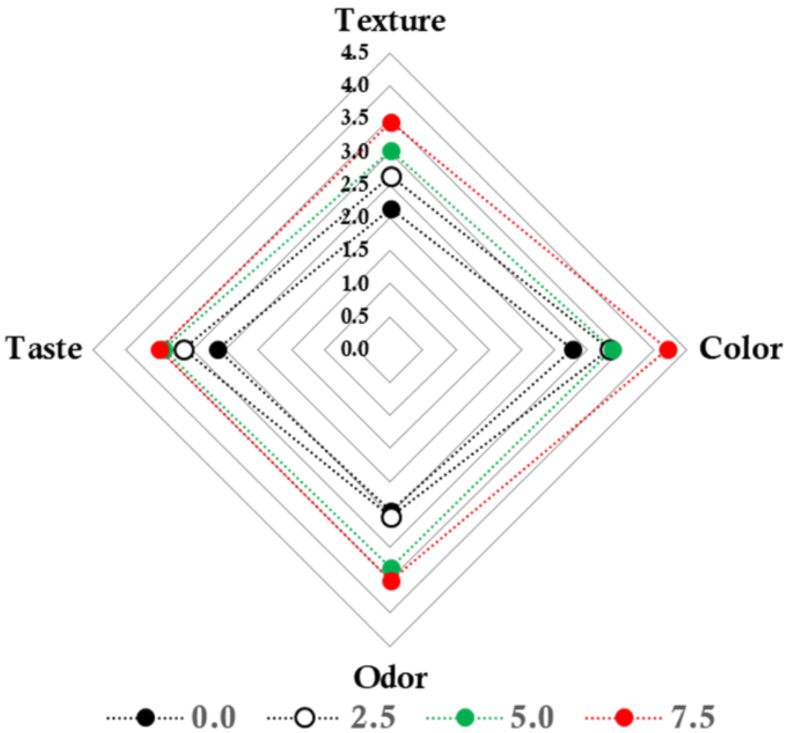

Figure 4. Radar plot showing the sensory qualities of mango yogurt fermented using LcS with the lowest score of 0 (poor) and the highest score of 5 (good)

The results of sensory analysis on mango yogurt based on sucrose variations indicate that the acceptance of this product is still moderate for the color, odor, taste, and texture categories as presented in Figure 4. This suggests the need for optimization in the finalization of yogurt before it is distributed as a commercial product to garner a more positive response from consumers. Conversely, a study investigating the sensory analysis of fermented milk using probiotics, discovered a notable impact on quality and acceptance among the panelists when sucrose was added at higher concentrations (Pato et al., 2017). In addition, the use of 15% skimmed milk and variations in sucrose content had a notable impact on all sensory aspects, including color, taste, and texture. In line with these findings, other study reported that no difference was observed between probiotics and dairy starters in terms of flavor, appearance, texture, and overall acceptability in fermented skimmed milk drinks using *L. casei* and *L. rhamnosus* (Shabbir et al., 2023). Overall, it has been determined that probiotic-enriched fermented milk possesses sensory qualities comparable to those prepared with commercial dairy starters.

CONCLUSION

The production of mango yogurt was undertaken through a straightforward approach involving the blending of Yakult® and mango juice with variations in inoculum load and sucrose concentration. In general, the total viable cells of probiotic bacteria met the standards in the 1:2 treatment with sucrose additions exceeding 5%. Nonetheless, further investigation into mango yogurt development is warranted, given the moderate sensory quality scores obtained.

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