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Enhancement of Probiotic Viability and Antioxidant Activity in Yogurt Treated with Corn Silk Extract

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Abstract

Yogurt is a widely consumed fermented milk product known for its numerous health benefits, while corn silk, the pistil of corn, is a byproduct rich in bioactive compounds and antioxidants. This study assessed the antioxidant activity of yogurt fortified with corn silk extract. Yogurt was prepared by fermenting cow's milk with a yogurt starter culture. The antioxidant activity of the resulting yogurt was evaluated using the DPPH method. The results demonstrated that the yogurt enriched with corn silk exhibited a total lactic acid bacteria (LAB) count of 1.2×10^8 cfu/mL, a pH of 4.4, a lactic acid content of 1.34%, and an antioxidant activity of 54%. In contrast, the control yogurt without corn silk had a LAB count of 1.9×10^7 cfu/mL, a pH of 4.5, a lactic acid content of 0.93%, and an antioxidant activity of 47%. Organoleptic evaluations revealed no significant differences in taste, aroma, texture, or overall acceptability between the two yogurt types. The addition of corn silk significantly increased the antioxidant activity, total microbial count, and lactic acid content of the yogurt. These findings suggest that corn silk extract can be a beneficial natural additive to enhance the antioxidant properties of yogurt, thereby improving its nutritional value.

Keywords: Antioxidant activity; cornsilk; DPPH method; fermented milk; lactic acid bacteria (LAB); yogurt.

INTRODUCTION

In recent decades, the prevalence of chronic non-communicable diseases (NCDs), including obesity, diabetes mellitus, and cardiovascular conditions, has risen significantly (Wilkins et al., 2019). Modern lifestyles, characterized by the excessive use of antibiotics, poor dietary choices, sedentary behavior, and stress, have contributed to imbalances in gut microbiota. These imbalances are increasingly recognized as key factors in the onset and progression of many NCDs (Gomaa, 2020; Illiano et al., 2020). Furthermore, a growing body of evidence links these diseases to deficiencies in antioxidant defenses, highlighting the importance of antioxidants in managing oxidative stress and preventing disease progression (Niemann et al., 2017).

In response, there is a growing interest in natural antioxidants derived from functional foods, with research focusing on combining different food sources to maximize their health benefits and multifunctionality. Yogurt, a widely consumed functional food, is valued not only for its pleasant taste and nutritional profile but also for its potential therapeutic benefits. It has been shown to support digestive health and may play a role in cancer prevention. Additionally, studies have demonstrated that yogurt fermented with *Lactobacillus* bacteria exhibits higher antioxidant activity than raw milk. Research has reported DPPH scavenging antioxidant activity in yogurt ranging from 47.85 to 60.67 mg/mL (54.26 ± 6.41 mg/mL) (Pereira et al., 2013). As such, yogurt represents a safe and effective source of natural antioxidants, offering significant potential for health promotion. El-Khadragy et al. (2019) further emphasized yogurt's role as a probiotic, with the potential to modulate oxidative stress and liver fibrosis in *Schistosoma mansoni*-infected rats.

Corn silk, the stigma of corn flowers, is a byproduct of corn production, typically discarded as waste during the processing of corn for food and animal feed. Recent studies, however, have revealed that corn silk possesses a variety of pharmacological properties, including antioxidant, anti-apoptotic, hypoglycemic, and anticancer activities (Li et al., 2023). For instance, corn silk has demonstrated DPPH scavenging antioxidant activity of 92.6% at a concentration of 1.6 mg/mL (Ebrahimzadeh et al., 2008). Furthermore, research by Guo et al., highlighted the potential of corn silk polysaccharides in reducing diabetes, pointing to its promise as a functional food ingredient (Guo et al., 2019).

Given the antioxidant-rich profile of corn silk, it holds considerable potential as an additive to enhance the nutritional value of yogurt. This study aims to explore the antioxidant activity and microbial characteristics of yogurt enriched with corn silk extract. It is anticipated that this research will not only introduce a novel functional yogurt variant but also contribute to enhancing the economic value of corn silk, a largely underutilized agricultural byproduct.

MATERIALS AND METHODS

Materials and equipment

The primary material used in this study was corn silk, which was sourced from Batang Hari Ogan Village, Tegineneng, Pesawaran. The bacterial starter cultures employed for yogurt production were *Lactobacillus bulgaricus* (FNCC 0041), *Streptococcus thermophilus* (FNCC 0015), and *Lactobacillus plantarum* (FNCC 0026), obtained from the FNCC collection at the Food and Nutrition Study Center, Gadjah Mada University. The medium used for culture rejuvenation and stock culture preparation was De Man, Rogosa, and Sharpe (MRS) agar (Oxoid). For cryopreservation, 20% (v/v) glycerol and 10% (w/v) skim milk were used as cryoprotectants. The MRS agar contained 100 ppm NaN_3 and 100 ppm $CaCO_3$. For acidity testing, 1% phenolphthalein (PP) indicator and 0.1 N NaOH were used. The materials for antioxidant testing included 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Merck) and ethanol.

The equipment used during the study included an autoclave (All American B0004146), an incubator (Memmert), a pH meter (Hanna), a vortex (Scilogex), a laminar airflow (Telstar), a freezer (Toshiba), an analytical balance (AND), a spectrophotometer (Milton Roy 21D), titration apparatus, and a micropipette (Eppendorf).

Starter culture preparation

To prepare the bacterial starter cultures, 1 mL of each bacterial culture was transferred into test tubes containing 10 mL of sterilized MRS medium, which was autoclaved at 121°C under 15 psi for 15 minutes. The inoculated MRS medium was then incubated at 37°C for 24 hours. To prepare the starter, 10% (v/v) of the inoculum was added to 100 mL of sterilized 18% (w/v) skim milk, which was sterilized at 110°C under 13 psi for 10 minutes. This mixture was incubated for 12 to 18 hours until curd formation was observed, resulting in the mother starter. A 10% (v/v) portion of the mother starter was further inoculated into 100 mL of sterilized 18% (w/v) skim milk and incubated for another 12 to 18 hours to produce a bulk starter. The starter was inoculated directly into milk for fermentation or stored at 10°C for future use.

Cornsilk preparation

Corn silk was first sorted, washed, and dried to a moisture content of ≤ 10% using an oven at 50°C for 150 minutes. To prepare the corn silk extract, 5 g of the dried corn silk was added to 200 mL of distilled water. The mixture was heated to a boil for 5 minutes and then filtered, following the method outlined by Caleja et al. (Caleja et al., 2016).

Milk fermentation

Fresh cow's milk was pasteurized at 85°C for 30 minutes and cooled to 40°C. Two groups of fermented milk were prepared: the control group (without corn silk addition) and the experimental group (with corn silk addition). For the control, 100 mL of pasteurized milk was mixed with 100 mL of skim milk, which was prepared by dissolving 3 g of skim milk powder in 100 mL of distilled water. For the corn silk-enriched yogurt, 100 mL of pasteurized milk was mixed with 100 mL of skim milk, where the skim milk was prepared by dissolving 3 g of skim milk powder in 100 mL of boiled corn silk extract. A 3% (v/v) starter culture was added to each group, and the mixture was incubated at 37°C for 18 hours. After fermentation, the yogurt was stored at 4°C for further analysis.

pH measurement

The pH of the yogurt samples was measured using a pH meter (Hanna). Prior to measurement, the instrument was calibrated with pH 4 and pH 7 buffer solutions. The sample was placed in a beaker, and the electrode was immersed. The pH value was recorded after the reading stabilized. The electrode was then rinsed with distilled water, dried, and recalibrated with the standard buffer solutions, as described by AOAC (AOAC, 2023b).

Acidity

Acidity was determined using Mann's Acid Test method. A 10 mL sample was placed in an Erlenmeyer flask, and 3 drops of phenolphthalein (PP) indicator were added. The sample was titrated with 0.1 N NaOH. Acidity was then calculated using the formula (AOAC, 2023a):

$$\text{Acidity (\%)} = \frac{\text{Volume of NaOH} \times 90}{\text{Volume of sample} \times 1000} \times 100\% \quad (1)$$

where 90 refers to the molecular weight of lactic acid.

Total lactic acid bacteria

The total LAB in the fermented milk was quantified using the pour plate method with MRS agar containing 100 ppm NaN_3 and 100 ppm $CaCO_3$. LAB, capable of dissolving calcium carbonate, formed clear zones on the agar, indicating their presence. Serial dilutions of the sample were prepared using 0.85% NaCl. A 1 mL aliquot from the selected dilution was inoculated onto 15 mL of MRS agar. The plate was spread with a sterile drygalski spatula, and after solidification, it was incubated at 37°C for 24 hours. Colonies were counted based on the clear zones formed, with the acceptable colony count ranging from 30 to 300, as described by Hosseini & Behbahani (Hosseini & Behbahani, 2021).

Antioxidant activity

Antioxidant activity was assessed using the DPPH method (Gurkan et al., 2019). A 1.0 mL sample solution (500 ppm in ethanol) was added to each test tube, along with 1 mL of ethanol (as the blank). To each tube, 3 mL of 0.16 mM DPPH solution in ethanol was added, and the mixture was vortexed. The tubes were incubated in the dark for 30 minutes. Absorbance was measured at 517 nm using a spectrophotometer. The antioxidant activity was calculated using the following formula:

$$\text{Antioxidant activity (\%)} = \left(1-\frac{\text{absorbance of the sample at 517 nm}}{\text{absorbance of the blank at 517 nm}}\right) \times 100\% \qquad (2)$$

Statistical analysis

This study employed a laboratory experimental design with descriptive analysis. Sensory testing was conducted using a hedonic test, where panelists rated the products based on aroma, sour taste, texture, and overall acceptability. The sensory data were analyzed using analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSIONS

The analysis of the yogurt drink, with and without the addition of corn silk, yielded the data summarized in Table 1.

Table 1. Total lactic acid bacteria (LAB), pH, acidity, and antioxidant activity of yogurt without and with corn silk addition.

Parameters	Yogurt without corn silk	Yogurt with corn silk
Total LAB (cfu.mL^{-1})	1.9×10^7	1.2×10^8
pH	4.5	4.4
Lactic acid (%)	0.93	1.34
Antioxidant activity (%)	47	54

Total lactic acid bacteria (LAB)

The total LAB count in yogurt without corn silk was 1.9×10^7 cfu.mL^{-1}, while yogurt with corn silk exhibited a significantly higher count of 1.2×10^8 cfu.mL^{-1}. Both values meet the Codex standard for yogurt, which requires a minimum of 10^7 cfu.mL^{-1}. The increased LAB count in yogurt with corn silk suggests that the addition of corn silk positively influences the fermentation process. This enhancement may be attributed to corn silk's high content of essential minerals such as Ca, Mg, Cu, Zn, K, Na, and Fe, as well as its complex carbohydrates and soluble fibers that serve as additional nutrients for the lactic acid bacteria (Raghuvanshi et al., 2024; Rahman & Wan Rosli, 2014). Corn silk can be a prebiotic, further promoting LAB growth during fermentation (Raghuvanshi et al., 2024). Moreover, *L. plantarum* in the starter culture is known to enhance the growth of both *Lactobacillus* species and *Streptococcus thermophilus*, and it also suppresses the growth of harmful bacteria like *Escherichia-Shigella*, which likely further contributes to the observed increase in LAB (Zhang et al., 2024).

pH and acidity

The pH of yogurt with corn silk was 4.4, slightly lower than the 4.5 recorded for the yogurt without corn silk. In addition, the yogurt with corn silk exhibited a higher total acidity (1.34%) than the control (0.93%). These differences can be attributed to the additional nutrients in corn silk, such as soluble fibers and sugars, which provide energy to the LAB, supporting their metabolic activity and consequently increasing the production of lactic acid. The higher acidity and lower pH observed in the yogurt with corn silk suggest an enhanced fermentation process, which likely contributed to the more pronounced sour taste of the yogurt. This relationship is consistent with the fact that LAB convert lactose into lactic acid, reducing the pH and increasing the acidity during fermentation (Wang et al., 2021).

Antioxidant activity

Fresh cow's milk typically has an antioxidant activity of approximately 38.7% (Balakrishnan & Agrawal, 2014). The fermentation process, however, can enhance this antioxidant capacity. In the present study, yogurt with corn silk demonstrated an antioxidant activity of 54%, compared to 47% in the yogurt without corn silk. This 7% increase indicates that the addition of corn silk boosts the antioxidant properties of the yogurt. The synergistic effect between the antioxidant compounds in corn silk and the metabolites produced by LAB, such as lactic acid, exopolysaccharides, and bioactive peptides, likely contributes to this enhancement (Gurkan et al., 2019). Therefore, corn silk not only enhances the nutritional quality of yogurt but also contributes to its functional health benefits, including increased antioxidant activity.

Organoleptic test

The organoleptic test assessed the taste, aroma, and overall acceptability of the yogurt, as presented in Table 2.

Table 2. Average organoleptic scores of yogurt with and without the addition of corn silk.

Treatment	Taste	Aroma	Acceptability
Cornsilk yogurt	4.00 ± 0.40	4.07 ± 0.27	1.15 ± 0.37
Yogurt control	4.00 ± 0.00	4.00 ± 0.00	1.07 ± 0.27

The taste test aimed to assess the level of acidity and the acceptability of fermented milk produced with and without the addition of corn silk. The acidity levels were categorized into five criteria: 1) very not sour, 2) not sour, 3) slightly sour, 4) sour, and 5) very sour. Results indicated no significant differences between the two treatments. Overall, the fermented milk products were rated as sour, a characteristic attributed to the activity of lactic acid bacteria. These bacteria metabolize lactose into lactic acid, lowering the milk's pH and creating the distinctive sour taste.

The aroma test evaluated panelists' preference and acceptability of the fermented milk drink's aroma. The criteria for aroma preference included: 1) very dislike, 2) dislike, 3) slightly like, 4) like, and 5) very like. ANOVA analysis of aroma characteristics revealed no significant differences between the treatments. The addition of corn silk extract had no impact on aroma acceptance, likely because the amount of corn silk added was minimal and did not significantly alter the aroma profile.

The overall acceptability of fermented milk products was analyzed, with results presented in Table 2. ANOVA analysis showed no significant differences ($p > 0.05$) in the acceptability of yogurt fermented with corn silk compared to yogurt without corn silk. Panelists provided neutral ratings for the acceptability of both samples.

CONCLUSIONS

Adding corn silk to yogurt positively influences the fermentation process, enhancing the growth of lactic acid bacteria (LAB) and boosting the antioxidant activity of the final product. The total LAB count in yogurt with corn silk was significantly higher than in the control, meeting the Codex standard for yogurt. The presence of corn silk contributed to an increase in acidity and a slight reduction in pH, indicating a more active fermentation and stronger sour taste. Additionally, the antioxidant activity of yogurt with corn silk was 7% higher than the control, demonstrating the nutritional benefits imparted by corn silk's bioactive compounds. Organoleptic testing revealed no significant differences in taste, aroma, or overall acceptability between the two yogurt variants, suggesting that corn silk's inclusion does not negatively impact consumer preference. Overall, adding corn silk improves the functional quality of yogurt, making it a valuable ingredient for enhancing both nutritional and health benefits.

Authors' Contributions: Arizki Tri Nopitariyani designed the study, performed the laboratory work, and drafted the manuscript. Dina Tri Marya supervised the research and finalized the manuscript. All authors reviewed and approved the final version.

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