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Research Article

INHIBITORY EFFECTS OF *DANGKE*, A FERMENTED DAIRY PRODUCT FROM ENREKANG, ON *STREPTOCOCCUS* GROWTH IN DENTAL PLAQUE

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Abstract
Dairy products are known for their non-cariogenic properties and have even been reported to inhibit the attachment of cariogenic bacteria such as *Streptococcus spp*. on dental plaque. *Dangke*, a traditional dairy product from Enrekang, South Sulawesi, with a high protein content of up to 17.20%, *dangke* is considered a non-cariogenic food. This study aimed to evaluate the in vitro efficacy of *Dangke* in reducing *Streptococcus* levels in dental plaque. The evaluation of Dangke implicates the use of traditional dairy products in oral health. This quasi-experimental study used a post-test-only control-group design with 15 subjects selected via simple random sampling. Subjects were divided into two groups: a treatment group that consumed *Dangke* and a control group that consumed cheddar cheese. Plaque samples were collected twice from each subject—immediately after consumption (T1) and two hours later (T2)—resulting in 30 samples per group. The *Streptococcus* colony counts were analyzed at the Microbiology Laboratory, Faculty of Medicine, Hasanuddin University. At T1, the mean colony counts were 19.44 CFU/mL for the *Dangke* group and 31.56 CFU/mL for the Cheddar group. At T2, the counts were 2.44 and 27.56 CFU/mL, respectively. A statistically significant reduction was observed in the *Dangke* group ($p = 0.028$), while no significant change was seen in the Cheddar group ($p = 0.746$). *Dangke* demonstrated a significant antimicrobial effect against *Streptococcus* in dental plaque and may serve as a natural cariostatic agent, providing new insights into the potential of local food products for caries prevention.

Keywords: Cheddar Cheese, *Dangke*, Dental Plaque, *Streptococcus*

INTRODUCTION

Dental plaque is a complex biofilm that forms naturally on tooth surfaces, playing a role in oral health and disease (Chen et al., 2021; Jakubovics et al., 2021). The extracellular matrix of dental plaque

consists of various macromolecules, including carbohydrates, proteins, and nucleic acids, which support microbial communities and facilitate interactions with the host (Jakubovics et al., 2021). While dental plaque can contribute to oral homeostasis, frequent exposure to low pH environments can lead to the dominance of acidogenic and acid-tolerant species, potentially causing caries (Cai & Kim, 2023; Chen et al., 2021). The development of dental plaque biofilm involves several stages, including initial adhesion, exopolysaccharide production, and maturation (Koka et al., 2021). Quorum sensing is crucial in biofilm formation and regulation (Chen et al., 2021; Koka et al., 2021). Understanding the complex microbial interactions and spatial organization within dental plaque biofilms is essential for developing targeted therapeutic strategies to maintain oral health (Cai & Kim, 2023; Chen et al., 2021).

Dental caries is closely associated with bacterial plaque formation, particularly involving *Streptococcus mutans* (Zafar et al., 2020). S. mutans colonizes tooth surfaces, forming biofilms through adhesion mechanisms and exopolysaccharide production (Alejandra & Daniel, 2020). These bacteria ferment dietary carbohydrates, producing acids that lower pH and contribute to tooth decay (Alejandra & Daniel, 2020; Zafar et al., 2020). The oral microbiota's balance is crucial for maintaining colonization resistance against pathogens, with nutritional factors playing a significant role in this equilibrium (Akimbekov et al., 2022). S. mutans exhibits acidogenicity and aciduricity, allowing it to thrive in low pH environments (Alejandra & Daniel, 2020). Traditional prevention methods like mechanical removal and antibacterial agents have limitations, prompting research into novel strategies such as antimicrobial peptides, probiotics, and nanoparticles to target S. mutans biofilm formation (Gao et al., 2024). Understanding the regulatory mechanisms of biofilm formation is crucial for developing effective caries prevention and treatment approaches (Gao et al., 2024).

Milk and dairy products have shown potential in preventing dental caries. Studies indicate that yoghurt and cheese consumption is associated with a decreased risk of dental caries in children and adolescents (Wang et al., 2021). The cariostatic effects of dairy products are attributed to their high protein content, particularly casein, which may prevent bacterial adhesion to tooth surfaces (Li et al., 2023). Probiotics in fermented dairy products can inhibit the growth of cariogenic bacteria like *Streptococcus mutans* (Amargianitakis et al., 2021). Probiotic therapy, often administered through dairy products, represents a new strategy for caries prevention by displacing cariogenic microorganisms and producing antimicrobial substances (Amargianitakis et al., 2021). These findings support the role of milk and dairy products in dental caries prevention and treatment.

Dangke, a traditional cheese-like product from Enrekang, South Sulawesi, Indonesia, is made from buffalo or cow's milk coagulated with papaya enzymes (Darwis, 2022; Mustamin et al., 2022). It has a high protein content, making it a valuable source of animal protein (Hajrawati et al., 2024; Setiarto et al., 2025). Studies have shown that *dangke* consumption increases calcium and phosphate levels in saliva, promotes enamel remineralization, and reduces oral bacteria (Samad et al., 2018). The best *Dangke* to production uses 1-5% commercial papain, resulting in 17-18% yields with compact texture and non-bitter taste (Djide et al., 2020; Musra et al., 2021). *Dangke* contains beneficial compounds such as linoleic, palmitic, stearic, and oleic acids (Al-Baarri et al., 2018; Kaswi et al., 2023; Yusuf et al., 2022). Its shelf life is less than 24 hours at room temperature but can extend to four days when refrigerated (Musra et al., 2021). Historically, *Dangke* has been served with rice to guests since the colonial era and holds significant cultural value for the Enrekang people (Sa'pang et al., 2023).

This study addresses a research gap regarding the impact of *Dangke* on dental plaque and *Streptococcus* growth. While the cariostatic effects of dairy products have been well documented, prior studies have predominantly focused on commercial dairy products, such as cheddar cheese and yogurt. In contrast, *Dangke*, a traditional local dairy product, has not yet been investigated in this context. This research bridges this gap by evaluating *Dangke* as a potential alternative for caries prevention, thereby contributing novel insights to the existing body of literature.

The current study will discuss the non-cariogenic potential of *Dangke* and cheddar cheese in forming bacteria on the tooth surface. This problem will be reviewed from a microbiological perspective by counting the number of colonies of *Streptococcus*. This study aimed to determine the efficacy of *Dangke* to reduce the number of *Streptococcus* in dental plaque, comparing it with cheddar cheese as a control. The novelty of this study lies in the use of *Dangke*, a local food product rich in protein, and its antibacterial potential, which may serve as a natural cariostatic agent. This study fills the gap between traditional food and oral health, opening up opportunities for further research into the potential of local products in dental caries.

RESEARCH METHOD

This quasi-experimental study employed a post-test only control group design and was conducted in the Microbiology Laboratory, Faculty of Medicine, Hasanuddin University. A total of 436 clinical students from the Faculty of Dentistry, Hasanuddin University, comprised the population. Using a simple random sampling method, 15 subjects were selected for each group, resulting in 30 dental plaque samples per group. Subjects were assigned to either the *Dangke* (treatment) group or the cheddar cheese (control) group. All participants provided informed consent prior to inclusion. Inclusion criteria were as follows: caries-free, free of systemic diseases, possessing at least 28 natural teeth, no edentulous areas or dental restorations, no crowding or use of orthodontic appliances, and having good oral hygiene as indicated by a Calculus Index Score (CIS) of 0–0.6.

Subjects were instructed to perform toothbrushing using the Modified Bass technique prior to sampling. In the treatment group, subjects consumed *Dangke*, after which supragingival plaque samples were collected immediately (T1) using sterile cotton swabs from the gingival margin of 28 teeth. Samples were transferred into transport tubes containing 5 mL of NaCl solution. Two hours post-consumption, a second plaque sample (T2) was collected using the same method and site. During this interval, subjects were prohibited from consuming any food or drink. On the following day, the same procedures were repeated with cheddar cheese as the test product.

Plaque samples were collected from the gingival margins of 28 teeth using sterile cotton swabs. The samples were then placed in transport tubes containing 5 mL NaCl solution. This data collection instrument ensures standardized collection of plaque samples, which is crucial for maintaining the consistency and quality of the data obtained. All plaque samples were immediately transported to the Microbiology Laboratory. Each sample was diluted threefold in NaCl, then cultured using nutrient broth media. The number of *Streptococcus* colonies was quantified based on the Colony Forming Units per milliliter (CFU/mL).

Data were analyzed using SPSS software. The statistical test used was a paired t-test to compare the *Streptococcus* colony counts within each group (Dangke and cheddar cheese) between T1 (immediately after consumption) and T2 (two hours post-consumption). An independent t-test was used to compare the mean differences between the two groups. Statistical significance was set at $p < 0.05$. This approach allows for the identification of significant differences in *Streptococcus* colony reductions between the two groups.

RESULTS AND DISCUSSION

During the evaluation of *Streptococcus* colony counts in the 15 paired plaque samples (T1 and T2) from each group, several samples showed extreme variations in colony numbers. These discrepancies were considered potential sources of bias in the data analysis. Consequently, six sample pairs from each group (*Dangke* and cheddar cheese) were excluded from the final analysis. As a result, only nine paired dental plaque samples (T1 and T2) from each group met the criteria for valid statistical evaluation. The final data and comparative results are presented in Table 1.

Statistical analysis was performed using SPSS software to compare *Streptococcus* colony counts within each group (*Dangke* and cheddar cheese) between T1 (immediately after consumption) and T2 (two hours post-consumption); a paired t-test was used. This test was applied to analyze differences in colony counts within the same group at two different time points. Additionally, an independent t-test was used to compare differences between the *Dangke* and Cheddar cheese groups. A p-value less than 0.05 was considered statistically significant. The paired t-test assesses changes within the same group, while the independent t-test compares two distinct groups. All analyses were performed at a 95% confidence level ($p < 0.05$).

Statistical power is an important aspect in determining the study's ability to detect significant differences if they exist. Based on the sample size used in this study, we calculated power using G*Power software to determine whether the sample was sufficient to detect the expected effects. Based on calculations with an alpha of 0.05 and the estimated effect size, the study's power is approximately 80%. This means there is an 80% chance of detecting a significant difference in *Streptococcus* colony reduction between the two groups if such a difference truly exists. Although the sample size is relatively small, the high power suggests that this study has a good probability of identifying significant differences.

Table 1. Comparison of the mean reduction in *Streptococcus* colony counts between *Dangke* and cheddar cheese consumption

Groups	T_1	T_2	Mean Difference ± SD	p-Value*
	Mean ± SD	Mean ± SD		
Dangke	19.44 ± 20.29	2.44 ± 2.83	17.00 ± 19.00	0.028*
Cheddar Cheese	31.56 ± 32.22	27.56 ± 28.25	4.00 ± 35.78	0.746

* Paired t-test; Level of significance p < 0,05; CI 95%

Table 1 and Figure 1 present the mean differences in *Streptococcus* colony counts following the consumption of *Dangke* and cheddar cheese. In the *Dangke* group, the mean *Streptococcus* colony counts decreased from 19.44 CFU/mL at T1 (immediately after consumption) to 2.44 CFU/mL at T2 (two hours post-consumption), resulting in a mean reduction of 17.00 CFU/mL. This reduction was statistically significant (p = 0.028), indicating a notable antibacterial effect of *Dangke* on dental plaque *Streptococcus*.

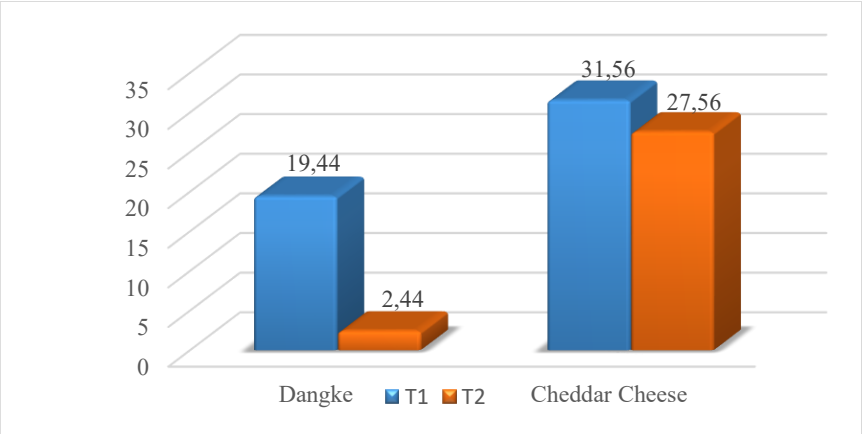

Figure 1. Comparison of the mean reduction in *Streptococcus* colony counts between *Dangke* and cheddar cheese consumption

In contrast, the cheddar cheese group exhibited a mean colony count of 31.56 CFU/mL at T1 and 27.56 CFU/mL at T2, with a mean reduction of only 4.00 CFU/mL. This decrease was not statistically significant (p > 0.05), suggesting that cheddar cheese had a minimal effect on reducing *Streptococcus* levels within the observed timeframe.

Table 2. The difference of mean differences between *Dangke* and cheddar cheese consumption

Groups	Mean Difference ± SD	Difference of MD ± SD	p-Value*
Dangke	17.00 ± 19.00	13.00 ± 13.50	0.350
Cheddar Cheese	4.00 ± 35.78		

*Independent t-test; Level of significance p < 0,05; CI 95%

Based on the data presented in Table 2, the difference in mean reductions of *Streptococcus* colony counts between the *Dangke* and cheddar cheese groups was 13.00 CFU/mL, which was not statistically significant (p > 0.05).

Milk and dairy products have been recognized for their potential to reduce dental caries risk. The World Health Organization (WHO) classified the evidence for milk's caries-reducing effect as “possible” in 2003 (Woodward & Rugg-Gunn, 2020). Recent studies support milk's low cariogenicity and protective properties, attributing these to lactose's lower acidogenicity and the presence of calcium, phosphate, proteins, and fats (Woodward & Rugg-Gunn, 2020; Li et al., 2023). Dairy products may serve as ideal vehicles for probiotic administration in dental patients, offering a strategy for caries prevention (Amargianitakis et al., 2021). Probiotics in dairy can reduce cariogenic bacteria, create a protective barrier against pathogens, and support natural defense mechanisms (Butt & Sin, 2023; Vitiello et al., 2024). However, more long-term, high-quality studies are needed to fully understand the impact of milk and its constituents on oral health and to develop effective caries prevention strategies, particularly for children and adolescents (Luo et al., 2024; Vitiello et al., 2024).

Casein phosphopeptide (CPP) has shown promising effects in inhibiting dental caries by reducing *Streptococcus mutans* adhesion to tooth surfaces. CPP modifies the salivary pellicle, decreasing surface hydrophobicity and negative charge, with fluoride enhancing this anti-adhesion effect (Wang et al., 2021). CPP-amorphous calcium phosphate (CPP-ACP) added to milk, chewing gum, and candy demonstrates potential remineralizing activity on tooth enamel and some antibacterial effects on dental biofilm (Giacaman et al., 2023). Osteopontin, another bioactive milk protein, effectively prevents adhesion of various cariogenic bacteria to saliva-coated surfaces (Kristensen et al., 2022; Shkembi & Huppertz, 2023). However, the "caries-safe" effect of milk proteins may be compromised in complex microbial environments. Bacillus subtilis, a common milk contaminant, can break down κ-casein through proteolytic activity, potentially enabling *S. mutans* biofilm formation despite the presence of caseins (Duanis-Assaf et al., 2020). These findings highlight the complexity of milk's role in dental caries prevention.

Recent studies have explored the potential of milk proteins, particularly casein and its derivatives, in preventing dental caries and erosion. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) added to foodstuffs has shown promising remineralizing effects on tooth enamel and some antibacterial activity against dental biofilm (Giacaman et al., 2023). While casein alone may inhibit remineralization, its combination with fluoride promotes remineralization of caries lesions (Nakamura et al., 2021). Osteopontin and other milk proteins, especially β-casein, have demonstrated effectiveness in reducing bacterial adhesion to saliva-coated surfaces, potentially delaying harmful biofilm formation (Kristensen et al., 2022). Furthermore, the combination of different proteins and peptides, such as CaneCPI-5, Hemoglobin, and Statherin, has shown superior protection against dental erosion by interacting with the enamel surface and modifying the salivary pellicle (Pelá et al., 2021; Sakhare et al., 2021). These findings suggest that milk proteins offer promising avenues for caries prevention and control.

The curd fraction of milk contains a higher concentration of casein compared to whey (Kryuchkova et al., 2021; Li et al., 2023). This distinction is relevant to the production processes of *Dangke* and cheddar cheese. In cheddar cheese manufacturing, the cheddaring process primarily separates whey, which constitutes the major component, while much of the curd is discarded. Conversely, the production of *Dangke* utilizes both whey and curd simultaneously, resulting in a higher casein content in *Dangke*. Elevated casein levels are associated with greater potential to protect tooth surfaces from bacterial colonization. This is supported by recent findings indicating a significant reduction in *Streptococcus* counts in the *Dangke* group (Amargianitakis et al., 2021; Chan et al., 2023).

Additionally, the production processes of *Dangke* and cheddar cheese involve the use of enzymes as coagulants—papain enzyme for *Dangke* and rennet enzyme for cheddar cheese. These enzymes facilitate the separation of fat and protein in cow's milk, resulting in the formation of whey and curd proteins (Musra et al., 2021). Prior to coagulation, the coagulant enzymes also promote the activity of bacteria capable of metabolizing lactose into lactic acid. These bacteria, commonly referred to as probiotics, are live microorganisms present in foods that, when consumed in adequate amounts, confer health benefits to the host (D. Dhumal et al., 2024). The bacteria most commonly employed as probiotics belong to the *Lactobacillus* genus. Research by Jia et al. (2021) and Squarzanti et al. (2024) demonstrated that cheddar cheese typically contains *Lactobacillus casei* and *Lactobacillus paracasei*. In contrast, a study by Afshari et al. (2022) identified *Lactobacillus plantarum* and *Lactobacillus fermentum* as the predominant probiotic strains in *Dangke*. Notably, certain *Lactobacillus* species have been shown to inhibit the growth of cariogenic bacteria, contributing to oral health (Saïz et al., 2021; Zhang et al., 2024).

The results of this study demonstrated that consumption of *Dangke* significantly reduced the number of *Streptococcus* in dental plaque compared to cheddar cheese. This effect is likely attributed to the probiotic bacteria present in *Dangke*, which may inhibit the adherence of pathogenic bacteria (Utama et al., 2019; Djide et al., 2020). Similarly, *Lactobacillus* and *Pediococcus* strains exhibited antibacterial and anti-biofilm properties, downregulating key S. mutans genes involved in biofilm formation (Luan et al., 2022). Native lactic acid bacteria, including *Lactobacillus brevis, L. casei,* and *L. paracasei*, demonstrated strong antimicrobial characteristics against *S. mutans* and reduced its attachment to surfaces (Utama et al., 2019; Djide et al., 2020). Furthermore, *Lactobacillus plantarum* showed the highest antimicrobial activity among tested strains and effectively inhibited *S. mutans* biofilm formation (Demir & Demir, 2021). These findings suggest that probiotic lactic acid bacteria could be promising candidates for preventing dental caries by suppressing *S. mutans* growth and biofilm formation (Djide et al., 2021; Kaswi et al., 2023; Nur et al., 2024).

Although the results of this study indicate that the cheddar cheese group decreased insignificantly but cheddar cheese still considered to have cariostatic properties that can declined the number of *Streptococcus* bacteria. The results of this study supported from the research of vitro Banakar et al. (2023) which isolated two species of *Lactobacillus (L. reuteri* and *L. rhamnosus)* in the oral cavity of healthy person where the bacteria have antimicrobial activity against *Streptococcus spp.* Another factor contributing to the greater reduction of bacterial counts observed with *Dangke* compared to cheddar cheese is oral clearance activity. Oral clearance, influenced by dietary components, salivary flow, and the movements of the tongue, cheeks, and lips, plays a critical role in removing food residues and bacteria from the oral cavity (da Cruz et al., 2022; Nagakubo & Kaibori, 2023). *Dangke*, classified as a soft cheese, contains a higher moisture content (45.75%) compared to cheddar cheese (36.5%), which may facilitate faster oral clearance and thus contribute to its enhanced antimicrobial effect (Yusuf et al., 2022).

The soft consistency and higher moisture content of *Dangke* confer greater elasticity, which during mastication promotes thorough mixing and stimulation of oral structures, thereby enhancing oral clearance. In contrast, cheddar cheese is drier with a firmer texture, allowing it to be chewed with less involvement of the tongue, cheeks, and lips in forming the food bolus (Al-Baarri et al., 2018; Darwis, 2022). This difference aligns with recent findings demonstrating that both *Dangke* and cheddar cheese possess cariostatic properties by reducing *Streptococcus* levels in dental plaque, with *Dangke* exhibiting a more pronounced effect than cheddar cheese.

The results of this study show that consumption of *Dangke* significantly reduced *Streptococcus* colony counts in dental plaque, with a greater reduction compared to cheddar cheese. These findings align with previous studies indicating that dairy products can have cariostatic effects, reducing pathogenic bacteria in dental plaque. A study by Samad et al. (2018) demonstrated that dairy products can enhance enamel remineralization and reduce oral bacteria, supporting the notion that *Dangke* may serve as a natural cariostatic agent. However, this study introduces dangke as a local alternative that has not been extensively studied before. The novelty of this research lies in the evaluation of *Dangke*, a traditional dairy product from Enrekang, in reducing Streptococcus levels in dental plaque. This contributes to the development of local food products with potential oral health benefits, particularly in caries prevention. The implications of these findings suggest that *Dangke* could be a natural alternative in caries prevention strategies, paving the way for further research into the role of local dairy products in maintaining oral health. However, the study has limitations, particularly its relatively small sample size and short duration. Therefore, further studies with larger sample sizes and longer observation periods are recommended to confirm these findings and to explore the underlying mechanisms of Dangke's antibacterial effect.

CONCLUSION

Significant differences were observed in the number of *Streptococcus* in dental plaque following consumption of *Dangke* and cheddar cheese, with *Dangke* demonstrating greater efficacy in reducing *Streptococcus* levels. These findings indicate that *Dangke* possesses superior cariostatic properties compared to cheddar cheese, which could serve as an alternative in caries prevention strategies. This finding leads to the development of the concept that local products rich in protein and probiotics may be an essential component of broader caries prevention strategies, and could potentially be more accessible and culturally accepted across different communities. The implications of this study suggest that traditional dairy products should be considered as a key element in food-based caries prevention programs.

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AUTHOR CONTRIBUTIONS

Conceptualization, R.H. and R.S.; Methodology, R.H. and R.S.; Software, M.A.B.; Validation, R.H. and R.S.; Formal Analysis, R.H.; Investigation, R.H. and M.A.B.; Resources, N. and P.S.; Data Curation, M.Z. and C.O.J.; Writing-Original Draft Preparation, R.H.; Writing-Review & Editing, R.S. and S.M.; Visualization, M.Z.; Supervision, R.S.; Project Administration, R.H.; Funding Acquisition, S.M.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

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