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A Comparison of Yogurt and Probiotic Supplements Effects on the Hardness of Tooth Enamel: An In vitro Study

Perbandingan Pengaruh Yogurt dan Suplemen Probiotik terhadap Kekerasan Enamel Gigi: Studi in Vitro

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Abstract

The demineralization of tooth enamel, which can be triggered by acidic foods and drinks at pH < 5.5, can initiate dental caries. Yogurt and probiotic supplements are drinks that are often consumed to help digestive function and improve the immune system. However, both drinks have a low pH, thereby, a long time of consumption can trigger the demineralization process. This study aims to evaluate the enamel hardness after applying yogurt and probiotic supplements, which was carried out experimentally in a laboratory with a one-group post-test design. The sample used is 9 human maxillary premolars in each treatment group. The treatment group comprises the test material, such as yogurt and probiotic supplements, as well as one control group with artificial saliva. Dental samples were immersed in the test material for 96 hours, and the enamel hardness level was measured with a Micro Vickers Hardness tester. The T-paired test showed that soaking with yogurt does not significantly affect the hardness of the enamel, as indicated by a p-value of 0.016. However, the effect was significant in probiotic supplements, with p=0.004. The results showed that soaking with yogurt was not significantly different from probiotic supplements in reducing enamel hardness, as evidenced by the Post Hoc (LSD) test (p=0.464). Conclusively, yogurt decreased enamel hardness less than probiotic supplements.

Keywords: prebiotic, yogurt, probiotic, demineralization, hardness of tooth enamels

Abstrak

Karies gigi dapat diawali dari proses demineralisasi enamel gigi yang dapat dipicu oleh makanan dan minuman yang asam (pH < 5.5). Yogurt dan suplemen probiotik merupakan minuman yang sering dikonsumsi oleh masyarakat untuk membantu fungsi pencernaan dan meningkatkan sistem imunitas. Akan tetapi, kedua minuman tersebut memiliki pH yang rendah sehingga dapat memicu terjadinya proses demineralisai apabila dikonsumsi dalam jangka waktu yang lama. Tujuan penelitian ini adalah untuk mengevaluasi kekerasan enamel setelah pemberian yogurt dan suplemen probiotik yang dilakukan secara ekperimental laboratoris dengan rancangan *one group pretest-postest*. Sampel yang digunakan yakni 9 gigi premolar maksila manusia pada setiap kelompok perlakuan. Penelitian ini terdiri atas 2 kelompok perlakuan dengan bahan uji yakni yogurt dan suplemen probiotik dan 1 kelompok kontrol berupa saliva buatan. Sampel gigi direndam dengan bahan uji selama 96 jam. Tingkat kekerasan enamel diukur dengan Micro Vickers Hardness tester. Hasil uji statistik dengan uji *T-paired* menunjukkan bahwa perendaman dengan yogurt tidak berpengaruh signifikan terhadap kekerasan enamel (p=0.016). Sedangkan pada suplemen probiotik, efek yang ditimbulkan signifikan (p=0.004). Hasil penelitian ini menunjukkan bahwa perendaman dengan yogurt tidak berbeda signifikan dengan suplemen probiotik dalam menurunkan kekerasan enamel yang dibuktikan dengan uji *Post Hoc (LSD)* (p=0.464). Studi ini menyimpulkan bahwa yogurt menurunkan kekerasan enamel lebih sedikit daripada suplemen probiotik.

Kata kunci: prebiotik, yogurt, probiotik, demineralisasi, kekerasan enamel gigi

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INTRODUCTION

Caries are one of the top ten most commonly reported diseases in Indonesia.[1] The prevalence of national dental and oral problems increased to 57.6% in 2018 from the previous 53.2% in 2013.[2] Dental caries have a relationship with several factors, such as tooth condition, carbohydrate fermentation, and acid production from bacteria in the oral cavity.[3] It is caused by demineralization of the tooth, which is triggered by the production of organic acids by bacteria in dental plaque resulting from carbohydrate metabolism.[4,5]

One strategy to prevent caries is to inhibit the demineralization process in the tooth through the use of minerals containing bioactive glass nano-silica, such as bagasse ash.[6] It can also be prevented with casein phosphopeptide (CPP) ingredients such as yogurt. According to Ferrazano et al., casein phosphor-peptide (CPP) content in yogurt significantly detains mineral decomposition and accelerates the mineral formation of tooth enamel.[7] However, the low pH of yogurt allows for demineralization of enamel.[8]

Probiotic supplements are also believed to inhibit the demineralization of tooth enamel. Keller et al. examined the effect of *Lactobacillus reuteri* in probiotic supplements on early caries lesions and showed a tendency for less demineralization.[9]

Based on these data, yogurt and probiotic supplements have potential as tooth enamel remineralization materials. However, their low acidity allows demineralization of the tooth, which affects the hardness of the enamel.[10] This study is expected to evaluate tooth enamel's hardness after applying yogurt and probiotic supplements. Thus, the differrence in the effects of the two materials on tooth enamel can be determined.

MATERIALS AND METHODS

Research design. This was an experiments study used a one-group pretest-posttest design. Research sites. The hardness of the enamel surface of a tooth sample was measured at the Approved Training Body Laboratory, Politeknik Negri Medan, North Sumatera, Indonesia, from December 2020 to February 2021. Ethical clearance. Ethical approval was obtained from the Health Research Ethic Commission (KEPK) Faculty of Medicine, University of Sumatera Utara / H. Adam Malik Hospital Medan through Ethical Clearance Letter No:837/KEP/USU/2020.

Population and sample. The samples used include extracted human maxillary first and second premolars for orthodontic purposes and the manufacture of prostheses from dentist practices around the city of Medan, North Sumatra.

There were three treatment groups in this study. Tooth samples were soaked with yogurt in the first group. In the second, dental samples were soaked with probiotic supplements. The third was the control group, where the tooth samples were soaked with artificial saliva. Each group consisted of 9 samples of premolars with intact crowns.[11] The sample size was determined using Federer's formula and selected using non-probability sampling.[11]

Tooth sample preparation: The tooth sample is immersed in an artificial saliva solution to maintain health. The surface was cleaned with a bur brush, and the root was cut with a disc bur to the cementoenamel margin before being implanted in a block made of type 2 stone gypsum.

Preparation of the test material and immersion of the sample: A total of 100 ml KIN® original yogurt was poured into a beaker glass. The probiotic supplement solution was prepared by mixing 1 gram of the BioGaia Interlac® probiotic with 30 ml of aquadest and poured into a glass beaker. Furthermore, the artificial saliva solutions were prepared using the McDougall method (1948) namely MgCl (0.06 g/L), NaCl (0.47 g/L), KCl (0.57 g /L), $NaHCO_3$ (9.8 g/L), $Na_2HPO_4.12H_2O$ (9.3 g/L), and $CaCl_2$ (0.04 g/L). These substances were mixed with distilled water in an Erlenmeyer flask to a volume of 500 mL and stirred using a magnetic stirrer until all the ingredients were homogeneous. A total of 100 mL of the mixture was poured into a glass beaker. Each sample was immersed in the three solutions in a different container for 96 hours. The time used is based on the report of Sudjalim TR et al. that immersion of a dental specimen in a demineralized solution for 96 hours resulted in a 100 µm deep enamel lesion. This is comparable to the natural time on a human tooth for three months.[12] The acidity level of each solution was measured using a pH Meter Hanna 96107 before being immersed with the samples.

The result obtained an acidity level of each yogurt, probiotic supplement, and artificial saliva solution of 5.8, 6.2, and 7, respectively. Analysis of tooth enamel surface hardness: The Micro Vickers Hardness Tester was used to test the hardness of the enamel surface level of each tooth sample. Hardness testing was performed on the buccal surface. The sample is placed beneath the objective lens and below the diamond indenter. The tool is activated, and the indenter descends to apply 100 grams of pressure for 15 seconds and rise to leave an indent on the sample surface. Subsequently, the sample was adjusted to the object-

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tive lens and refocused to ensure visibility on the eyepiece. Alignment was performed at 3 points representing the upper 1/3, middle 1/3, and lower 1/3 section of the tooth mesial crown. The average value of the diagonal length measurement is then entered into the Vickers Hard-ness Number (VHN) formula.[13]

The data were analyzed using the Statistical Package for Social Science (SPSS). Paired T-test was used to determine the difference between sample hardness before and after the application of the test material. The Post Hoc test (LSD) was used to determine the difference between the treatments group.

RESULTS

The surface hardness of the tooth enamel was measured twice, namely before and after the sample was soaked with the test material for four days (96 hours). Figure 3 shows the results of measuring enamel hardness using the Micro Vickers Hardness Tester. The average value of the enamel surface hardness measurement in groups 1 and 2 is 48.03 and 79.19 VHN. Furthermore, the curve of enamel hardness decrease in the first group is generally below the second.

The paired t-test was used to determine the significant effect of reducing enamel hardness before and after soaking with the test material. The results are shown im Table 1.

Table 1 shows the significant difference ($p<0.05$) in the decrease in enamel hardness before and after soaking with yogurt in group 1. There is also a significant difference before and after soaking with probiotic supplements in group 2 ($p<0.05$).

Furthermore, a Post Hoc LSD test was conducted to determine the significant difference between groups 1 and 2, and the results are shown in Table 2. The results showed that the samples soaked with yogurt and probiotic supplements decreased significantly ($p<0.05$) compared to each control group. However, there were no significant differences between the results of groups 1 and 2. This implies that the reduction in enamel hardness caused by probiotic supplements was greater than in yogurt.

DISCUSSIONS

In this study, group 1 samples showed a significant decrease in hardness of 48.03 VHN ($p<0.05$). This result is consistent with Brancher et al. that yogurt's low pH induces demineralization of tooth enamel. Brancher et al. compared the degree of demineralization after administering yogurt and milk. The products were obtained from various yogurts and milk, divided into four groups according to their acidity and calcium content. Apparently, the differences in pH value have little effect on the demineralization of tooth enamel. The calcium content of a drink directly influences the release of tooth minerals. Furthermore, the more calcium a drink contains, the less demineralization occurs. This study compared yogurt and probiotic supplements. Demineralization caused by soaking with yogurt was less than probiotic supplements (pH 6.2), even though the pH of yogurt (pH 5.8) was lower. Additional studies are suggested to determine the calcium levels in the two drinks.[8]

The fermentation process of the bacterial culture in yogurt causes a decrease in pH to 3.8-4.5.[14] According to Zulkapli et al., some of the characteristics of yogurt include low pH and high buffer capacity, which have the potential to cause erosion of tooth enamel and affect its hardness.[7,15] Another study that supports this result is Moeiny et al., which proves that the surface hardness of tooth enamel can be reduced by fruit yogurt. Furthermore, the soaking process was shown to reduce the hardness of tooth enamel in the study of Mesquita-Gumares et al.[4,16]

The tooth sample of group 2 soaked with probiotic supplements led to a significant decrease in enamel hardness at 79.19 VHN. Probiotic supplements typically administered orally to humans in powder or beverage form contain probiotic strains.[17,18,19] In this study, it contained *Lactobacillus reuteri*, which is a lactic acid organism commonly found in the human digestive system. These bacteria can thrive at a pH of 5 or lower. They adhere to the tooth surface, form biofilms, produce acid from carbohydrate fermentation in the cavity, and degrade hydroxyapatite, which decreases enamel hardness.[20] Furthermore, the bacteria is a heterofermentative obligator that produces other products besides lactic acids, such as acetic acid and ethanol from glucose. This results from bacterial metabolism, which also triggers demineralization.[21,22] Haukioja et al. proved that *Lactobacilli* strains were associated with the development of dental caries. Acid production from carbohydrate fermentation produces a critical pH (<5.5), which induces enamel demineralization and decreases the hardness of tooth enamel.[23,24,25]

Enamel demineralization occurs through the diffusion of water-soluble ions or enamel into saliva due to the concentration differences between the acid on the surface and the solution for 96 hours.[26] Although enamel has a hard structure, it has permeable properties to ions and molecules derived from food and drink, which slowly dissolves inorganic elements of enamel and affects its hardness.[27] The mineral dissolves due to acid exposure to enamel, which originates from the fermentation of bacteria in the mouth as well as food and beverages that decreases the pH

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of the oral cavity.[28]

Probiotics are living microorganisms that are beneficial to hosts due to their ability to maintain intestinal microbes as well as healthy tooth and mouth.[29] They inhibit the formation of biofilms and release chemicals to interfere with harmful bacteria that can affect oral cavity health.[18] Kusumaningsih et al. also showed another benefit of probiotic bacteria, *L. reuteri*, which was increased Beta Defensin-2 (BD-2) in saliva. This increase can inhibit *S. mutans*, which triggers caries in the oral cavity.[30] The benefits of these products are also present in chewing gum, milk, ice cream, cheese, and others.[18]

This study shows that yogurt and probiotic supplements can reduce tooth enamel hardness. However, the effect of probiotic supplements on reducing enamel hardness is higher than in yogurt. Probiotic supplements did not affect inhibiting tooth demineralization, but yogurt had an inhibiting effect on demineralization because it contained CPP-ACP.[31]

The CPP content in yogurt can stabilize calcium, phosphate, and fluoride ions at high concentrations, which triggers remineralization.[32] It is derived from tryptic casein found in cow's milk protein, which can bind and stabilize amorphous calcium phosphate (ACP) in dental plaque. The protective effect produced by CPP occurs when there is a demineralizing substance, such as acidic pH levels, which causes CPP-ACP to release calcium ions. This is followed by an increase in the concentration of Ca cations and triggers a surfeited condition that restrains demineralization and increases enamel remineralization, thereby affecting its hardness.[7]

Enamel remineralization can affect the hardness of tooth enamel. In the process, mineral ions are returned to the hydroxyapatite structure. The ion lost in the process is recovered when the pH is around 7 (neutral) and the content of Ca^{2+} and PO_4^{3-} ions is adequate. Furthermore, the decomposed apatite product can become neutral through a buffering process, while the Ca^{2+} and PO_4^{3-} ions in saliva inhibit the dissolution process through the common ion effect. This process rebuilds partly dissolved apatite crystals.[32]

According to Ferrazano et al., the proteolytic activity of lactic acid bacteria produces casein phosphorpeptide (CPP) in yogurt. In addition, yogurt contains calcium which can withstand the erosive effect due to its low pH.[7] This is consistent with Singh et al., who proved that the CPP-ACP content in plain yogurt can inhibit enamel demineralization more effectively than 500 ppm NaF.[5]

Both yogurt and probiotics have their advantages and disadvantages. Yogurt is lighter in reducing the hardness of enamel despite its acidic nature. This is possible due to the presence of CPP-APP, which is not found in probiotic supplements. According to Kusumaningsih, probiotic supplements can prevent caries, but the prevention mechanism differs from yogurt. Probiotics prevent caries by inhibiting *S. mutans* bacteria due to increased BD-2 in saliva after consuming this product.[30] In this study, the procedures were conducted in vitro without any salivary reaction. Therefore, it is likely that an increase in BD-2 was not observed, similar to how probiotics prevent caries.

Yogurt and probiotic supplements can significantly reduce the hardness of tooth enamel. However, the ability of yogurt to reduce enamel hardness is lower than the probiotic supplements. Considering the several limitations of this study, further investigations are recommended to determine more about the properties and characteristics of both products.

TABLES

Table 1. Different enamel hardness before and after soaking yogurt and probiotic supplements (analysis with paired T-test).

Group	n	Mean ± SD	p-value
Yogurt (before soaking)	9	339.5 ± 38.60	0.016
Yogurt (after soaking)	9	291.5 ± 74.33	
Probiotic Supplement (Before soaking)	9	337.7 ± 44.81	0.004
Probiotic Supplement (After soaking)	9	258.5 ± 75.29	

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Table 2. Comparation of the tooth enamel hardness between the test groups and the control group (analysis with Post Hoc (LSD) test)

Group	Variable	Mean difference	p-value
Yogurt	Probiotic Supplement	31.16	0.464
	Control	-94.79	0.033
Probiotic Supplement	Yogurt	-31.16	0.464
	Control	-125.94	0.006

FIGURES

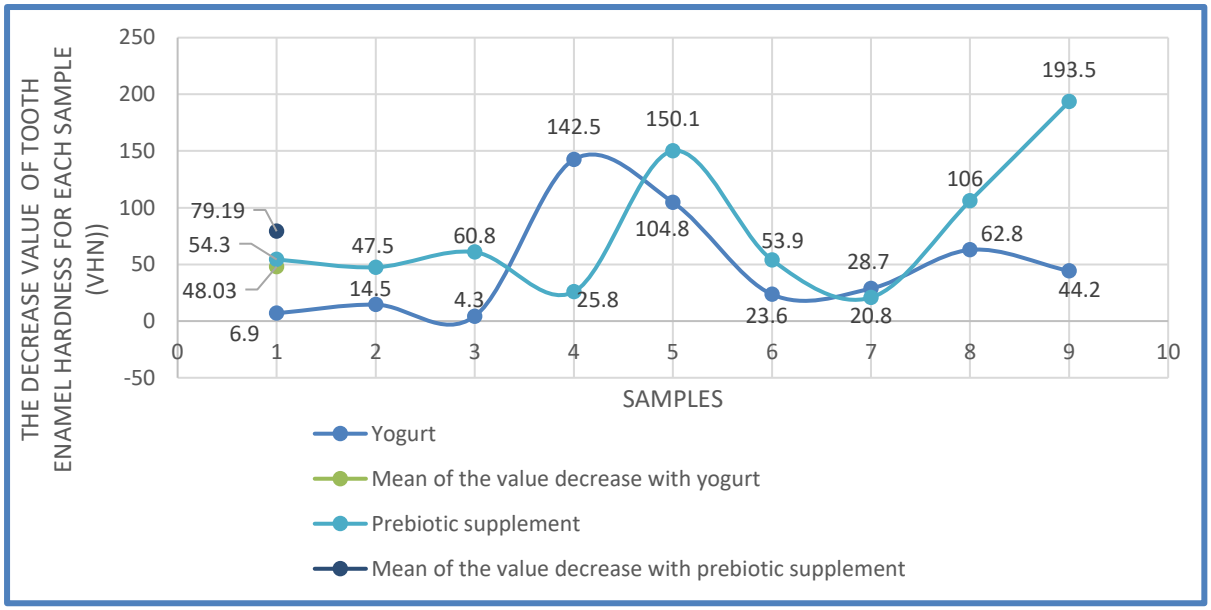

Figure 1. The results of measuring tooth enamel hardness. The value of decreasing enamel hardness in 9 samples of teeth that had been soaked for 96 hours in groups 1 and 2.

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