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Influence of Dangke (Cheese Typical Enrekang, South Sulawesi) Consumption to calcium and phosphate levels in saliva, Remineralization of enamel, Number and Type of Bacteria in Dental Plaque

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Abstract

A type of *quasi experimental* study, uses the design of *pre and post-test with control group design*. Sampling technique was done using *simple random sampling*. 16 sample consumed dangke and 16 consumed cheddar cheese as control. Twice daily dangke consumption of 50 grams for 7 days, calcium and phosphate levels of saliva samples were measured by spectrophotometer (*part per million*). the number of bacteria was calculated using *colony counter* with the unit of *Colony Forming Units* (CFU). Email remineralization was measured using *Scanning Electron Microscope (SEM)*.

The mean calcium level before treatment was 21.40 ppm increased to 55.17 ppm after consumption of dangke and 73.24 ppm after cheese consumption, with P=0.000 (P<0.05). The mean of phosphate concentration before treatment was 185,16 ppm increased to 598,45 ppm after consuming dangke (P=0.001) and 568,00 ppm after cheese consumption P=0.008), average enamel density before treatment was 17,62 µm and decreased in microporosity depth after being immersed in the dangke extract became 7.72 µm (P<0.05) while in control, immersed in an artificial saliva of 10.81 µm. Difference of microporosity depth before and after treated dangke was 9.98 µm and saliva control by 6.80 µm (P<0.005). Based on the result of t-paired test, the number of oral bacteria on dental plaque decreased after consuming dangke the reduction of Streptoccus pneumoniae, Streptococcus aureus, and Streptococcus sp. (P=0.000) means that there is a significant difference between the number of oral bacteria on dental plaque before and after consuming dangke.

Dangke has a significant influence in increasing the levels of calcium and phosphate in saliva, affecting remineralization by reducing the depth of enamel microporosity, as well as reducing the number of bacteria in dental plaque.

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Introduction

Dental caries is a process of progressive damage that is irreversible. This is due to various factors influencing hard-tooth tissue.[1] Based on RISKESDAS data in 2007 and 2013, the percentage of dental and oral health problems in Indonesia increased from 23.2% to 25.9%. The degree of dental decay measured by the DMF-T index in 2013 reached 4.6%, meaning that there were up to 460 pieces of dental damage per 100 people.[2]

Dental caries is a process that occurs on every tooth surface in the oral cavity due to dental plaque which is allowed to develop over a period of time. Dental caries is caused by the direct and indirect factors. Direct factors are host, agents or microorganisms, substrates or diet, and time. Indirect factors (a risk factor for caries) are caries history or experience.

Dental caries occur due to a localized chemical disintegration of tooth surfaces (enamel and dentine) caused by dental plaque and mediated by changes in salivary acidity.[3,4] Plaque formation is a natural and physiological process

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whereby a collection of microorganisms attaches to the surface of the tooth which is always metabolically active. Some of the inherent bacteria are capable of fermenting carbohydrates such as sucrose and glucose so as to result in acid. Reduced pH repeatedly can cause demineralization. The process of caries is influenced by host factors (tooth surface), microorganisms (bacteria that cause caries), substrate (fermentable carbohydrates), and time. Caries can occur if all of these factors. The effect of plaque on the periodontium plays a vital role in the initiation and progression of periodontal diseases.[5-8]

Demineralizing tooth is the dissolution of tooth enamel due to high acid concentration. Caries is a multifactorial disease that begins with the demineralization process of hard tooth tissue over a period of time.[9]

Oral cavity bacteria are scattered in certain places, eg some species of Streptococcus belonging to the normal flora of the oral cavity and using it to form lactic acid are found at different sites in the oral cavity. Streptococcus salivarius inhabits the surface of the tongue, S. mitism inhabits most of the mucosa in the cheeks and the Streptococcus sanguis involves dental surfaces.[10]

Dental caries is characterized by the solubility of a number of inorganic and organic materials. The process of dissolution of calcium phosphate minerals from enamel or dentine is called mineralized.[1]

The demineralization and remineralization cycle occurs alternately in enamel films, demineralization will occur under pH of the oral cavity below 5.5. Conversely, demineralization will cease and the process of remineralization may occur if the pH of the oral cavity increases or returns to a normal point and there is an increase in the amount of calcium and phosphate ions in the oral cavity.[1,10]

Dental caries is still a common dental and oral disease in Indonesia.[2] Thus a precautionary measure is needed to reduce the incidence of dental caries in Indonesia. An effective preventive measure is prevention prior to the clinical symptoms of an illness arising or known as a primary precaution. Primary prevention can be done by providing protection against teeth with anti-caries material.

Milk and other dairy products are known to have low cariogenic potential, even cariostatic properties.[10] Milk and other dairy products are common products consumed by the community and are good source of protein, containing carbohydrates, fats, vitamins and minerals including magnesium, iodine, potassium, calcium, phosphate and a number of bioactive peptides that have been shown to prevent tooth decay and maintain oral health, through email protection and anti dental effects.[11,12]

One of the most commonly processed dairy products is cheese. Cheese has two e-mail protection mechanisms, that is, by stimulating salivary flow thus improving the function of buffer to dental plaque and by increasing the levels of calcium and phosphate in plaque or saliva so as to inhibit demineralization and increase remineralization.[13] The content of phosphopeptide in cheese can bind with calcium thereby inhibiting the formation of caries lesions through tooth enamel decalsification. Whereas glycomacropeptide derived from casein in cheese contributes to the anticaries effect by inhibiting adhesion and bacterial growth forming plaque on the oral mucosa. Thus consuming milk and dairy products is very good in maintaining healthy teeth and the oral cavity of man.[14] Calcium and phosphate is an ion that has an important role in the oral cavity because it is one element of the compilers email. The enamel is the outermost layer of the toughest teeth, mostly composed by hydroxyapatite crystals, which have the chemical formula [Ca10 (PO 4) 6 (OH) 2]. The presence of calcium and phosphate ions in enamel is required as a constituent of hydroxyapatite molecules, whereas in plaque and saliva are required as buffers and play a role in the process of remineralization.[10,13]

Dangke is a local processed cheese made from fermented buffalo or cow milk traditionally originated from Enrekang Regency, South Sulawesi. Dangke is known as soft cheese (soft cheese), containing 45.75% water, made by heating to boiling and added papaya sap (enzyme papain) as coagulant.[15,16] Dangke has a mineral content of 2.32% and protein content 17.20% which is not much different when compared with protein content in cheddar cheese which is about 10-30%.[16]

Materials and methods

Type of quasi experimental research that uses the desig of *pre and post-test with control group design*

Levels of calcium and phosphate in saliva

Research on the influence of dangke consumption on calcium and phosphate levels in saliva with a sample of preclinical students of Hasanuddin University Dentistry Faculty who meet criteria that do not use orthodontic appliance, have no systemic disease, and have good oral hygiene. Number of samples treatment and control were 32 people. Salivary sampling was performed before admission, after consumption of dangke, and after consumption of cheddar cheese, twice a day amounting to 50 grams. After a seven-day wash-out period, samples were asked to consume cheese for three consecutive days and saliva was again taken on the third day after ingestion of cheese. Subjects were asked to collect saliva by spitting method by collecting saliva on the basis of mouth and then poured into labeled sample bottles. The sample bottle containing saliva was then inserted into a cooling box to avoid contamination which may cause changes in the composition of salivary inorganic components. All e collected samples were taken to the Laboratory of Agricultural Technology Development Center (BPTP) and measured calcium and phosphate levels using Atomic Absorption Spectrophotometers ((SSA)

Number and type of bacteria on dental plaque

Calculation of the number and types of bacteria in dental plaque was done in the Laboratory of Microbiology of Hasanuddin University Hospital. Hasanuddin University. The plaque sample was removed by cutton swab by applying cutton swab on the labial surfaces of the maxillary and mandibular anterior teeth, as well as the buccal surface and lingual surfaces of the maxillary posterior and mandibular teeth, fed to a transport medium already labeled. put into a cooling box, calculated the number and type of oral bacteria found on dental plaque using a colony counter with the unit of *Colony Forming Units* (CFU).

Remineralization of tooth enamel

Research on the influence of dangke on tooth enamel remineralization (in vitro). A total of 10 mandibular permanent premolars free of caries were cut in the preparation laboratory of the Unhas Faculty of Dentistry to obtain a sample of the crown of the tooth thus there were 30 pieces of tooth. Each tooth piece was etched with etching of 37% phosphorous acid for 60 seconds on the buccal and palatal parts resulting in demineralization and deeper micropore- ticity of the enamel. 10 samples of this etching result will be taken as pre-test data. Thereafter 10 other pieces of tooth were soaked in 100% and 10% dangke extract soaked in artificial salivaas a 7 day control. Replacement of dangke extract is done every eight hours and saliva replacement every 24 hours. The treated sample was measured using Scanning Electron Microscope (SEM) to see the depth of enamel microporosity. Email remineralization occurs when the depth of the microporosity of email is reduced.

Results

Intervention	Before (ppm) Mean±SD	After (ppm) Mean±SD	*P*
Dangke	185.16±184.38	598.45±228.87	0.001
Cheese 1	185.16±184.38	568.00±490.71	0.008
Cheese 2	598.45±228.87	568.00±490.71	0.469

Table 1. Differences in Calcium and Salivary Levels Before and After Treatment.

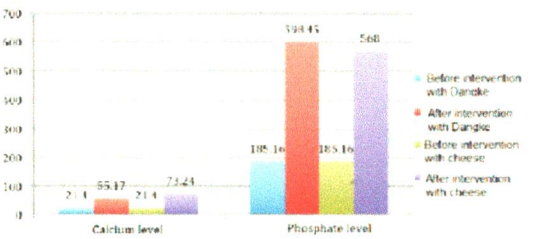

Figure 1. Diagram of Differences Calcium and Phosphate Levels Before and After Treatment.

Intervention	Before (ppm) Mean±SD	After (ppm) Mean±SD	*P*
Dangke	185.16±184.38	598.45±228.87	0.001
Cheese 1	185.16±184.38	568.00±490.71	0.008
Cheese 2	598.45±228.87	568.00±490.71	0.469

Table 2. Difference in Phosphate and Salivary Levels Before and After Treatment.

Remineralization of tooth enamel

Intervention	N	Before (µm)	After (µm)	*P**
		Mean±SD	Mean±SD	
Dangke	10	17.62±1.11[a]	7.72±0.79[a]	0.000
Saliva	10	17.62±1.11[a]	10.81±0.9[a]	0.000

Table 3. Average Depth of Email Microporosity Before and After Soaking in Dangke And Salivary.

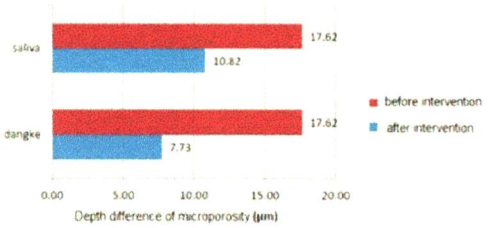

Figure 2. Depth Difference of Microporosity Before and After Soaking in Dangke And Salivary.

Intervention	N	Reducing the depth of microporosity before and after intervention	*P* value*
		Mean±SD	
Dangke	10	9.89±1.19	0.000
Saliva	10	6.80±1.53	

Table 4. Depth Difference of Microporosity Before and After Soaking in Dangke and Salivary.

Type of bacteria	Before	After	Number of before	Number of after	*P* value
	n	n	Mean (CFU/mL)	Mean (CFU/mL)	
Streptococcus pneumoniae	13	12	$9x10^5$	$1.53x10^5$	0.000
Staphylococcus aureus	2	3	$9x10^5$	$3.2x10^1$	0.000
Streptococcus sp.	1	1	$9x10^5$	$6x10^1$	0.000
Total	16	16			

Table 5. Distribution of the Number AND Type of Bacteria Before and After Consuming Dangke.

Type of bacteria	Before	After	Number of before	Number of after	*P* value
	n	n	Mean (CFU/mL)	Mean (CFU/mL)	
Streptococcus pneumoniae	13	8	$9x10^5$	$9x10^5$	-
Staphylococcus aureus	2	4	$9x10^5$	$9x10^5$	-
Streptococcus sp.	1	5	$9x10^5$	$9x10^5$	-
Total	16	17			

Table 6. Distribution of the Number and Type of Dental Plaque Before and After Consuming Cheddar Cheese.

Type of bacteria	The difference score before and after consumption of dangke	The difference score before and after consumption of cheese	*P* value
	Mean (CFU/mL)	Mean (CFU/mL)	
Streptococcus pneumoniae	$8.8x10^5 \pm 3.0x10^5$	0.000±0.000	0.000
Staphylococcus aureus	$8.9x10^5 \pm 7.1x103$	0.000±0.000	0.000
Streptococcus sp.	$8.9x10^5 \pm 0.000$	0.000±0.000	0.000
Total			

Table 7. Difference of the Number and Type of Bacteria Before and after Consuming Dangke And Cheddar Cheese.

Discussion

Levels of calcium and phosphate in saliva

The treatment given in this research was dangke consumption (*soft cheese*) and*cheddar cheese* (*hard cheese*) which was a dairy product. Dairy products such as cheese have low cariogenic potential and prevent the occurrence of demineralization of enamel by increasing the levels of calcium and phosphate in plaque or saliva leading to remineralization. In general, normal levels of calcium in saliva range from 1-2.5 mmol/L or 40-100 ppm, whereas normal levels of phosphate in saliva range from 2-22 mmol/L or 62-682 ppm.[17,18]

Dairy products are known to have low cariogenic potential because they contain bioactives *casein phosphopeptides-amorphous calcium phospahate* (CPPACP). CPP is fed by a casein protein in milk and has an amazing ability to stabilize calcium and phosphate in solution and can substantially increase calcium and phosphate in plaque. CPP-ACP works as a buffer, so it can help maintain pH in plaque, reduce demineralization and increase remineralization.[19] This is in line with research conducted by Katharina (2000), that milk can reduce mineral dissolution in enamel because the content of casein in milk can be absorbed rapidly to the surface of the enamel and provide resistance to the acid. The substance of casein in milk in modulating adhesion between strains of cariogenic microorganisms, Streptococcus mutans with hydroxyapatite in enamel.[20]

Test results of*t-paired* (table1) and test of*Wilcoxon Signed Ranks* (table2) showed a significant difference between calcium and phosphate levels in saliva before and after dangke consumption. Research conducted by Yadav (2013) describes three types of dairy

products, cheese shows the highest increase in pH compared to milk and yogurt. This proves that cheese has better anti-cariogenic properties than other dairy products.[21] The findings are also consistent with research conducted by Ravishankar (2012) that dairy products like cheese and yogurt without added sugar (sucrose) are cariostatic because it can increase calcium and phosphate levels in plaque.[12] A research done by Sonmez (2007) showed that the consumption of*white cheese* for 1 minute can increase plaque pH, and when white cheese is consumed 5 minutes accompanied by gargling with 10% sucrose solution, plaque pH increased more rapidly.[22]

The results show that dangke has an influence on increasing calcium and phosphate levels in saliva thus it can reduce caries risk. This finding is in accordance with research conducted Nur (2015) that probiotic bacteria contained in dangke such as Lactobacillus plantarum and Lactobacillus fermentum play a role in inhibiting the growth of cariogenic bacteria.[23]

This study explains that regular dangke consumption has a good influence on oral health because it can form deposits of minerals that will impact on increasing email remineralization.

Remineralization of tooth enamel

Calcium and phosphate are important elements as material for the remineralization process. Dairy products such as cheese can prevent caries development, prevent demineralization, increase pH and salivary flow rate, and increase the concentration of calcium in dental plaque to support remineralization. In the saliva there are also calcium and phosphate ions to allow the process of remineralization.[24,25]

Immersion in artificial dangke and saliva showed a significant decrease in enamel tooth enamel integrity. In table 3 shows the mean value of microporosity depth at the dangke treatment from 17.62 µm to 7.72 µm whereas in the sample soaked in saliva there was a decrease from 17.62 µm to 10.81 µm. From the data seen a change in porosity depth that indicates the occurrence of remineralization tooth enamel. This is in line with the research undertaken by Widyaningtyas (2014) on the analysis of increased enamel remineralization after soaking in pure soy milk using SEM, showed the enamel microporosity depth of the sample soaked in saliva greater than the depth of enamel microporosity soaked in pure soy milk. However, in the study it was concluded that the two treatments, namely pure soy milk and saliva, could improve the remineralization of tooth enamel, with the influence of greater pure soy milk.[26]

Nutrient content in dangke is 45,75% water, 32,81% fat, 17,20% protein, and 2,32% mineral. Fat and protein content in the diet will protect teeth, as well as dairy products can increase calcium and phosphate, and eating cheese can prevent the decrease in salivary pH. Eating these foods can improve remineralization, stop demineralization and prevent caries in teeth.[27]

The difference in microporality depth to the immersion treatment in the dangke is greater than that in saliva (Table 4). This can be due to the lack of calcium and phosphate in the saliva. The entry of minerals into the microporosity of enamel resulting in remineralization.[12] Based on research conducted byHedge, et al (2014) Saliva should be saturated against calcium and phosphate to give remineralization effect. Applications of Milk, cheese and GC tooth mousse can saturate enough amounts of calcium and phosphate.[13]

A research byHasanah (2014) explained that elevated levels of phosphate ions in artificial saliva in the teeth were applied CPP-ACP (*Casein Phosphopeptides Amorphous Calcium Phosphate*). Phosphate is the compound of tooth enamel with presentation in enamel about 55,5%. The enamel hydroxyapatite crystals, which have the chemical formula [Ca10 (PO 4) 6 (OH) 2] are composed of calcium and phosphate. The calcium ions and phosphate ions in the oral cavity can diffuse into the enamel to form hydroxyapatite crystals and cover the demineralized region. The CPP contained in milk and cheese has the ability to bind and stabilize calcium ions and phosphate ions in solution, and bind them in tooth enamel. In neutral pH, free calcium and phosphate ions form crystalline structures. However, the CPP keeps the calcium and phosphate ions in an amorphous state, thus calcium and phosphate ions can enter the tooth enamel by diffusion. The calcium and phosphate ions from the ACP will then diffuse into the tooth and surrounding environment, and the remineralization process will occur.[15]

Number and type of bacteria

Dangke showed considerable influence in decreasing the amount of oral bacteria in dental plaque compared to cheddar cheese. Of the chemical composition and nutritional value of dangke, dangke contains 32.81% fat and 17.20% protein.[28] The fat acts as an antimicrobial agent, and proteins also help inhibit bacteria in dental plaque and can reduce the role of bacteria as well as reduce the production of acids . Proteins can also prevent caries by absorbing tooth enamel surfaces.[29] In terms of the amount of bacteria at the time after consuming dangke, it decreases in number compared to consuming cheddar cheese.

Seen in Table 5, 8 types of bacteria identified at the time after consuming dangke and cheddar cheese are Streptococcuspneumoniae, Staphylococcus aureus, and Sreptococcus sp. However, Staphylococcus epidermidis bacteria were identifiedW at the time after consuming dangke, not identified at the time before and after consuming cheddar cheese. If identified specifically, many other types of bacteria are identified in each sample.

Dangke is made from cow's milk using coagulant from papaya sap (papain enzyme). Papain enzymes are often presented as active ingredients for tooth paste preparations. Papain in toothpaste can clean out the food remnants attached to the teeth. The remainder of this food often creates a stench if it is left too long.[30] Although in this study the cheddar cheese has not changed, but the cheddar cheese assessed still has a cariostatic nature that can reduce the number of oral bacteria

Conclusions

Dangke has a significant influence in increasing the levels of calcium and phosphate in saliva, forming tooth enamel remineralization by reducing the depth of enamel microporosity, as well as decreasing the number of bacteria in dental plaque.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

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