ORIGINAL ARTICLE

Differences in enamel surface hardness in soaking carbonated drinks and application of duck eggshell paste remineralization material

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ABSTRACT

Introduction: Demineralization can cause the enamel's surface hardness to decrease. Carbonated drinks are acidic drinks that can lower the pH of the oral cavity and cause damage to the enamel. Lost tooth minerals can be restored with remineralizing materials, one of which contains calcium. One of the natural materials that can help remineralize is calcium carbonate ($CaCO_3$), which can be found in several animal shells, such as marine materials, pearl snails, and eggshells. Duck eggshell is one of the household wastes that is high in calcium and consists of 94% calcium carbonate. The purpose of this study is to analyze differences in enamel surface hardness in soaking carbonated drinks and the application of duck eggshell paste remineralization material. **Methods:** This research employed a true experimental laboratory with a pretest-posttest research design with a control group design. The study sample consisted of eight post-extraction premolars that matched the inclusion criteria. The samples were divided into two groups, which were previously soaked in carbonated drinks for 15 minutes. Group I: duck eggshell paste; and Group II: pasta without duck eggshell (control), which was applied for 3 minutes twice a day for 14 days. Hardness measurement using a Vickers hardness tester. **Results:** Based on data analysis using a paired t-test, enamel surface hardness after immersion in carbonated drinks revealed a mean value of 327.50, SD=23.33. Whereas the enamel surface hardness increased after application of duck eggshell paste (mean=467.50, SD=14.15) p=0.001. The control group also increased after the application of pasta without duck eggshell (mean=429.40 SD=29.01) p=0.002. **Conclusion:** There is a difference in the hardness of the enamel surface after being soaked in carbonated drinks and after the application of duck eggshell paste. These findings could give potential natural source implications for protecting the teeth.

KEYWORDS

Demineralization, enamel surface hardness, carbonated drinks, duck eggshell paste, remineralization.

INTRODUCTION

One of the beverages that are often consumed around the world is carbonated drinks.[1] Carbonated drinks are the main source of sugar in many countries.[2] The effect of soft drinks on dental caries is explained by their sugar content and acidity. This drink contains phosphoric acid, sugar, caffeine, coloring, and flavoring. Phosphoric acid is an active ingredient in carbonated drinks. Generally, carbonated drinks have an acidic pH of 1.8-3.5 which can cause damage to enamel, namely demineralization.[1,2]

The enamel is the outer part of the anatomical crown of the tooth, which is above the dentin. When compared to other tissues, enamel is the hardest and strongest tissue.[3] Enamel basically consists of 70% organic matter, 30% organic matter, and water.[4] Damage to enamel is characterized by the dissolution of mineral ions such as calcium, phosphorus, and phosphate, which are arranged in hydroxyapatite crystals, which is called the demineralization process. Tooth minerals lost as a result of this process can be restored with a remineralization process.[5] In the remineralization process, minerals in the oral cavity, especially calcium and phosphate, will bind to each other, so that the hydroxyapatite crystals formed will cover the areas that have undergone demineralization and increase the hardness of the enamel surface.[6]

The hardness of the enamel surface is affected by the demineralization and remineralization processes of the enamel.[4] Enamel hardness is one of the physical properties that is influenced by the amount of inorganic materials, such as calcium. Soluble calcium and a low pH cause the hardness of the enamel surface to decrease, making it susceptible to tooth decay.[7] Decreased enamel surface hardness due to the demineralization process can be improved by using remineralizing materials containing calcium, which can increase the enamel surface hardness so that it can help restore mineral ions.[8]

To maintain an ideal oral environment, and effectively to clean, remove and prevent plaque and increase remineralization is done by brushing teeth. especially using toothpaste that contains calcium.[9] Toothpaste is a preparation in the form of a paste or gel which has the function of cleaning, maintaining oral health and improving aesthetics.[10] The basic ingredients used in toothpaste such as abrasive, humectants, flavors and sweeteners, active ingredients, gels and binders, therapeutic agents and surfactants.[11] One of the efforts to reduce the negative impact caused is to use natural ingredients that function as abrasive and therapeutic materials that can help the remineralization process and reduce side effects on the body.[7]

One of the natural ingredients that contain high calcium is duck eggshells. Duck eggshells contain 94% calcium carbonate. The content of calcium carbonate in duck eggshells can be used in remineralizing early lesions on tooth enamel.[9] Calcium particles allow calcium to penetrate deeper into the teeth so that they can trigger remineralization. Utilization of duck eggshells is by processing them into toothpaste because the calcium carbonate contained in duck eggshells can be used as an abrasive ingredient in toothpaste.[12]

Research conducted by Tangboriboon et al,[13] hydroxyapatite obtained from duck eggshells has good potential for biomaterial applications such as bone and artificial teeth, especially as a remineralizing agent in toothpaste. The use of duck eggshells to replace calcium carbonate in toothpaste formulations includes research conducted by Syurgana et al[12], with duck eggshell toothpaste formulations of 25, 1 Na CMC and 35% glycerin showing good physical stability. Research conducted by Haghgoo et al,[14] used eggshell concentrations of 3% and 10% compared to nano hydroxyapatite. The results showed an increase in enamel hardness at concentrations of 3% and 10% compared to nano hydroxyapatite and concluded that 3% eggshell concentration was the ideal concentration as a remineralizing agent. Follow-up research conducted by Yaberi and Haghgoo et al[15], the results of the present study showed that microhardness of the enamel demineralized from exposure soft drink significantly increased after treatment with either 10% nano hydroxyapatite solution or 3% eggshell extract solution, but in this study, eggshells were only used as hydroxyapatite extract and were not added to toothpaste formulations.[15]

Based on the description above, there has been no research using duck eggshell paste with 40% formulation as a remineralizing agent and the effect of giving duck eggshell paste on enamel surface hardness after soaking with carbonated drinks. The purpose of this study is to analyze differences in enamel surface hardness in soaking carbonated drinks and application of duck eggshell paste remineralization material.

METHODS

This research is a type of true experimental laboratory research, namely testing conducted in the laboratory. This research was conducted at the Microbiology Laboratory, Faculty of Medicine, Andalas University, Baiturrahmah Pharmacy Laboratory, and Metallurgy Laboratory, Mechanical Engineering Study Program, Faculty of Engineering, Andalas University.

The samples in this study were human premolars obtained from several dental practices and community health centers in Padang City that met the study inclusion and exclusion criteria. Inclusion criteria consisted of no carious lesions; Teeth with intact crowns; The tooth has no restoration. Exclusion criteria consisted of Crown tooth fracture; There is an anomaly in the enamel. The sample size is determined using the formula for determining the experimental test o Daniel sample according to Daniel and Cross et al.[16] So that, the number of sample: $n=z^2x\sigma^2/d^2$, $n= (1,96)^2$, $n=3,84\approx4$. In this study the sample will be divided into 2 groups: Group I: 4 samples for the demineralized treatment group were then given a paste containing calcium carbonate from duck eggshells; Group II: 4 samples for the control group were demineralized then given calcium carbonate toothpaste without duck eggshells.

The samples were divided into 2 groups, each group consisting of 4 samples, namely the treatment group that applied duck eggshell paste and the control group that was applied without duck eggshell paste. Sample preparation began by embedding the sample in a 2 cm diameter PVC pipe with epoxy resin. Samples were demineralized with carbonated drinks (Coca-Cola®) by soaking for 15 minutes and the initial enamel hardness was measured using a Vickers hardness tester at the Metallurgical Laboratory, Faculty of Engineering, Andalas University.[15] Application of the paste in the control and treatment groups for 3 minutes 2 times a day within 14 days, after which a hardness test was carried out again to see differences in tooth enamel hardness before and after being given treatment. Research preparation stages start from collecting samples and collecting duck eggshells.

The formulation of duck eggshell toothpaste was made according to the calculations in the Table. The research was conducted by cleaning the shells of duck eggs, then boiling the shells for 15 minutes and drying them in an oven for 30 minutes. Grind the duck eggshells with a mortar until they become powder and blend it to get a finer powder. After that, the powder was sieved using a 100-mesh sieve to obtain fine powder of duck eggshells. Duck eggshell paste is made from 100 grams of duck eggshell powder. Then develop NaCMC in a mortar stamper with warm water. In a different container, mix glycerin with methyl paraben, stir until homogeneous. Duck eggshell powder is put into the mortar stamper then added to a mixture of glycerin, methyl paraben and crushed until homogeneous. The mixture of ingredients in the mortar is added with *Sodium lauryl sulfate* and ground until homogeneous. Add menthol then grind until homogeneous. Expanded NaCMC is added to the mixture in the mortar and crushed until homogeneous so that a mass of toothpaste is formed for the control

group,[17] the paste without duck eggshell used the same formulation as the duck eggshell paste, the difference was that the active ingredient used calcium carbonate powder.

Table 1. Duck Eggshell toothpaste formula[24]

No	Composition	Amount (%)	Explanation
1.	Duck eggshell powder	40	active substance
2.	Na.CMC	1	Bond
3.	*Gliserin*	20	Humectan
4.	*Metil Paraben*	0.1	Preservatives
5.	*Natrium lauryl sulfate*	2	Foaming
6.	*Menthol*	1	Fragrance
7.	*Aquadest*	Ad 100	Solvent

Demineralization process by immersing the sample using carbonated drinks. First, measuring the pH of carbonated drinks using a pH meter. Soaking for 15 minutes. Then rinse the sample with aquadest. Soaking in 15 minutes is equivalent to 150 days of consuming carbonated drinks.[17] Measure the initial surface hardness of each sample using the Vickers hardness tester as the initial data. Samples were immersed in artificial saliva and stored in an incubator at 37° for 24 hours.[15]

In this study, for fourteen days each sample group was immersed in artificial saliva to simulate the actual condition of the oral cavity. The artificial saliva used for soaking the samples was replaced every 24 hours. Each sample was given treatment according to the group, namely Group I: The sample was applied to a paste made from duck eggshells with a 40% formulation by smearing it using a micro brush evenly on the buccal surface and left for 3 minutes then rinsed. Samples were immersed in artificial saliva and stored at room temperature. The procedure was repeated 2 times a day and was carried out for 14 days in a row. Group II Samples were applied to the paste without duck eggshells by smearing it using a micro brush evenly on the buccal surface and left for 3 minutes then rinsed. Samples were immersed in artificial saliva and stored at room temperature. The procedure was repeated 2 times a day and was carried out for 14 days in a row. Measure the hardness of the enamel surface in the sample after administering the test material for 14 days using the Vickers hardness tester.

RESULTS

The average results of enamel surface hardness in treatment and the control groups can be seen in Table 2. The premolars in the control group that were applied to the toothpaste without duck eggshells had an average enamel surface hardness of 307.25 VHN, after the paste was applied the average enamel surface hardness was 429.50 VHN.

Table 2. The average enamel surface hardness in treatment and control groups.

Group	Mean ± SD	Minimum (VHN)	Maximum (VHN)
Control			
Before (pre-test)	307.25±38.07	277.00	363.00
After (post-test)	429.50±29.01	401.00	463.00
Treatment			
Before (pre-test)	327.50 ±23.33	295.00	348.00
After (post-test)	467.50 ±14.15	453.00	482.00
Difference			
Control	122.25±22.79	100.00	149.00
Treatment	140.00±16.83	126.00	163.00

There was an increase in tooth enamel surface hardness in the control group after application of toothpaste without duck eggshells. The premolar teeth in the treatment group that were applied with duck eggshell paste had an average enamel surface hardness of 327.50 VHN. After being given the treatment the average enamel surface hardness became 467.50 VHN. There was an increase in the hardness of the enamel surface after the application of duck eggshell paste.

Bivariate analysis was used to analyze the effect of duck eggshell paste application on the enamel surface hardness. The Shapiro Wilk normality test found that the data were normally distributed because the p value obtained was more than 0.05. Based on the normality test. It can be interpreted that the data meets the requirements for a paired t-test to see significant differences in mean before and after being given treatment in each group. The difference in the increase in tooth enamel hardness values between the control and treatment groups was seen by the independent t-test with a significant level of 95% ($p < 0.05$)

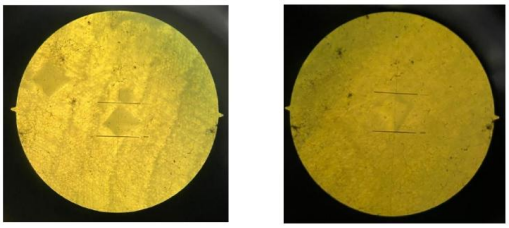

Figure 1. Measuring the surface hardness of enamel using Vickers hardness tester before application of duck eggshell paste

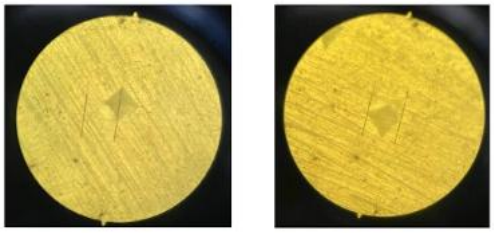

Figure 2. Measuring the surface hardness of enamel using Vickers hardness tester after application of duck eggshell paste

Table 3. Average tooth enamel surface hardness before and after application of paste without duck eggshell

Enamel surface hardness	n	Mean±SD (VHN)	*p*-value
Before Treatment	4	307.25±38.07	0.002
After Treatment	4	429.50±29.01	

The results of the study after the paired t test in the control group (n=4) showed a value of p=0.002 (p <0.05), meaning that there was a significant difference in the value of tooth enamel surface hardness before and after application of toothpaste without duck eggshells.

Table 4. Average tooth enamel surface hardness before and after using duck eggshell based paste

Enamel surface hardness	n	Mean±SD (VHN)	*p*-value
Before treatment	4	327.50 ±23.33	0.001
After treatment	4	467.50 ±14.15	

The results of the study after the paired t test was carried out in the treatment group (n=4) showed a value of p=0.001 (p<0.05), meaning that there was a significant difference in the value of tooth enamel surface hardness before and after application of duck eggshell paste.

Table 5. The results of the average difference in the tooth enamel surface hardness in the control group with the application of pasta made from duck eggshells and the control group with the application of paste without duck eggshells.

Enamel surface hardness	n	Mean±SD (VHN)	*p*-value
The control group used pasta without duck eggshells	4	122.25±22.79	0.257
The treatment group used duck eggshell paste	4	140.00±16.83	

Based on Table 5, after the independent t-test was carried out, it showed a value of p=0.257 (p>0.05), meaning that there was no significant difference between the control group and the treatment group.

DISCUSSION

This research was conducted to determine the effect of the application of duck eggshell paste on the hardness of the enamel surface. Samples that met the inclusion criteria were divided into two groups, each consisting of four samples that were applied with duck eggshell paste and the control group that was applied with paste without duck eggshell. The two groups were soaked in carbonated drinks (Coca-cola®) with a pH of 2.96 for 15 minutes to obtain a demineralizing effect.

The choice of immersion duration of 15 minutes in this study was adapted from exposure simulations to human physiological conditions when consuming soft drinks, which is 6 seconds per day and is equivalent to 150 days of drinking carbonated drinks. The frequency of carbonated drink consumption every six seconds per day for 150 days resulted in a repeated decrease in pH, consequently accelerating the demineralization process. Based on the *Stephan curve*, the decrease in pH begins after 1-3 minutes of exposure to acids and reaches a critical pH within 10-15 minutes, then the dissolution of mineral ions will occur which is characterized by a decrease in the hardness of the

enamel surface.[18] The choice of carbonated drinks as demineralizing materials is because they are one of the drinks that is widely consumed and liked by all people.[1,2] Carbonated drinks contain phosphoric acid, tartaric acid, lactic acid and maleic acid and generally have a pH below 5.5 which can cause damage to enamel.[2] In accordance with the research of Yaberi and Haghgoo, 2018 soaking teeth with carbonated drinks for 15 minutes can result in a demineralization process and reduce the hardness of the enamel surface.[15] In this study, for fourteen days each sample group was immersed in artificial saliva to simulate the actual condition of the oral cavity. The artificial saliva used for soaking the samples was replaced every 24 hours. Saliva solution contains calcium and phosphate minerals which can help the enamel remineralization process.[19,20] Samples were immersed in artificial saliva and stored in an incubator at 37 °C for 24 hours at the Microbiology Laboratory, Faculty of Medicine, Andalas University. The next day each sample was given treatment according to the group. The application of paste to each group was carried out for 3 minutes twice a day in the morning and evening within 14 days, after which the enamel surface hardness was measured again in the three groups to see differences in tooth enamel surface hardness before and after being treated.

The results of the paired t test in Table 4 for the control group, namely before and after the application of paste without duck eggshells, showed a significant increase in enamel surface hardness with $p<0.05$, namely $p=0.002$. The results of measuring the average value of enamel surface hardness after being soaked in carbonated drinks and before application of pasta without duck eggshells was 307.25 VHN, after applying paste without duck eggshells the average enamel surface hardness became 429.50 VHN, which means an increase in enamel violence. The increase in enamel surface hardness occurred because pasta without duck eggshells contain calcium carbonate as the main constituent of the paste.

Calcium carbonate in toothpaste is in the form of a white solid which can polish and remove stains and plaque and helps increase the thickness of the toothpaste.[17] The smallest structure of calcium carbonate can be used as a source of calcium and phosphate to maintain mineral ions in tooth enamel. The deposition of these ions on the enamel surface can help the remineralization process.[21]

The results of the paired t-test in Table 5 for the treatment group, after the application of duck eggshell paste showed $p <0.05$, namely $p=0.001$, which means that there was a significant increase in the tooth enamel surface hardness before and after application. The results of measuring the average surface hardness of the enamel after being soaked in a carbonated drink and before the application of duck eggshell paste was 327.50 VHN. The results of the average measurement of enamel surface hardness after application of duck eggshell paste became 467.50 VHN, which means an increase in enamel hardness.

The increase in enamel surface hardness is caused by the calcium content found in duck eggshells which can increase the hardness of tooth enamel by means of calcium entering the teeth so that it can trigger remineralization which is characterized by an increase in enamel hardness.[22] Supported by research conducted by Haghgoo et al,[14] which showed an increase in enamel surface hardness of 132 VHN after using eggshell paste. Research conducted by Yaberi and Haghgoo et al[15], namely the application of eggshells with an application time of 10 minutes showed an average increase in enamel surface hardness to 101 VHN, previously the sample was soaked in a carbonated drink for 15 minutes.

Research conducted by Yurisya et al showed that the average surface hardness of enamel after being soaked in chicken eggshell solution for 14 days was 499.90 VHN. Compared to the average hardness of the enamel surface after application of duck eggshell paste for 14 days, namely 467.50 VHN, the difference is not that great, due to the remineralization process that took place for 14 days. The content in chicken eggshells and duck eggshells which are high in calcium carbonate can help the remineralization process thereby increasing the hardness of the enamel surface

Duck eggshells besides containing high calcium also contain other elements such as phosphate and strontium which have a good effect on tooth structure.[14] The palisade layer on duck eggshells is the thickest layer where calcite, which is a crystal form of calcium carbonate, grows and becomes the main component of eggshells.[23] In the study of Tang Boriboon, hydroxyapatite obtained from duck eggshells has good potential for biomaterial applications such as bone and dentures, especially as a remineralizing agent in toothpaste.[3]. In this study, the formulation of duck eggshell paste was 40%, this was based on the fact that duck eggshell powder was used as the main ingredient (abrasive material) for the paste and the abrasive material formulation in toothpaste was generally 30-40% of the toothpaste content. 40% formulation is used so that the resulting toothpaste is more effective and works better.[12,24]

The mechanism of the remineralization process begins with calcium and phosphorus mineral ions deposited on the surface layer of the microporosities, then the minerals diffuse into the enamel microporosities. The minerals that enter spread in all directions between the enamel crystals and then are absorbed by hypo mineralized email, namely email that has previously undergone a demineralization process.[25] The enamel microporosity that occurs will be filled with calcium and phosphorus contained in the duck eggshell, because the enamel microporosity is only filled with mineral ions that have the same ionic radius as the missing mineral ionic radii.[17]

Based on the independent t-test in Table 6, it shows the results of the average difference in the application of paste in the duck eggshell paste treatment group and the control group without duck eggshell pasta with $p = 0.257$ ($p > 0.05$) which means there is no significant difference significant between the treatment group and the control group. The contents of duck eggshell paste and duck eggless pasta both contain calcium carbonate as the main ingredient of the paste; this means that duck eggshell paste and pasta without duck eggshell are both effective in increasing the hardness of the enamel surface. The content of calcium carbonate in duck eggshell paste and pasta without duck eggshell will inhibit the decomposition process of hydroxyapatite and cause the formation and

replenishment of some of the dissolved hydroxyapatite because of the demineralization process[17]. Both groups of samples in this study after the application of duck eggshell paste and paste without duck eggshells, the samples were immersed in artificial saliva which helps the remineralization process. Artificial saliva contains the same minerals as normal saliva's inorganic components such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^- and phosphate.[25]

Based on this research, it was found that duck eggshell paste can be used as a remineralization material to overcome the decrease in surface hardness after being soaked in carbonated beverages. Duck eggshell paste fulfills the characteristics of an ideal remineralization agent such as being able to diffuse into the sub-surface and channel calcium and phosphate into the enamel subsurface.

Some suggestions from researchers are, the future research can see the effect of duck eggshell paste application on changes in porosity in tooth enamel using a Scanning Electron Microscope (SEM) and It is necessary to conduct a feasibility test for duck eggshell paste so that it can be used and marketed.

CONCLUSION

Duck eggshell paste is effective in increasing the hardness of the enamel surface and could be used as a remineralizing agent and there is no significant difference in duck eggshell paste and paste without duck eggshell, but both are effective in increasing enamel surface hardness.

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