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The effect of time in the exposure of theobromine gel to enamel and surface hardness after demineralization with 1% citric acid

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Abstract. Theobromine is one of the alkaloid compounds that can be found in cacao (*Theobroma cacao*). It is said that theobromine can prevent enamel demineralization. The aim of this research was to evaluate the effect of different exposure times to 200 mg/L theobromine gel on enamel microhardness after demineralization in 1% citric acid. Twenty-eight specimens of human premolar teeth were divided into four groups and were immersed in 1% citric acid (pH 4) for 2.5 minutes. Then 200 mg/L theobromine gel was exposed to the specimens for 16 minutes (n = 7), 48 minutes (n = 7), and 96 minutes (n = 7). Enamel microhardness (KHN) values were tested using the Knoop Microhardness Tester (Shimadzu, Japan) using a 50-gram load for 5 seconds. A statistical test was performed using the Friedman test, Wilcoxon test, Kruskal–Wallis test, and Mann–Whitney test. The results showed a significant decrease, of microhardness values after demineralization with 1% citric acid. There was also a significant increase in hardness ($p < 0.05$) after exposure of the demineralized specimens to theobromine gel for 16 minutes (32.3%), 48 minutes (39.8%), and 96 minutes (43.7%). It can be concluded that exposure to 200 mg/L theobromine gel for 16, 48, and 96 minutes increased enamel microhardness.

1. Introduction

Dental caries is a multifactorial disease that occurs with involvement of four important factors, which are microorganisms, substrate, host and tooth, and time. Substrate in the form of carbohydrates will be broken down by microorganisms, and the metabolic process creates an organic acid. The formation of the organic acid then causes a decrease of pH level and an increase of H^+ . If this process continuously happens, the transfer of hydroxyl ions from the enamel will cause further damage to the structure of the enamel. That process is called demineralization [1]. Eventually, ongoing demineralization will cause porosity of the enamel surface and lead to caries [1]. Remineralization, the reverse process of demineralization, replaces mineral salt in the tooth enamel. Demineralization can be stopped and remineralization begin if the saliva's pH is restored to normal levels along with a restoration of calcium (Ca^{2+}) and phosphate (PO_4^{3+}) ions in the oral cavity. An increase of saliva in the oral cavity brings the pH back to its normal level gradually, and then (PO_4^{3+} and (Ca^{2+}) can form hydroxylapatite crystals and cover the demineralized area [2].

One of the tooth remineralization agents that has been proven in preventing caries is fluoride. Systemic fluoride were developed as an alternative to addition of fluoride intake in the form of tablets, salt, milk, or water. Fluoride intake by fluoridation of water which targets the whole people is one of cost-effective method [3]. However, fluoride also has harmful effect for human health when being



consumed excessively. An excessive intake of fluoride can cause fluorosis, tooth damage, dark stripes on teeth, and tooth loss, as well as a decrease of intelligence in children on low fluoride dosages, early aging, spontaneous abortion, and brittle bones. High fluoride dosages can irritate the digestive tract [4]. Because of these characteristics, several industries formulate dental health products without fluoride. An alternative that is used is cacao, or chocolate. Chocolate is a food processed from cacao seed (*Theobroma cacao*). Because of its sweet taste, many people think chocolate is key factor in causing dental caries. In reality, the tooth damage that occurs is likely caused by the high sugar content in the chocolate product [5].

Sadeghpour (2007) had argued that theobromine, which is one of the alkaloid compounds in chocolate, can be used to prevent enamel demineralization [6]. Theobromine, with the chemical formula 3,7 dimethylxanthine, is a white crystal powder and is differentiated from caffeine by only one methyl group. Sadeghpour's research indicates that theobromine can stimulate new enamel growth. Sadeghpour states that theobromine causes calcium and phosphate from the saliva to merge into a crystal unit that is four times bigger than hydroxylapatite. The combination of mineral placement as new enamel growth takes place can effect a change in enamel hardness [6]. Toothpaste containing theobromine has already been developed and marketed, but is not yet known worldwide. Gels containing theobromine are not yet available. Gel is a colloid suspension that comes from a solid that has been partially dissolved [7]. Gel has several advantages, including less usage compare to mouthwash, stability, and stickiness so that the active content can work longer [7]. This study sets out to test use of a topical theobromine gel for 16 minutes, 48 minutes, and 96 minutes on a demineralized enamel surface to examine its effect on enamel hardness as well as its suitability as one method of increasing oral hygiene and protection against caries. We set out to determine if the presence of mineral deposits on an enamel surface during the remineralization process will affect enamel surface hardness. This research tested the effect of 200 mg/L theobromine gel, used for three different durations, 16 minutes, 48 minutes, and 96 minutes, on human tooth enamel that had been demineralized with 1% citric acid.

2. Materials and Methods

The sample used consisted of 28 specimens of human premolar enamel. Part of a premolar tooth crown was cut by machine and then cast inside the ring and filled with decorative acrylic resin. Afterward, all the specimens were measured for initial hardness using a Knoop Microhardness Tester (Shimadzu, Japan). The specimens were divided into four groups. All were immersed in 1% citric acid with a pH of 4 for 2.5 minutes. One group (n = 7) would later be used for descriptive comparison. The remaining three groups were then exposed to theobromine gel, 200 mg/L, for different durations, specifically, 16 minutes (n = 7), 48 minutes (n = 7), and 96 minutes (n = 7). After each handling, the hardness of the specimens was measured. The three durations of gel exposure are used as an analogy for exposures of 1 month, 3 months, and 6 months, with the calculation of each application being 4 minutes every week. The weekly gel use assumes use in an individual with a low caries level, which is one to two cavities each year [2].

The data analysis was done using SPSS. The Friedman test was used first to measure the significance of increasing or decreasing enamel surface hardness within the exposure groups, and the Wilcoxon test was then performed on each specimen as well. The Kruskal–Wallis test was used to see the significance of increases or decreases of enamel surface hardness between the exposure groups and was followed by a post hoc using the Mann–Whitney test.

3. Results and Discussion

3.1 Results

The results of changes to enamel surface hardness after immersion in citric acid and exposure to theobromine gel for three different durations can be seen in Table 1.

Table 1. The mean score of microenamel hardness before and after immersion in 1% citric acid and exposure to theobromine gel

Hardness score	Length of exposure			p-values
	1 month (16')	3 months (48')	6 months (96')	
Initial Hardness	404.57±7.97	407.43±5.15	411±4.54	0.164
Hardness after demineralization with 1% citric acid	261.71±11.19	247.71±7.65	247.57±9.09	0.048
Hardness after exposure to 200 mg/L theobromine gel	387.14±11.03	411.71±10.11	440.29±7.38	0.000
p-values	0.002	0.005	0.001	

Table 1 shows the difference between the mean score of enamel surface hardness before and after immersion in 1% citric acid followed by theobromine gel exposure for three different durations of 16 minutes, 48 minutes, and 96 minutes. After immersion in 1% citric acid, decreases of enamel hardness resulted in mean score changes as follows: in the group exposed for 16 minutes, enamel hardness went from 404.57±7.97 KHN to 261.71±11.19 KHN, a 35.3% decrease. In the 48 minute group, it went from 407.43±5.15 KHN to 247.71±7.65 KHN (39.2% decrease), and in the group exposed to theobromine for 96 minutes, it dropped from 411±4.54 KHN to 247.57±9.09 KHN (39.7% decrease). After the immersion in 1% citric acid, the samples were exposed to the gel and then hardness was again measured. An increase in enamel surface hardness was seen in the 16-minute group, going from 261.71±11.19 KHN to 387.14±11.03 KHN (32.3% increase); the 48-minute group, going from 247.71±7.65 KHN to 411.71±10.11 KHN (39.8% increase); and the 96-minute groups, going from 247.57±9.09 KHN to 440.29±7.38 KHN (43.7% increase). A Friedman test was used to measure the significance of decreasing or increasing enamel surface hardness in the three exposure group (16 minutes, 48 minutes, and 96 minutes). Results can be seen on Table 1.

Table 2. Results of Wilcoxon statistical test measuring the significance of handling in three exposure groups (16 minutes, 48 minutes, 96 minutes)

Groups	Significance Score	
16-minute group	Initial to demineralization	0.018
	Demineralization to gel	0.018
	Gel to initiation	0.042
	Initial to demineralization	0.018
48-minute group	Demineralization to gel	0.018
	Gel to initiation	0.310
	Initial to demineralization	0.018
96-minute group	Demineralization to gel	0.018
	Gel to initiation	0.018

A Wilcoxon test was conducted following the Friedman test to measure the significance of the initial hardness scores and scores after demineralization and gel exposure. Results are seen on Table 2. The 16-minute and 96-minute groups both had significant changes in enamel surface hardness ($p < 0.05$) after demineralization and remineralization. However, the 48-minute group showed an insignificant change in enamel surface hardness. To measure the significance of decreasing or increasing hardness scores between the three exposure groups, the Kruskal–Wallis statistical test was used (Table 1). The significance score on the initial test was $p < 0.05$, meaning there was no significant difference between initial enamel hardness in the three groups. The mean significance score of enamel hardness after demineralization was $p < 0.05$, indicating a significant difference of enamel hardness between the groups. Finally, the mean significant score on enamel hardness after gel exposure was $p < 0.05$, again indicating a significant difference of enamel hardness among the three groups. A post

hoc analysis was done using a Mann–Whitney test. The results (Table 3) show there were significant differences between the 16-minute group when compared to the 48-minute group and the 96-minute group when compared to the 16-minute group.

Table 3. Result of post hoc analysis using Mann–Whitney test to measure significance of decrease and increase in hardness scores between groups after demineralization and theobromine gel exposure

	Groups	Significance Score
Hardness after demineralization with 1% citric acid	16 minutes to 48 minutes	0.025
	48 minutes to 96 minutes	1.000
	96 minutes to 16 minutes	0.048
Hardness after theobromine gel exposure	16 minutes to 48 minutes	0.002
	48 minutes to 96 minutes	0.002
	96 minutes to 16 minutes	0.002

3.2 Discussion

The demineralization process was simulated by the using 1% citric acid, pH 4, based on previous research demonstrated that citric acid causes faster erosion at low pH levels [2]. Citric acid is also twice more destructive to enamel than chloric acid or nitric acid because of its high affinity to calcium [8]. The pH used in the study simulates the cariogenic process, and the 150 seconds acid exposure was aimed to imitate real situation when patient consuming sugar-containing products and fails to remove dental plaque [9]. This is supported by research from Attin, showing citric acid's potential to cause demineralization because citric acid works as chelator agent, binding calcium to the enamel surface [10]. Previous research shows that immersion in citric acid, pH 4, *in vitro* for 150 seconds is the same as 40 minutes of exposure to orange juice *in vivo*. Additionally, there is a significant change of hardness score [11]. The three exposure groups showed a significant decrease after immersion in the citric acid. All three groups had a mean hardness score decrease of 35% after demineralization. This was due to erosion caused by the acid on the surface of the enamel that later caused dissolving of calcium and phosphate ions on the enamel's surface [12]. In turn, the ion dissolution caused gapping between crystals, which is an irreversible process and can cause porosity of the enamel [12], and the increasing porosity results in the decreased hardness score.

The use of theobromine as a remineralization agent was based on research by Nakamoto that showed theobromine and fluoride to be two substances that can elevate apatite crystal size [13], which is related to enamel surface hardness. Nakamoto further stated that theobromine is safer because of its low toxicity level when compared to fluoride [13]. The theobromine gel concentration of 200 mg/L was selected based on research by Sadeghpour on the effectiveness of theobromine at different concentrations, ranging from 1 mg/L to 500 mg/L [5]. His research demonstrated a significant increase in enamel surface hardness scores at concentrations of 100 mg/L to 500 mg/L. Further, Kargul and Nakamoto studied the effects of theobromine on enamel surface hardness using two different concentrations, 100 mg/L and 200 mg/L [5], and results showed 200 mg/L theobromine to be more effective at increasing enamel surface hardness [5].

After exposure to 200 mg/L theobromine gel, an increase of hardness in the demineralized enamel surfaces of all three exposure groups occurred. The 16-minute exposure group increased 32.3%, the 48-minute group increased 39.8%, and the 96-minute group increased 43.7%. Supporting this conclusion, the Friedman statistical test showed a significant difference ($p < 0.05$) in all three groups. This increase in enamel hardness corresponds with Nakamoto's statement that theobromine can increase apatite crystal size, which he verified with X-ray diffractometry [13]. The increase of crystal size is allegedly due to an interstitial reaction of theobromine inside the crystal micro-tunnel. Indirectly, it increases crystal density and apatite crystal microstain. The enhancement of stretch is in line with Coulomb's law, which states that the tensile strength between atoms will make the apatite

harder to break down. Macroscopically, this can be seen as the increase in enamel surface hardness [14]. The three theobromine gel exposure times selected in this study, 16 minutes, 48 minutes, and 96 minutes were based on representing gel exposure for 1 month, 3 months, and 6 months. Six months was selected because Gunawan (2006) showed that fluoridation occurs after 6 months of fluoride usage [15]. The duration for one exposure is 4 minutes every week, assuming use in an individual with low caries risk [2].

The Wilcoxon statistical test showed a significant difference ($p > 0.05$) from initial hardness in the 16- and 96-minute groups after gel exposure. In the 16-minute group, theobromine increased enamel surface hardness after demineralization, but did not restore it to its initial hardness. The 48-minute group also had an increase in enamel surface hardness. Lastly, the 96-minute group had an increase in enamel surface hardness that exceeded the initial hardness. This indicates that 200 mg/L theobromine gel might cause remineralization in a demineralized enamel surface. We assume that the 16-minute group wasn't restored to initial hardness because of less exposure time of the theobromine gel to the tooth enamel surface so that the change of crystal size was not optimized. The post hoc Mann-Whitney test showed a significant difference in change of hardness between the groups. The best result was obtained in the 96-minute group, probably because of the longer exposure. In this case, the change of crystal size is likely to be maximized compared to exposures of 16 and 48 minutes. As explained before, in demineralization with 1% citric acid, the porosity of the enamel prism layer is increased due to the erosive characteristics of citric acid [12]. The opening of the enamel prism layer (porosity) will, in turn, increase contact with and penetration of theobromine gel on the enamel surface, which causes elevation of apatite crystals so that the enamel will be hardened and can withstand acid [5].

4. Conclusion

Based on this research, it can be concluded that 200 mg/L theobromine gel can increase the enamel surface hardness that being demineralized with citric acid 1% with the exposure duration 16 minutes, 48 minutes, and 96 minutes. The exposure with theobromine gel for 48 minutes and 96 minutes, theobromine increases the enamel hardness that being demineralized by citric acid 1% until reach the initial hardness. Several suggestions that can be used for further research or comparison research are the research about the effectivity of 200 mg/L theobromine gel with topical fluoride gel to increase the enamel hardness and also further research about theobromine interstitial reaction inside the hydroxylapatite crystal.

References

- [1] Kidd EAM 2008 *Dental Caries: The Disease and Its Clinical Management 2nd Edition* (Oxford: Blackwell Munksgaard).p. 4-9.
- [2] Mount G J and Hume W R 2005 *Preservation and Restoration of Tooth Structure*. 2nd ed. (Queensland: Knowledge Books and Software).p. 25-27
- [3] Espelid, I. 2009. Caries preventive effect of fluoride in milk, salt, and tablets: A literature review. *European Archives of Paediatric Dentistry*, 10 (3), 149-156.
- [4] Aravind A, Dhanya RS, Narayan A, Sam G, Adarsh VJ, Kiran M 2016 Effect of fluoridated water on intelligence in 10-12-year-old school children *Journal of International Society of Preventive and Community Dentistry* 6 237-242.
- [5] Kargul, B, Özcan M, Peker S, Nakamoto T, Simmons WB, Falster AU 2012 Evaluation of Human Enamel Surface Treated with Theobromine: A Pilot Study *Oral Health Prev Dent* 10 275-282.
- [6] Sadeghpour A A 2007 *Neural Analysis of Theobromine vs. Fluoride on The Enamel Surface of Human Teeth: An Experimental Case Study With Strong Implications for The Production of A New Line of Evolutionary and Natural Non-Fluoride Based Dentrifices*. [Dissertation] (New Orleans: Department of Electrical Engineering and Computer Science of Tulane University for The Degree of Doctor of Philosophy).

- [7] Allen LV, Ansel HC 2014 *Ansel's Pharmaceutical Dosage Form and Drug Delivery Systems, 10th Edition*. (Philadelphia: Lippincott Williams and Wilkins).p. 316-320.
- [8] Ilyas M. Perbedaan kadar kalsium dalam saliva sebelum dan sesudah mengkonsumsi minuman ringan yang mengandung asam sitrat. *JITEKGI*, 2006; 3 : 96-9.
- [9] Rahardjo A, Karina K, Fadhilah A, Eriwati YK, Triaminingsih S, Maharani DA 2013. Caries-preventive Effect of 1300ppm Fluoride and Carageenan Containing Toothpaste *Journal of Dentistry Indonesia* **10** 1-4.
- [10] Attin T, Weiss K, Becker K, Buchalla W, Wiegand A 2005 Impact of Modified Acidic Soft Drinks on Enamel Erosion *Oral Diseases* **11** 7-12.
- [11] Barbour ME, Parker DM, Allen GC, Jandt KD 2003 Human enamel dissolution in citric acid as a function of PH in the range $2.30 \leq PH \leq 6.30$ - a nanoindentation study *European Journal Of Oral Sciences* **111** 258-262.
- [12] Karlinsey RL, Mackey AC, Blanken DD, Schwandt CS 2012 Remineralization of eroded enamel lesions by simulated saliva in vitro *Open Dent. J.* **6** 170-6.
- [13] Nakamoto T, Falster AU, Simmons WB 2016 Theobromine: A Safe and Effective Alternative for Fluoride in Dentifrices *Journal of Caffeine Research* **6** 1-9.
- [14] Amaechi BT, Porteous N, Ramalingam K, Mensinkai PK, Ccahuana Vasquez RA, Sadeghpour A, Nakamoto T 2013 Remineralization of Artificial Enamel Lesions by Theobromine *Caries Res*, **47** 399-405.
- [15] Gunawan H A 2006 *Pengaruh Perubahan Kristal Apatit, Tingkat Retensi Dan Intrusi Fluor Terhadap Kelarutan Email Setelah Perlakuan Larutan Ikan Teri Jengki (S insularis)* Dissertation Paper. (Jakarta: Universitas Indonesia).