

Case Report

The effect of theobromine and NaF 2% exposure to enamel surface hardness after immersing in orange juice beverage

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ABSTRACT

Background: Consumption of orange juice which had acidic quality will cause a demineralization on enamel. The most effective way to prevent the demineralization process was by involving remineralization agents. Fluoride was effective to improve remineralization but has toxicity effect and caused fluorosis at certain dose. Another alternative for remineralization agent without providing side effects is theobromine contained in cocoa peel extract (*Theobroma cacao*).

Purpose: To explain the effect of theobromine and NaF 2% exposure on enamel surface hardness after immersion in commercial orange juice. **Methods:** Twenty-seven cattle incisors were cut into a square shape 1x1 cm and then planted in a round resin mold then divided into three groups. All three groups were immersed in orange juice beverage for 60 minutes. The control group was immersed in distilled water, group I was given theobromine 200 mg / L, and group II was given 2% NaF after exposure for 96 minutes. Surface hardness measurements were carried out using the Wolpert Micro Vickers Testers tool. **Results:** There was a significant difference ($p < 0.05$) between surface hardness of tooth enamel from the control group and theobromine group and there was also a significant difference between the control group and the NaF group. However, there was no significant difference ($p > 0.05$) in the NaF group with theobromine group.

Conclusion: The effect of exposure to theobromine and NaF 2% on surface enamel hardness after immersion in orange juice beverage has the same result.

Keywords: Enamel surface hardness; theobromine; fluoride; commercial orange juice

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INTRODUCTION

Consumption of fruit juices which has acidic pH such as orange, lemon, and grapes has become a lifestyle. Orange juice is a nutritious and acidic beverage¹. Acid that continues to attack the teeth will cause a demineralization on enamel surface to soften and forms porosity on enamel that causes caries. Dental caries is a multifactorial disease which is a major problem in oral health and experienced by 95% of the world's population and 90,05% incidence in Indonesia. Enamel consists of 95% mineral hydroxyapatite formed as calcium phosphate crystal, 1% organic materials and 4-5% water from weight percentage^{2,3,4}. If there is a destruction in crystal hydroxyapatite, the atomic bonds become loosens and enamel hardness decreases^{1,5,6}.

The acidic exposure can reduce mineral content on the enamel which effects crystal hydroxyapatite to become smaller and damaged the surface enamel structure^{6,7}. Enamel has lack of ability to improve its structure thus it is important to prevent enamel demineralization⁹. One way to increase

hardness and repair damage from enamel demineralization is by remineralization agents¹⁰.

Fluoride is the most effective caries prevention agent. Fluoride can reduce caries by preventing demineralization and remineralization on early caries lesions¹¹. However, the fluoride safe doses are still discussed, because fluoride has toxicity and causing fluorosis^{9,12}.

Another alternative agent that can be used without side effects is theobromine which contained in cocoa fruit extracts (*Theobroma cacao*)¹³. The size of hydroxyapatite crystals can be increased by theobromine exposure up to four times larger^{7,8}. Currently Indonesia is the third largest cocoa producer with 777,750 MT/year¹⁴. Considering this, the accumulation of cocoa pod husk waste has high quantity so innovation of the management is needed.

Irawan *et al.*¹⁵ proved that theobromine concentration of 200 mg/L after demineralization with 1% citric acid can increase enamel hardness. The research uses theobromine 200 mg/L because this concentration is effective in increasing enamel hardness¹⁵. The application of topical

gel usually at intervals of 3 to 12 months, so it has a low risk of fluorosis¹⁶.

Theobromine and fluoride have the ability to conduct a remineralization process on enamel. This research is to explain the effect of theobromine and NaF 2% exposure on surface enamel hardness after immersion in orange juice beverages.

MATERIALS AND METHODS

This research is an experimental laboratory in vitro with pre-test and post-test only group control design. The procedure of this consists of (1) the producing of cocoa-peel-based Theobromine extract at BPKI Ketintang Surabaya and the making of NaF 2% gel at Organic Chemistry Lab, Faculty of Science and Technology, Universitas Airlangga Surabaya. (2) The preparing of enamel samples, (3) Samples treatment and tooth enamel surface hardness test at metallurgical laboratory, Faculty of Industry Technology, ITS Surabaya.

Cocoa-pod-rind-based theobromine extract were extracted from cocoa peel using maceration method and ethanol 70% as solvent. The theobromine concentration used was 200 mg/L. The NaF used was a 2% sodium fluoride gel with pH 7. The orange juice used was Buavita® brand orange juice with pH of 3.7. The exposure time required for applying topical gel using analogy to exposure for 96 minutes for 6 months, with the calculation of application for 4 minutes every week.

The sample used was bovine incisors, which were separated between crowns and roots. The crowns of the tooth were cut in a square shape of 1 x 1 cm planted in a round resin mold with a diameter of 2 cm and a width of 1.5 cm. The sample was divided into three groups which are control group where tooth was immersed in orange juice for 60 minutes, group I where tooth was immersed in orange juice for 60 minutes and theobromine 200 mg/L gel topically applied with exposure time of 96 minutes, and group II where tooth was immersed in orange juice for 60 minutes and NaF 2% topically applied with exposure time of 96 minutes.

The tooth enamel surface hardness test is conducted before and after treatment to observe indentation power carried out on the labial surface of each sample with load of 100 g which measured using a Vickers Microhardness Tester

(*Wolpert Micro Vickers Tester*; Model 402-MVD, Made in China) in *Vickers Hardness Number* (VHN). Research results is analyzed using *Shapiro-Wilk* normality test, *Levene homogeneity test*, *One-Way ANOVA* and significance test using *Tukey HSD*.

RESULTS

This research is to obtain the data of tooth enamel surface hardness at each indentation, then mean value and standard deviation were measured of each group. Data were obtained from measurements of surface hardness of enamel in several groups, the control group, the treatment group I (theobromine 200 mg/L) and group II (NaF 2%). The mean value results of the study can be seen in Table 1.

The normality test used the *Shapiro-Wilk Test* to determine whether the data obtained is normally distributed or not. In the normality test in the initial hardness control group, $p = 0.356$, after demineralization, $p = 0.442$, and after remineralization, $p = 0.523$. In theobromine group, the initial violence results $p = 0.839$, after demineralization, $p = 0.403$, and after remineralization $p = 0.715$. In the NaF group the initial hardness $p = 0.357$, after demineralization $p = 0.159$, and after remineralization $p = 0.551$. From the results of the normality test, it can be concluded that all groups have p values greater than 0.05 ($p > 0.05$) which indicates the data are normally distributed.

The homogeneity test used *Levene's Test* to determine whether the obtained data has the same variance. Based on the homogeneity test data calculation of hardness after remineralization, the results obtained $p = 0.803$ ($p > 0.05$), it can be stated that the data variance between groups are the same (homogeneous) and the *One-Way Anova* test is valid to test groups relationship.

Table 2. The significance differences of tooth enamel surface hardness between groups

Groups	Control	Theobromine	NaF
Control		$p = 0.037^*$	$p = 0.046^*$
Theobromine			$p = 0.994$
NaF			

note: * Significant difference ($p < 0.05$)

Table 1. Mean value and standard deviation of tooth enamel hardness of each group.

Groups	n	Mean value (VHN)			Standard Deviation
		Initial Hardness	Post Demineralization	Post Re-mineralization	
Control	9	365	251.2	251.6	24.278
I (Theobromine)	9	363.1	242.8	284.3	28.500
II (NaF)	9	390.6	253.3	283.0	25.746

The next test is the One-Way ANOVA test to determine the average differences between all sample groups. The results of the One-Way ANOVA test showed $p = 0.022$ ($p < 0.05$), it can be stated that each group gives a significant difference. The analysis was continued with *Post-Hoc Multiple Comparison* Test with the *Tukey HSD* method to determine whether there were any differences between the sample groups.

There was a significant difference between the surface hardness of tooth enamel in the control group and theobromine group, and there was also a significant difference between the surface hardness of tooth enamel in the control group and the NaF group. But there was no significant difference in the NaF group with theobromine group.

DISCUSSION

In this research, all treatment groups experienced a decrease in the enamel surface hardness value. This is in accordance with research by De Melo *et al.*¹ which proved that enamel surface hardness was decreased after immersion with orange juice beverage. The differences in organic and inorganic minerals contained in the teeth affect surface hardness values among three groups¹⁷.

The process of demineralization that occurs continuously can dissolve the mineral content in enamel, therefore, the enamel porosity increases. This causes the caries to happen⁴. The citric acid contained in orange juice beverage has a high affinity for calcium and it will destruct enamel structure⁶. Early identification of the loss of enamel's mineral content can help caries development prevention^{4,8}. One way to increase hardness and repair damage from enamel demineralization is by the process of remineralization agent⁷.

Nakamoto *et al.*¹⁸ proved in their research that theobromine with a concentration of 200 mg/L increased enamel surface hardness. In addition, Herisa *et al.*¹⁹ also proved that 96 minutes of theobromine exposure can increase enamel surface hardness. Theobromine exposure can cause a remineralization process that can increase enamel surface hardness. The size of hydroxyapatite crystals can increase due to theobromine exposure thereby inhibiting the dissolution of apatite from the enamel surface. The process that occurs between theobromine and hydroxyapatite is called an interstitial reaction^{13,15}.

In interstitial reaction, small theobromine ions ($C=170$ pm, $N=152$ pm, and $H=152$ pm) can pass through tunnels in the hydroxyapatite structure that changes in the physical properties of apatite¹³. Theobromine can increase enamel surface recrystallization because of the increased merging of calcium and phosphate to form supersaturation on the enamel surface^{15,20}. The bond that occurs is the hydroxyl group (OH) in hydroxyapatite crystals that binds directly with theobromine ($C_7H_8N_4O_2$) to form Theobromine apatite ($Ca_{10}(PO_4)_6(OHC_7H_8N_4O_2)$). The new form of hydroxyapatite crystal increases the size of the crystal up to

four times larger thereby increasing bond strength between atoms and tooth resistance to acids.

Theobromine contained in cocoa peel extract (*Theobroma cacao*) can be used as an alternative material to increase enamel surface hardness without giving side effects¹³. The use of theobromine as an anticaries is believed to be safer and cheaper^{7,9}. In a study conducted by Nakamoto *et al.*¹⁸, it has been proven that theobromine has a better ability as a substitute for fluoride because of its superiority which is not toxic to the body.

The NaF group showed an increase in enamel surface hardness. This is in accordance with the research of Liwang *et al.*²⁰ which proves that distribution of fluorine after demineralization can increase enamel surface hardness. Fluoride exposure can cause the exchange of hydroxyl (OH) ions with fluorine ions to form fluorapatites. In analogy, if there is an XY bond and meet the Z group, the substitution reaction will produce an XZ structure because the Y group exchanges with the Z group. Fluorine can merge with the crystal lattice structure and attach to the surface of the crystal because it has the ability to diffuse so that fluoride can exchange with hydroxyl groups on the surface. Fluorine ions can migrate into the crystal body and joined with hydroxyapatite which cause a change in the shape and physical properties of the crystal. In addition, fluorine can also be exchanged with hydroxyl groups but not as a whole, this forms Fluor hydroxyapatite (FHA)¹³.

Fluoride is effective in preventing caries because it can inhibit the process of demineralization and remineralization. Fluor binds to apatite by forming fluorapatite and calcium fluoride. In addition, fluoride can also inhibit the work of enzymes from a bacterium through its antimicrobial activity. However, the safe dose of fluoride use and its toxicity is still discussed⁹. Fluoride ingested in a certain amount and in a short time can cause acute toxicity that can cause poisoning.

A possible toxic dose from fluoride use is 5 mg/kg of body mass¹². The toxic dose of sodium fluoride is not more than 120 mg or 264 mg in one exposure. Four main effects of fluoride toxicity are burning sensation in tissues, impaired nerve function, cell poisoning, and impaired cardiac function²¹. Susik *et al.*²² in their research on the effects of fluoride on the oral mucosa of Wistar rats have proven the existence of cellular changes and dysplastic changes due to the use of 100 ppm sodium fluoride for 42 days.

The results of this research among the control group, theobromine group, and NaF group have different values of enamel surface hardness. Theobromine group has the highest value, then the NaF group and the control group. Descriptively, theobromine group has a higher value than the NaF group. However, the data analysis results did not show a significant difference between theobromine group and NaF group.

This research is in accordance with the research of Irmaleny *et al.*⁷ which proves that theobromine and fluoride can increase enamel surface hardness but do not have a significant difference in value because they have the same ability in the remineralization process. In his research used

theobromine 1000 mg/L solution and 1000 ppm fluoride solution for 15 minutes. This research is not correlate with Nasution and Zawil¹³ research which proves that fluoride can increase enamel surface hardness better than theobromine. In his research used theobromine 2% and fluoride 2% for 5 minutes 24 times. This proves the difference in results is influenced by the concentration and exposure time of theobromine and fluorine on the enamel surface.

CONCLUSION

The effect of exposure to theobromine and NaF 2% on enamel surface hardness after immersion in orange juice beverage has the same result.

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