

<p>Research Report</p>

Differences in Surface Hardness of Bovine Enamel after Exposure of Theobromine, Alkaloid Cocoa Pod Extract and Acidulated Phosphate Fluoride

Tamara Yuanita, Nanik Zubaidah and Jesica Ceren

Department of Conservative Dentistry,
Faculty of Dental Medicine, Universitas Airlangga,
Surabaya, Indonesia

ABSTRACT

Background: Acid diet could process demineralization on enamel surface. Process remineralization needed to prevent caries occurrence. The excessive fluorine usage could induce adverse side effects, therefore the development of natural resources such as theobromine and alkaloid cocoa extract as a remineralization is needed. **Purpose:** To discover the differences of bovine teeth enamel surface hardness after exposure to theobromine, alkaloid cocoa pod extract and acidulated phosphate fluoride. **Methods:** Twenty-eight tooth crowns of bovine insisivus cut into the shape of the square then planted on the resin mold round shape and divided into four groups. The control group without treatment, the group I was exposed APF, the group II was exposed 1000 mg/L alkaloid cocoa pod extract, group III was exposed 200 mg/L Theobromine 200 mg/L. Surface hardness measured using Wolpert Wilson Vickers Microhardness Tool. **Results:** The group of theobromine produces the highest enamel surface hardness. The enamel surface hardness in alkaloid group of cocoa pod extract was higher compared to the APF group and the control group. **Conclusion:** The hardness surface of bovine tooth enamel after exposure to theobromine and alkaloid cocoa pod extract is harder than exposure to acidulated phosphate fluoride.

Keywords: Theobromine; Alkaloid; Cocoa Pod Extract; Acidulated Phosphate Fluoride; Enamel Surface Hardness

Correspondence: Tamara Yuanita, Department of Conservative Dentistry, Faculty of Dental Medicine, Airlangga University. Jl. Mayjen Prof. Dr. Moestopo 47, Surabaya, Indonesia, 60132. Email: tamara-y@fkg.unair.ac.id,

INTRODUCTION

Caries is a multifactorial disease that occur due to interactions between sugars in food, biofilms and tissues in oral cavity. Caries is an accumulation of demineralization and remineralization cycles that occur on the enamel surface¹. Enamel is the outermost and hardest part of tooth that contains mostly hydroxyapatite crystals. The hydroxyapatite crystal amount affects the enamel thickness². These crystals are permeable to ions and molecules that come in direct contact with enamel surface. When the acid encounters the enamel, demineralization occurs in the results of a decrease in the enamel surface hardness. This is the caries initial stage³. The enamel ability to resist acid is affected by the enamel chemical composition which varies in each individual and other factor that affect tooth structure⁴.

Robinson, Brookes & Kirkham⁵ stated that after enamel fully formed, enamel become the only dental tissue that does not have ability to regenerate. This supports the importance of preventing enamel demineralization. one of ways to increase the surface hardness and repair the damage from

enamel demineralization is by remineralization through the assistance of remineralizing agents^{6,7}.

The most often used remineralizing agent is fluorine as active ingredients to prevent caries development by forming fluorapatite and calcium fluoride which inhibit the work of bacterial enzymes through its antimicrobial properties^{8,9}. The fluorine can be given topically and systemically. APF (*acidulated phosphate fluoride*) with 1.23% concentration is commonly used due to stability and availability. Phosphoric acid in APF could etch the tooth enamel, then fluoride ion replaces the mineral hydroxyapatite to form a more stable, and more resistance to acids¹⁰.

A high intake of fluoride is leading to its adverse effects such as dental fluorosis, skeletal fluorosis, bone fractures and toxicity¹¹. It is needed a research to find natural substances that work in bone remineralization while preventing bacterial growth and have minimal side effects.

Cacao pod (*Theobroma cacao L.*) is a natural resource that can be used as a remineralization agent. Cocoa contain secondary metabolite in the form of purine alkaloid which derive from xanthine such as theobromine, caffeine, and theophylline⁹. Amaechi *et al.*¹² stated that theobromine in

cocoa fruit has better effect in remineralization process and enamel hardening. Theobromine makes calcium and phosphate in saliva to combine into crystals that are sized four times larger than hydroxyapatite crystals¹³. Qasthari, Irawan & Herda¹⁴ research stated that theobromine gel with concentration of 200 mg/L for 48 minutes and 96 minutes can increase enamel hardness. Duraisamy *et al.*¹ also supported the statement, in his research stated that 200 mg/L theobromine has great potential to increase enamel remineralization process.

Theobromine, alkaloid extract from Cocoa Pod and APF gel has ability to enhance enamel remineralization. This research aimed to discover the differences between the enamel surface hardness of bovine teeth after theobromine, cacao-pod alkaloid extract and acidulated phosphate fluoride gel.

MATERIALS DAN METHODS

This was an in-vitro laboratory experimental research with pre and post-test control group design. The workflow of this research were consisted of (1) The making of Cocoa-pod theobromine extract at BPKI Ketintang Surabaya, (2) The making of enamel samples, (3) Sample treatment and hardness test of tooth enamel surface in Metallurgy Laboratory, ITS Faculty of Industrial Technology, Surabaya.

The theobromine concentration used was 200mg/L. The concentration of extract alkaloids obtained from cocoa pods was 1000 mg/L. APF is a fluorine agent with a composition of 1.23% fluorine ions. The carbonated drink used in this research had acidic pH.

Samples were using 28 bovine incisors; crown is separated from its roots. The crown is shaped into 1 x 1 cm square, planted in a 2 cm diameter round resin mold and a width of 1.5 cm. The sample separated into four groups; (1) The control group was immersed in carbonated drink for 75 minutes, (2) The Group I was immersed in carbonated drink for 75 minutes then then exposed to theobromine 200 mg/L

0.1 gram for 5 minutes, (3) The Group II was immersed in carbonated drink for 75 minutes then exposed to cocoa-pod alkaloid extract 1000 mg/L for 5 minutes, (4) The Group III was immersed in carbonated drink for 75 minutes then exposed to APF gel for 5 minutes.

The measurement of enamel hardness was done pre and post treatment to discover the indentation power on labial surface of each sample with load of 200 g using Vickers Microhardness Tester in Vickers Hardness Number (VHN). The results were statistically analyzed using Shapiro-Wilk normality test, homogeneity test using Levene Test, One-Way ANOVA, and significance test using Tukey HSD.

RESULTS

In this research, the data were obtained in the form of enamel hardness on each indentation, then averaged and the standard deviation of each group was count. The enamel hardness measurement data were collected then divided into several groups, namely the control group (no treatment applied), the treatment group I (APF), group II (cocoa-pod alkaloid extract 100 mg/L) and group III (Theobromine 200 mg/L). The average value is shown on Table 1.

The normality test was using The Shapiro-Wilk Test, the results from each group had value of $p > 0,05$ in conclusion, the data are normally distributed. Then the statistical analysis continued with One-Way ANOVA test and the results obtained $p = 0,475$ ($p > 0,05$), it can be concluded that there was no significant difference between enamel surface hardness on initial group. The homogeneity test was conducted using Levene's Test and obtained results of $p = 0,085$ ($p > 0,05$), therefore it can be stated that data variances between groups are homogeneous.

One-Way ANOVA test was conducted to discover the average differences among all groups of samples after remineralization. Analysis followed with Post-Hoc Multiple Comparison Test using the Tukey HSD method to discover whether there are any differences between the sample groups.

Table 1. Average value and standard deviation of enamel surface hardness post remineralization on each group

Groups	n	Average Value (VHN)			Standard Deviation
		Initial Hardness	Post-Demineralization	Post-Remineralization	
Control	7	330.78	220.4	221.21	8.44
I (Theobromine)	7	329.46	217.56	281.37	15.82
II (Alkaloid)	7	324.69	224.1	270.66	19.24
III (APF gel)	7	331.36	218.96	244.29	8.21

Table 2. The significant differences of enamel surface between group treatment post remineralization.

Group	Control	Theobromine	Alkaloid	APF
Control		P = 0.000*	P = 0.000*	P = 0.022*
Theobromine	P = 0.000*		P = 0.479	P = 0.000*
Alkaloid	P = 0.000*	P = 0.479		P = 0.008*
APF	P = 0.022*	P = 0.000*	P = 0.008*	

Notes: *: Significant difference ($P < 0.05$)

The test results showed a significant difference between control group and theobromine group, control group and alkaloid group, control group and APF gel group, theobromine group and APF gel group, and alkaloid group to APF gel group. In the other hand, there is no significant difference between theobromine group and alkaloid group.

DISCUSSION

The research results, all groups experienced enamel hardness reduction after immersion in carbonated drinks. In certain level of acidity, it can chemically dissolve the enamel mineral content, weakening teeth, increase sensitivity, and susceptible to damage leading to dental caries^{14,15}. Demineralization prevention on the enamel surface is the key effort to avoid dental caries⁹.

In this research APF Group showed an increase in enamel surface hardness, this shows a lower value compared to theobromine group and alkaloid group but higher to the control group. APF gel has been used extensively in daily practice due to anticariogenic capabilities that have been proven in laboratory, clinical and epidemiological studies¹⁶. Corresponding to the latest research by Cury & Delbem¹⁷ they proved a significant increase in enamel surface hardness after APF gel exposure compared to the control group, and there was no significant difference between the duration of exposure for 1 minute and 4 minutes. Maden *et al.*¹⁸ in his research stated that APF gel can reduce the minerals amount of minerals that are soluble in enamel that have been exposed to acidic drinks. The increase in enamel surface hardness is caused by the acidic properties of APF gel which has a pH among 3.6-3.9. The low pH in solution has a strong influence on the CaF₂ formation, and also the low pH of the solution can slightly increase the enamel micro porosity and precipitate large amounts of CaF₂ on the tooth surface^{4,16}. Acidic pH of APF gel can help increase the compounding of fluoride into the enamel and negative fluoride ions become reactive in acidic media, thereby increasing the CaF₂ formation. Formation of a CaF₂ layer on tooth surface that acts as a fluoride reservoir when in acidic atmosphere, fluoride is released from CaF₂ to form fluorapatite or fluor hydroxyapatite which makes minerals in enamel susceptible to dissolve. However, The formed fluorapatite can increase the tooth enamel hardness, although, the increase is not significant than theobromine group and alkaloid group².

In this research, the alkaloid group showed an increase in enamel hardness, where it was lower than theobromine group but higher when compared to the APF group and control group. The dominant alkaloids in cocoa pod extracts are theobromine, caffeine and theophylline, which derive from xanthine⁹. In the methylxanthine compound there are many phenolic compounds which have a hydroxy group (OH). Phenolic provides a beneficial antioxidant effect, research has shown that phenolic compounds are the main source of natural antioxidants in plants. In addition to

phenolic compounds, there are many methyl compounds and amine compounds which can also support the antioxidant effect of the cocoa fruit alkaloid extract. Antioxidants will play a role in increasing the value of the bond strength in enamels¹⁹. In the study of Nari-Ratih & Widyastuti²⁰ stated that free radicals have an important role in the onset and dental caries growth. Antioxidants can inhibit caries growth or delay the caries expansion. Thereby, the antioxidant content in alkaloids can increase the enamel surface hardness by increasing the enamel bond strength value.

The theobromine exposure in this research went through the apatite-forming-system and methylation of partially alkylated xanthine substituted to produce a product called theobromine apatite Ca₁₀(PO₄)₆(OHC₇H₈N₄O₂), with this formula, the apatite crystals produced with a size four times larger²¹. In the apatite crystal cell unit there is a micro tunnel with a diameter of ± 176 pm, making it possible for smaller ions to pass through the micro tunnel²². Some ions in theobromine have smaller diameters than micro tunnels, namely C=170 pm, N=152 pm, and H=152 pm. Other ion substitution in the apatite crystal will change the physical properties of the apatite. The compact arrangement of apatite crystals increases the attraction between the atoms²². Thus, it can be stated that the application of a larger crystal structure will become denser and increase the strength between the atoms. Therefore, an acid which has greater strength is needed to attract an ions from apatite crystals cause solubility and decrease in enamel hardness²³.

Alkaloids and theobromine are alternative ingredients that can be used to replace fluoride, by providing a better remineralization effect and minimum side effects²². In research by Nakamoto, Falster & Simmons²⁴ stated that theobromine does not provide toxic effects to body, this statement supported with study by Kayaputri *et al.*²⁵ which claimed that cacao seed coat extract is not potentially toxic. Cacao-pod alkaloid extract and theobromine can efficiently increase enamel surface hardness with affordable price⁹.

From this research it was found that every treatment group and control group had different values of enamel hardness measurement. Descriptively, theobromine group has the highest value, followed by the alkaloid, the APF and the control group. The data analysis results showed that there were significant differences between theobromine and alkaloid groups on the APF and control groups, this explained that the remineralization effect of the alkaloid and theobromine are better than the APF. But there is no difference between theobromine and alkaloid group, it showed that the remineralization effect of the given alkaloids and theobromine is comparable. This is in line with research conducted by Permatasari *et al.*⁹ stated that alkaloid with the concentration of 1000 mg/L which contain theobromine, caffeine and theophylline has the ability to increase enamel surface hardness value and did not differ significantly from single theobromine with 500 mg/L concentration. The enamel surface hardness of bovine incisors after exposure from theobromine, coco-pod alkaloid extract is harder than exposure to acidulated phosphate fluoride (APF).

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