The Effects of Toothpastes Containing Theobromine and Hydroxyapatite on Enamel Microhardness after Immersion in Carbonated Drink

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Abstract. This study aimed to analyze and compare the effects of toothpastes containing theobromine and hydroxyapatite on enamel microhardness after immersion in a carbonated drink. The remineralizing abilities of both toothpastes were also evaluated and compared. The crowns of 21 maxillary first premolars were immersed in a carbonated drink for 10 min. The specimens were divided into three groups as follows: those brushed with toothpastes containing theobromine \((n = 7)\) and hydroxyapatite \((n = 7)\), and those brushed with aquadest \((n = 7)\) for 9 min and 20 s each. The enamel hardness was measured after each treatment. A significant decrease in enamel hardness (average, 114 ± 12 KHN) was noted in all specimens after immersion in the carbonated drink. Kruskal–Wallis and Mann–Whitney tests revealed significant changes in hardness values after brushing with the theobromine- and hydroxyapatite-containing toothpastes. Enamel microhardness values were increased by 42 ± 9 KHN in the theobromine group and 55 ± 6 KHN in the hydroxyapatite group. Furthermore, enamel hardness in the theobromine group was significantly higher than that in the hydroxyapatite group \((p < 0.05)\).

1. Introduction

The enamel undergoes natural cycles of demineralization and remineralization; increased demineralization of the tooth surface results in the development of dental caries. Dental caries is characterized by a continuous loss of minerals from the crown or root of a tooth and is stimulated by bacteria. The five main factors that affect the occurrence of caries include plaque accumulation, carbohydrate intake, consumption of acidic foods and beverages, natural factors such as saliva and pellicle, and fluoride levels, with the first three being the driving factors of caries [1].

The enamel is demineralized when the pH in the oral cavity reaches 5.5. Carbonated beverages contain acids that can lower the pH in the mouth, and Coca-Cola® is one of the most popular carbonated drinks available in the market. Based on a study by Devlin et al, Coca-Cola® is a strong acidic beverage with a pH of 2.48 [2] and causes the most tooth decay when compared with other drinks such as Gatorade, Mountain Dew, and Sprite [3].

The critical pH threshold of 5.5 for demineralization can be lowered by fluorides [1]. Several dental hygiene products containing fluoride as the active ingredient are available in order to prevent
the development of dental caries [4]. However, the disadvantages of fluorides include fluorosis, bone fracture, toxicity, and gastric irritation when used in excess.

Recently developed toothpastes containing theobromine, an alkaloid present in chocolate or Theobroma cacao, can trigger remineralization without causing any toxicity [5-7]. Likewise, another toothpaste containing synthetic hydroxyapatite crystals has been developed to improve remineralization because the structure of the crystals is similar to that of human teeth.

This study aimed to examine the effects of the theobromine and hydroxyapatite toothpastes on the enamel surface after immersion in carbonated beverages and to compare the remineralization abilities of both substances.

2. Materials and Methods

This experimental laboratory study was conducted at the Laboratory of Dental Materials, Faculty of Dentistry, Universitas Indonesia and the Metal Laboratory, Faculty of Mechanical Engineering and Aerospace, Institut Teknologi Bandung, during September 2014 to November 2014. The number of specimens (n = 21) included in this study was determined using the G*Power version 3.1.9.2 for MAC OS X. Maxillary premolars with caries-free buccal surfaces that were indicated for extraction for orthodontic purposes were used.

The extracted teeth were washed with water and stored in 70% ethanol until use for the experiments. The crown of the tooth was separated from the root using a carborundum disk. The specimen was placed in a PVC pipe with a sticky paper attached to it (Figure 1). Decorative acrylic resin was then poured into the pipe. The surface of the specimen was smoothened with a grinding machine using silicon carbide paper (2000 grit) and polished with a polishing machine using 1 μm water-based alumina suspension. Care was taken not to exceed a thickness of 100 μm of enamel during the finishing and polishing of the enamel surface.

![Figure 1. Mounting the specimens.](image)

Initial enamel surface hardness tests were carried out with a Zwick Roell ZHμ Microhardness Tester using the Knoop method (50 g load for 15 s with five indentations at different locations). The specimen was immersed in Coca-Cola® (pH 2) for 10 min, washed with aquadest, dried, and then retested for hardness.

The specimens were brushed using a semi-rotational electric toothbrush at a pressure of 150g150 g. The amount of toothpaste used was controlled at a ratio of 1:33, where 3 g of paste was used in 10 mL of aquadest. The specimens were divided into the following three groups based on the type of toothpaste used: Group 1, brushing with Theodent-classic® toothpaste for 9 min 20 s (mimicking brushing twice a day for 1 month with each brushing period lasting for 10 s); Group 2, brushing with Pepsodent Sensitive-Expert Enamel Shield® toothpaste for 9 min 20 s followed by application of treatment described in Group 1; and Group 3 (controls), brushing with aquadest for 9 min 20 s
followed by application of treatment described in Group 1. The specimens were tested for enamel hardness after brushing.

Differences in mean values between the treatment groups were analyzed using Kruskal–Wallis and Mann–Whitney tests.

3. Results
Table 1 present the differences in the initial hardness, hardness after demineralization, and post-brushing hardness values between the Theodent, Pepsodent, and control groups. A decrease in hardness values after demineralization for 10 min in Coca-Cola® was noted in Group 1 (112 ± 12 KHN; 31.38%), Group 2 (114 ± 12 KHN; 30.92 %), and Group 3 (113 ± 10 KHN; 30.29%); however, the differences in these values among the three groups were not statistically significant (p > 0.05).

Table 1. Enamel hardness values in the three treatment groups

| Groups          | Enamel Micro Hardness ± SD (KHN) |          |          |          |
|-----------------|----------------------------------|----------|----------|----------|
|                 | Initial                          | Demineralization | Post-brushing |
| Control (n = 7) | 374 ± 9                          | 261 ± 16  | 273 ± 15  |
| Theodent (n = 7)| 362 ± 15                         | 250 ± 17  | 289 ± 23  |
| Pepsodent (n = 7)| 369 ± 15                        | 255 ± 14  | 310 ± 16  |

SD, standard deviation

As shown in Table 2, a significant increase in hardness (p < 0.05) post brushing was noted in Group 1 (42 ± 9 KHN; 16.94%), Group 2 (55 ± 6 KHN; 21.57%), and Group 3 (12 ± 4 KHN; 4.65%). The differences in values between the three groups were found to be statistically significant (p < 0.05).

Table 2. Change in enamel hardness post brushing in the three groups.

| Groups          | Ademineralization (KHN)  | Abrushing (KHN) |
|-----------------|--------------------------|-----------------|
| Group 3         | 113 ± 10                 | 12 ± 4          |
| Group 1         | 114 ± 12                 | 42 ± 9          |
| Group 2         | 114 ± 12                 | 55 ± 6          |

a. Decrease in enamel hardness after demineralization in Coca-Cola®
b. Increase in enamel hardness after brushing

Normality test (Shapiro–Wilk test) revealed that the data were normally distributed (p > 0.05) except for the Pepsodent demineralization group (Table 3); therefore, the non-parametric Kruskall–Wallis and Mann–Whitney tests were used to ascertain differences in mean values of hardness between two groups after demineralization and brushing.

Table 3. Results of Mann–Whitney test between groups.

| Group              | Mean value |
|--------------------|------------|
| Control-Theodent   | *          |
| Control-Pepsodent  | *          |
| Theodent-Pepsodent | *          |

*p significant (p < 0.05)
Friedman and Wilcoxon's signed-rank tests were used to ascertain differences in mean values of hardness among the groups after demineralization and brushing.

4. Discussion

The enamel hardness of 21 maxillary premolars planted in decorative acrylic resin was tested using the Knoop method. The specimens were immersed in Coca-Cola® (pH 2) in an incubator at 37 °C for 10 min based on a study conducted by Ponduri et al, wherein an individual is assumed to spend approximately 10 min to drink 250 mL of Coca-Cola® [8].

The decrease in hardness value after soaking in Coca-Cola® for a period of 10 min in all group were 114 ± 11 KHN (30.86%) of the initial hardness value. This may be attributed to the acidic content (phosphoric acid) of Coca-Cola®, which may have triggered the demineralization of the tooth enamel [2,9]. A previous study demonstrated a decrease of 70 KHN (19.66%) in tooth enamel hardness value after immersion in Coca-Cola® for 5 min [10]. Tantbirojn et al. evaluated the changes in hardness of bovine enamel specimens and reported a decrease of 100 KHN after immersion in Coca-Cola® for 8 min with removal from the solution at intervals of 2 min [11]. The differences in the decreased hardness values between the two aforementioned studies and the current study might be due to the different durations of immersion of the specimens in Coca-Cola®.

Brushing treatment in each group involved the use of a Pierrot's electric toothbrush (150 g load for 9 min and 20 s) and 3 g of toothpaste mixed with 10 mL of distilled water. Wiegand et al. (2007) compared the rate of enamel loss after brushing with a load of 150 to 450 g and reported that brushing with a pressure of 150 g caused the least amount of enamel loss when compared with the other load groups [12]. Therefore, in order to avoid changes in hardness values owing to brushing technique, 150 g loads were selected for the current study. A brushing time of 9 min and 20 s was chosen because it is equivalent to the time used for brushing for 28 days, twice a day for 10 s (with each tooth surface exposed to tooth brushing for no longer than 10 s) [5,11]. The toothpastes were mixed with aquadest at a standard ratio of 1:3 [13].

Brushing is known to remove the demineralized soft enamel layer and expose the underlying hard enamel layer, thereby increasing the hardness of the enamel [14]. There was a significant increase in hardness values in the control group after brushing with aquadest (12 ± 4 KHN; 4.65%). Likewise, the specimens in Group 1 (Theodent-classic toothpaste) also demonstrated a 16.94% (42 ± 9 KHN) increase in enamel hardness. Theodent-classic® toothpaste contains theobromine, which enhances remineralization because of the presence of calcium acetate [5]. Syafira et al. (2012) tested the increase in enamel hardness after immersion in various concentrations of theobromine solution and reported an increase of 24 KHN (7.59%), 41 KHN (12.97%), and 85 KHN (26.89%) at concentrations of 100, 500, and 1000 mg/L, respectively [16]. In the present study, we used the Theodent-classic toothpaste, with an increase in hardness equivalent to theobromine solution at a concentration of 500 mg/L, and showed an increase which is significant. The findings of the current study are in accordance with those reported by Amaechi et al. (2013), who compared the changes in enamel hardness values between theobromine solution and a common fluoride toothpaste; theobromine solution (151.9 mg/L) increased the enamel hardness by 38 KHN after 28 days of immersion relative to the artificial saliva group in their study [5]. In the current study, 28 days brushing period was used with the Theodent-classic® toothpaste, which contains calcium (Ca) and phosphate (PO₄) both of which are known to be present in saliva.

Specimens in Group 2 (Pepsodent Sensitive-Expert Enamel Shield® toothpaste) demonstrated a 21.57% (55 ± 6 KHN) increase in enamel hardness. Pepsodent Sensitive-Expert Enamel Shield® toothpaste contains both hydroxyapatite and sodium monofluorophosphate both of which act as remineralizing agents [16]. Hydroxyapatite in toothpastes can fill small demineralized defects on the tooth surface because it contains Ca and PO₄, the components required for remineralization [17]. Sodium monofluorophosphate containing fluorides bind to hydroxyapatite crystals and form氟roapatite crystals that have a lower critical pH of 4.5 [1]. Kliss et al. (2006) reported an increase in enamel hardness values (94 ± 11 KHN; 74%) after remineralization with a toothpaste containing
hydroxyapatite; the difference in hardness values between the study by Kliss et al. and the current study may be attributed to the different methods of remineralization. Kliss et al. used the pH cycling method with 120 min of remineralization time, whereas in the present study, a remineralization time of 9 min and 20 s was used [18]. The results of the present study are in accordance with that reported by Huang et al. (2009), who compared remineralization with hydroxyapatite (1% and 5%) for 12 days against enamel hardness. Huang et al. demonstrated an increase in hardness from 190 to 236 KHN in the 1% hydroxyapatite group (increase of 46 KHN) and from 190 to 263 KHN in the 5% hydroxyapatite group (increase of 74 KHN) [16]. In the current study, the increase in hardness values was higher in the Pepsodent group (Group 2) than in the Theodent group (Group 1). This might be due to the presence of both hydroxyapatite and fluoride in the Pepsodent Sensitive-Expert Enamel Shield® toothpaste. Remineralization by theobromine is expected to increase when Ca and PO₄ are added because theobromine cannot remineralize the tooth directly but can only induce the agents of remineralization [5-7].

5. Conclusion
A significant increase in enamel hardness was observed after brushing with the Theodent (theobromine) and Pepsodent (hydroxyapatite) toothpastes. Enhancement of enamel hardness in specimens treated with hydroxyapatite-containing toothpaste was greater than of those treated with theobromine toothpaste.

6. References
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