The influence of brushing with theobromine and sodium monofluorophosphate toothpaste on enamel surface resistance to roughness after demineralization

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Abstract. Herein, the influence of brushing with theobromine and sodium monofluorophosphate toothpaste on enamel surface roughness resistance after demineralization was determined. Overall, 21 human premolar enamel specimens were ground, polished, divided into three groups, and brushed for 5 minutes each with theobromine toothpaste, with sodium monofluorophosphate (MFP) toothpaste, or without toothpaste (control group). Next, specimens from the three groups were immersed in 20 ml of Coca-Cola® for 75 minutes. Surface roughness was measured before brushing, after brushing, and after immersion. Repeated ANOVA tests in the control group showed a non-significant (p > 0.05) increase in roughness after brushing and a significant increase (p < 0.05) in roughness after immersion. The theobromine group showed a significant (p < 0.05) increase in roughness after brushing and non-significant (p > 0.05) increase after immersion, and the MFP group showed a significant (p < 0.05) increase in roughness after brushing and after immersion. One-way ANOVA test showed that the theobromine group had the lowest increase in roughness (p > 0.05) after immersion, but it was not statistically significant compared to brushing using MFP. Therefore, brushing with theobromine toothpaste maintains enamel surface roughness after demineralization in Coca-Cola® and is slightly better than brushing with MFP toothpaste.

1. Introduction
The frequency of amino acid consumption is a main factor in caries formation. Over time, a diet high in acid can cause erosion on the surface of the teeth where it permanently dissolves the minerals of the tooth surface. The loss of minerals will increase the tooth surface roughness and decrease the tooth enamel roughness on the inner layer. Therefore, dental plaque can easily attach, and if not cleaned, the bacteria will grow and initiate the development of dental caries [1].

Demineralization occurs because the mineral component of enamel is very reactive with hydrogen ions with pH < 5.5 [2]. Carbonated beverages, energy drinks, and fruit juice are known to have pH < 4.0, speeding up the erosion process and caries initiation, depending on the frequency and duration of exposure to acid [3].

A carbonated beverage is a nonalcoholic drink containing a high level of acid that can increase demineralization and cause tooth erosion. Since 2007, the worldwide consumption of carbonated beverages reached approximately 552 billion liters, which meant that every person consumed approximately 83 liters per year. By 2012, every person consumed 95 liters annually [4]. This data shows that the level of carbonated beverage consumption is high, and these beverages are preferred in
the society. One of the most common carbonated beverages is Coca-Cola®. Based on research in the last few years, each person consumes 89 cans of Coca-Cola® annually [5]. Lussi et al. found that Coca-Cola® has the lowest pH among most carbonated beverages [6]. Coca-Cola® has pH of approximately 2.4, which is very reactive to dental erosion and is capable of increasing the tooth surface roughness [6, 7, 8].

Theobromine is one of the active ingredients found in toothpaste, and it can remineralize and prevent surface caries in tooth enamel. Currently, non-fluoride toothpaste products with theobromine have already been developed in the US. In Indonesia, one toothpaste with the active ingredient of sodium monofluorophosphate (MFP) has been developed that is reported to promote remineralization and maintain the strength of tooth enamel.

Previous research has discussed both active ingredients related to their potential to initiate remineralization. One newly published study about caries prevention found theobromine better than fluoride in remineralization and increasing the tooth surface roughness [9].

The effectiveness of toothpaste with the active ingredients of theobromine and sodium MFP in remineralization and maintaining the tooth surface roughness when exposed to Coca-Cola® has not yet been determined. The present research examined the influence of brushing with theobromine and sodium MFP toothpaste on enamel surface roughness resistance after demineralization when dipped in Coca-Cola®. In addition, the current study investigated which toothpaste was more effective in maintaining the enamel surface after exposure to a demineralizing liquid.

2. Method
This experimental laboratory research was conducted at the Dental Materials Laboratory, Faculty of Dentistry, Universitas Indonesia from September to November 2014. This research used 21 premolar teeth with smooth buccal surfaces without any cracks, anomalies, or color changes. The teeth had been extracted as a part of orthodontic treatment and had ethics approval. Enamel roughness was tested after treatment for three groups of specimens. Each group was brushed for 5 minutes with (A) without toothpaste (Aquabidest), (B) sodium MFP (Pepsodent Sensitive Expert® toothpaste), or (C) theobromine (Theodent® toothpaste). Each group comprised seven specimens.

The 21 premolar enamel areas were cut away from the roots (Accotum-2, Stuers). The crown portion (except for the largest and broadest buccal surface) was placed into acrylic resin that had been mixed with hardener. The buccal surface was smoothed (LabPol 21, Stuers) with emery paper (800 and 2000 grit) to get a flat and smooth enamel surface for all specimens. Abrasion with emery paper eliminated a maximum of 100 µm of enamel surface. The specimens were then polished using alumina 1µm in a polishing machine.

Each group of specimens was brushed and immersed into Coca-Cola® to continue erosion. Before the treatment, enamel surface roughness of every specimen was measured. Each specimen was brushed for 5 minutes using an electric toothbrush (Pierrot). Toothpaste was mixed with Aquabidest solution at 1:1 ratio, and the toothbrush load on the tooth was 150 grams. After brushing, each group of specimens was washed and dried, and the enamel surface roughness was measured again. Finally, all the groups were immersed in Coca-Cola® (20 ml; pH ± 3) for 75 minutes, washed with Aquabidest, and dried. Finally, the enamel surface roughness of each specimen was measured using a Surftest SJ-301 surface roughness tester (Mitutuyo, Japan). Every specimen was measured three times, and the mean values were determined. Measurement results for enamel surface roughness were tested statistically using repeated ANOVA and one-way ANOVA with 95% degree of confidence and p < 0.05. Previously, data normality was tested using Saphiro–Wilk test (n < 50).

3. Results
The tooth surface roughness results can be seen in Table 1.

### Table 1. Tooth surface roughness measurement of mean values.

| Groups                  | Surface roughness/Ra ± SD (µm) (× 10⁻³) | Before treatment (1) | After brushing (2) | After immersed into Coca-Cola® (3) |
|-------------------------|------------------------------------------|----------------------|--------------------|-----------------------------------|
| Aquabidest control (A) | 53.4 ± 4.72                              | 55.9 ± 5.55          | 101.6 ± 4.24       |
| Pepsodent Sensitive Expert® (B) | 53.4 ± 4.72                              | 72.1 ± 2.85          | 84.7 ± 6.16        |
| Theodent® (C)           | 53.3 ± 3.90                              | 71.3 ± 7.02          | 78.0 ± 7.62        |

### Table 2. Repeated ANOVA test results considered significant at p < 0.05.

| Variables                | Mean Difference | p value |
|--------------------------|-----------------|---------|
| Aquabidest (A)           |                 |         |
| A1 with A2               | −0.002          | 0.152   |
| A1 with A3               | −0.048          | 0.000*  |
| A2 with A3               | −0.046          | 0.000*  |
| Pepsodent Sensitive Expert® (B) |               |         |
| B1 with B2               | −0.019          | 0.000*  |
| B1 with B3               | −0.031          | 0.000*  |
| B2 with B3               | −0.013          | 0.005*  |
| Theodent® (C)            |                 |         |
| C1 with C2               | −0.018          | 0.003*  |
| C1 with C3               | −0.025          | 0.001*  |
| C2 with C3               | −0.007          | 0.112   |

Description: A, B, C = brushing groups (Aquabidest, Pepsodent S.E toothpaste, Theodent toothpaste); 1, 2, 3=before brushing, after brushing, after immersed into Coca-Cola; *significant difference

The repeated ANOVA test results of Aquabidest control group in Table 2 followed by post hoc pairwise cipher comparisons test revealed that there was no significant difference (p > 0.05) between early Ra (A1) and after brushing Ra with Aquabidest (A2). On the other hand, there is a significant difference (p < 0.05) between the early Ra (A1) and after immersed into Coca-Cola® Ra (A3) and after brushing Ra with Aquabidest (A2) with after immersed into Coca-Cola® Ra (A3). The Pepsodent Sensitive Expert group (B) showed a significant difference in the roughness value of all groups (p < 0.05). The Theodent® group (C) showed a significant difference (p < 0.05) in the roughness values for the groups before brushing (C1) and after brushing (C2) and before brushing group (C1) and after immersed into Coca-Cola® group (C3). However, there was no difference (p > 0.05) between the after brushing group (C2) and the after immersed in Coca-Cola® group (C3).

### Table 3. One-way ANOVA test results considered significant at p < 0.05.

| Treatment   | Mean Difference | p value |
|-------------|-----------------|---------|
| Early (1)   |                 |         |
| A1 with B1  | 0.00000         | 1.000   |
| A1 with C1  | 0.00014         | 0.998   |
| B1 with C1  | 0.00014         | 0.998   |
| Brushing (2)|                 |         |
| A2 with B2  | −0.01629        | 0.000*  |
| A2 with C2  | −0.01543        | 0.000*  |
| B2 with C2  | 0.00086         | 0.953   |
| Immersing (3)|               |         |
| A3 with B3  | 0.01686         | 0.000*  |
| A3 with C3  | 0.02357         | 0.000*  |
| B3 with C3  | 0.00671         | 0.132   |
Based on the one-way ANOVA test results with Tukey post hoc test, Table 3 shows that the roughness value of these three groups before the treatment (A1, B1, and C1) was not statistically different ($p > 0.05$). For the after brushing values (A2, B2, and C2), the mean value of surface roughness between control group (A2) and Pepsodent Sensitive Expert® groups (A2 and B2) and Theodent® groups (A2 and C2) were significantly different ($p < 0.05$). The mean roughness values for B2 and C2 were not significantly different ($p > 0.05$). When immersed in Coca-Cola® (A3, B3, and C3), there was a significant difference between the mean roughness surface value of control group and Pepsodent Sensitive Expert® group (A3 and B3) and Theodent® group (A3 and C3), with $P < 0.05$. Therefore, there was no difference between the mean roughness surface value of B3 and C3 ($P > 0.05$).

4. Discussion

The results of this research show that brushing premolar specimens with Aquabidest increases the enamel surface roughness (Ra) of the tooth, although it is not a significant difference. This shows that the pressure of the electric toothbrush on the tooth surface (150 mg of mass) allows a limited number of abrasive enamel minerals. However, the increasing roughness value indicates that the effects of the toothbrush and the pressure while brushing affect the minerals released from enamel and increase the enamel surface roughness. This is supported by John M. Powers in his book stating that the roughness and stiffness of a toothbrush will only have small abrasive strength, minimizing the value of abrasiveness from the brushing process. However, the abrasive force will increase when the toothbrush is used simultaneously with toothpaste [10]. Sandeep Kumar [11] shared that the design of a toothbrush, the frequency and duration of brushing, pressure while brushing, and the abrasive agents in toothpaste can improve the degree of abrasiveness on the tooth surface.

Brushing with Pepsodent Sensitive Expert® and Theodent® toothpaste shows the increasing Ra, which is significantly different compared to brushing without toothpaste. The increasing of enamel surface roughness after brushing with both toothpastes is caused by the abrasive agent called hydrate silica. According to Silje Storehagen and Nanna Ose (2003), the degree of abrasiveness depends on the strength of the agents, the form of abrasive particles, and the concentration of abrasive agents within the toothpaste. Abrasive agents in the toothpaste are not as hard as enamel but are harder than dentin, so that they can increase the enamel surface roughness [12]. Abrasive agents have functions for abrading, grinding, or polishing that can lift dental plaque, remove stains, and enrich the thickness of the toothpaste. In addition, Pepsodent Sensitive Expert® and Theodent® toothpastes contain other abrasive agents. Pepsodent Sensitive Expert® toothpaste contains mica particles and alumina, which clean the tooth surface. According to the British Dental Health Foundation, mica particles and alumina are mild abrasives that easily lift plaque from the tooth surface. Theodent® toothpaste also contains the whitening agent sodium bicarbonate, which is abrasive because it removes internal stains [12, 13, 14].

The first hypothesis that brushing with Theodent® toothpaste can maintain enamel surface roughness after demineralization in Coca-Cola® is accepted because there is an increase in roughness, although not significantly different, and it was lower than the group without toothpaste and the Pepsodent Sensitive Expert® group. Increasing of roughness shows that Theodent® paste provides resilience to enamel so that the minerals are not easily dissolved by acid attacks. This is possibly caused by the function of theobromine as a catalyst that can increase the size of hydroxyapatite crystals [15,16]. According to Armman Sadeghpour, the increasing size of the hydroxyapatite crystal increases the durability of enamel against acid. This opinion was stated in his research about the application of theobromine on the molar teeth of rats, showing that there is a correlation between the increasing size of hydroxyapatite crystals and increasing durability of enamel against acid compared with control group. In addition, Theodent® toothpaste also contains Rennou®TM, a mineral that consists of calcium and phosphate ions, which are major components of hydroxyapatite as active ingredients that serve as remineralization agents and for caries prevention [14].
Amaechi BT et al. noted that the theobromine component in Theodent® toothpaste is better than fluoride for remineralization and increasing enamel surface hardness [9]. Sadeghpour and Nakamoto (2011) and Amaechi et al. reported that theobromine can stimulate recrystallization and increase enamel surface hardness more effectively than sodium fluoride toothpaste [9, 14]. Moreover, Sadeghpour and Nakamoto used the treatment on the surface of specimens which had already been remineralized then demineralized again. When the amount of calcium lost from each group was measured, teeth with theobromine lost 8% less calcium than teeth treated with fluoride [9].

The second hypothesis that brushing with Pepsodent Sensitive Expert® toothpaste can maintain enamel surface roughness after demineralization with Coca-Cola®, is rejected because there is a significantly different increase in roughness. The active agents in Pepsodent Sensitive Expert® toothpaste have not yet been able to protect tooth surface from erosion by Coca-Cola®. However, when compared to the group without toothpaste, the difference in the two groups is significantly different. Brushing without toothpaste shows the highest increase of roughness value. Moreover, the difference in increased roughness compared with Theodent® shows that the two groups are not statistically different. The agent in the toothpaste is still able to provide protection on the enamel substance so the minerals are not released aggressively from the tooth surface. It may be caused by the formation between calcium on hydroxyapatite and fluoride on sodium monofluorophosphate that forms a CaF₂ layer, which provides protection to the minerals, so that enamel is durable despite being attacked by acid [12, 17]. This statement is also supported by the research by T.S. Carvalho and A. Lussi that measured the durability of toothpaste with fluoride and without fluoride against 1% citric acid with pH 3.6 [17]. The results show that durability declines significantly for both groups when demineralization occurs; however, the toothpaste with fluoride loses the enamel substance and has decreased surface microhardness that is lower than that in the toothpaste without fluoride. Although the toothpaste with fluoride showed resistance against the erosion by acid, fluoride could not provide full protection to maintain tooth enamel and protect from demineralization when pH is < 4.5 [12, 17].

The third hypothesis that there are differences between Theodent® and Pepsodent Sensitive Expert® toothpaste in maintaining enamel surface roughness against demineralization with Coca-Cola® is rejected because the changes in roughness for both toothpastes were not statistically different. Both toothpastes have the ability to maintain the minerals of the teeth and protect against demineralization, although the ingredients in Pepsodent Sensitive Expert® toothpaste have not yet been able to maintain the minerals better than Theodent® toothpaste.

If the changes in the roughness value of both brushing groups with toothpaste are compared to the brushing group without toothpaste (control), the increasing roughness of both groups brushing with toothpaste has lower value and is significantly different compared with the group brushing without toothpaste. This may be happening because of the minerals releasing very progressively from the hydroxyapatite bond in Coca-Cola® because its pH is very low, that is less than the critical pH 4.5–5.5, and there is no protective agent to maintain the minerals of enamel from demineralization.

Increasing enamel surface roughness after being immersed in Coca-Cola® is caused by acids such as phosphate acid, citric acid (flavoring), and carbonic acid, which is derived from the reaction between carbon dioxide and water. These acids make Coca-Cola® more reactive with the minerals on the tooth surface [18]. In addition, a diet with high consumption of phosphate and low consumption of calcium can cause decalcification of the bones, and the homeostasis of calcium in the body becomes unstable. Instability of calcium ions will cause the release of calcium ions from the apatit crystals [7, 18].

5. Conclusion
Both the toothpaste with theobromine and the toothpaste with sodium (MFP) are more able to provide protection after demineralization with Coca-Cola® for 75 minutes compared to the control group brushing without toothpaste, although the release of minerals in both groups still occurs so that the surface roughness increases. The toothpaste with theobromine can maintain enamel surface roughness better than the toothpaste with sodium monofluorophosphate.
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