Effects of theobromine toothpaste on prevention of enamel discoloration from coffee

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Abstract. This study investigated whether tooth brushing with toothpastes containing theobromine could prevent enamel discoloration due to coffee consumption. Thirty-five bovine incisor teeth were divided into groups that included brushing and no brushing treatments. Two brushing treatments consisting of two types of toothpaste: Theodent®, which contains theobromine, and Pepsodent®, which contains fluoride were crossed two brushing durations: 4 minutes 40 seconds and 20 minutes. Following the treatments, tooth specimens were immersed in coffee for 45 hours and the color change was measured using a Vita EasyShade spectrophotometer. Significant changes occurred in the color levels between the brushing and no brushing groups (ΔE* values; P < 0.05), but there was no significant difference between the types of toothpaste used. These results indicate chemicals in both toothpastes can effectively reduce enamel discoloration due to coffee consumption. Future studies should consider the importance of natural substances, like theobromine, for tooth whitening.

1. Introduction

The causes of tooth discoloration can be characterized as either intrinsic or extrinsic based on their etiology. Intrinsic factors causing discoloration include medications, such as tetracycline, genetic disorders, or trauma, which can cause teeth to be non-vital [1]. Meanwhile, extrinsic factors cause greater absorption of foreign materials in enamel pores, including smoke, cationic agents, like chlorhexidine, or foods high in tannin content, such as tea and coffee [2].

A primary cause for discoloration in Indonesia is the high frequency of coffee intake. Indonesia is among the eighth largest coffee consumers in the world, and as of 2012, it was the second largest consumer in Asia after Japan [3]. According to the literature, coffee is the second most frequently consumed beverage after water, and people consume approximately five cups of coffee per day, which means tooth discoloration is a real concern for those who regularly drink coffee [4,5].

Coffee contains large amounts of polyphenolic substances, such as tannin and chlorogenic acid, which tend to produce dark colors [6]. Coffee contains 22 types of acid also, including citric acid, acetic acid, as well as other acids with heavy molecules that contribute to the demineralization of enamel that causes more color absorption to teeth [7]. Therefore, it is necessary to identify products that can be applied to teeth to reduce absorption of these molecules and reduce discoloration.

Sadeghpour and Nakamoto (2011) demonstrated a substance in chocolate called theobromine can help calcium and phosphate remineralize tooth enamel. Theobromine is non-toxic and natural, making it a...
potential alternative to fluoride as the active ingredient in toothpaste [8]. Previous studies found the application of two remineralization agents (Casein Phosphopeptide Amorphous Calcium Phosphate or CPP-ACP and fluoride) to enamel surfaces decreased enamel permeability, which reduced absorption of staining materials [9,10]. The ability of theobromine to help calcium and phosphate in the remineralization process also can cause smaller pores in enamel, which should allow it to prevent or to decrease discoloration. Therefore, in this study, we determined the effects of theobromine in preventing discoloration after immersion of the teeth in coffee solution.

2. Methods
This study was performed at the Laboratory, Faculty of Dentistry, Universitas Indonesia from September to November 2014. Thirty-five bovine incisors were cleaned using twice distilled water, and a portion of the root was cut to ease pulp extirpation. Each tooth was given a 3 x 4 mm label on the middle third of the tooth and covered with nail polish. To prevent color penetration to the tooth from coffee solutions, nail polish was applied to all the tooth surfaces, including the root.

To obtain the baseline colors of teeth prior to coffee application and toothpaste treatment, specimens were placed above a pot cover, which was given a micro wax and layered with white printer paper. The color of the specimen surface was measured using a Digital Colorimeter (VITA EasyShade®) as levels of L* (brightness), a* (red/green), and b* (yellow/blue). Measurements were conducted daily between 13:00-17:00.

After the baseline measurements, two treatment groups were defined: those with and without tooth brushing. The brushing treatment was divided further into two groups that differed in the type of toothpaste used: a theobromine-containing toothpaste (Theodent Classic®) and a control, fluoride-containing toothpaste (Pepsodent®). The tooth specimens were fixed with a micro wax that was shaped like a bowl with a ± 5 cm diameter. Two hundred grams load were placed on the holder side. Brushing was performed with each type of toothpaste for two durations: either 4 min 40 sec or 20 min. These durations are equivalent to brushing for one week and one month, respectively. Seven teeth were included in each of the five treatment groups. To obtain the color estimates after brushing, the color measurements were taken again on all specimens, as described above.

A coffee solution was made by dissolving coffee powder in boiling water (1 g/75 ml). The solution was filtered, and its pH and temperature was measured. The specimens were fully immersed in coffee solution, and the container was shut. All the specimens were placed in an incubator at 37°C. Given that tooth staining tends to occur after six months of coffee consumption, teeth remained immersed in the coffee solution for 45 h. Subsequently, the final tooth color was measured using the same methods as above. We defined the value of ΔE* as the degree of color change from baseline to final measurements of L*, a*, and b*. After the data were collected, we tested for differences among the brushing treatments and the brushing durations using the non-parametric Kruskal-Wallis and Mann-Whitney statistical tests.

3. Results
The color of the bovine incisors changed following brushing (Table 1). The largest difference occurred with 20 minutes of brushing with Pepsodent, and the smallest difference occurred with 20 minutes of brushing with Theodent.
Table 1. Color changes mean value of bovine incisors after brushing and immersion in coffee solution.

| Toothpaste Type and Brushing Duration | Color Changes |                  |                  |                  |                  |
|--------------------------------------|---------------|------------------|------------------|------------------|------------------|
|                                      |               | ∆L*1             | ∆a*1             | ∆b*1             | ∆E*1             |
|                                      |               | ∆L*2             | ∆a*2             | ∆b*2             | ∆E*2             |
| Without brushing                     | -             | -                | -                | -                | 38.04            |
|                                      | 3.10          | -2.54            | -2.56            | 5.71             | -15.69           |
| Theodent 4 minutes 40 seconds         | 4.47          | -3.56            | -2.31            | 6.87             | -18.87           |
|                                      | 3.17          | -0.20            | -1.83            | 4.42             | -26.27           |
| Pepsodent 4 minutes 40 seconds        | 4.57          | -3.04            | -1.61            | 7.61             | -26.07           |
| Theodent 20 minutes                  | -             | -                | -                | -                | -15.69           |
|                                      | 3.10          | -2.54            | -2.56            | 5.71             | -15.69           |
| Pepsodent 20 minutes                 | -             | -                | -                | -                | -15.69           |

Minus (−) sign showed a decrease in value

- ∆L*1 = Changes in L* value which occurred before and after brushing.
- ∆L*2 = Changes in L* value which occurred after brushing and after immersion in coffee solution.
- ∆a*1 = Changes in a* value which occurred before and after brushing.
- ∆a*2 = Changes in a* value which occurred after brushing and after immersion in coffee solution.
- ∆b*1 = Changes in b* value which occurred before and after brushing.
- ∆b*2 = Changes in b* value which occurred after brushing and after immersion in coffee solution.
- ∆E*1 = Changes in E* value which occurred before and after brushing.
- ∆E*2 = Changes in E* value which occurred after brushing and after immersion in coffee solution.

Table 2. Significance of color difference (∆E*) from Mann Whitney.

| P        | Without Brushing | Theodent | Pepsodent |
|----------|------------------|----------|-----------|
|          |                  | 4 minutes| 20 minutes| 4 minutes| 20 minutes|
|          |                  | 40 seconds| 20 minutes| 40 seconds| 20 minutes|
| Without Brushing | 1               | 0.002*   | 0.018*   | 0.018*   | 0.180    |
| Theodent 4 minutes 40 seconds | 0.002* | 1         | 0.002*   | 0.898    | 0.013*   |
|          | 20 minutes       | 0.018*   | 0.002*   | 0.406    | 0.848    |
| Pepsodent 4 minutes 40 seconds | 0.018* | 0.898    | 0.406    | 1        | 0.110    |
|          | 20 minutes       | 0.180    | 0.013*   | 0.848    | 0.110    |

Significance level 0.05.
The * sign showed significant difference

There were significant color changes in all the groups after the immersion of bovine incisors in coffee solution. The biggest change occurred in the group without brushing, followed by the group that brushed with Pepsodent for 20 minutes, the group that brushed with Theodent for 20 minutes, the group that brushed with Pepsodent for 4 minutes 40 seconds, and last was the group that brushed with Theodent for 4 minutes 40 seconds.
Table 2 describes significant ΔE* color changes that occurred in all the groups. The group that brushed with Pepsodent for 20 minutes showed no significant difference in color change relative to the control group that did not brush.

In this study, the brightness of the bovine incisors was measured before brushing, after brushing, and after immersion in coffee solution. Brightness increased in all the groups after brushing with both type of toothpastes (Table 3). After immersion in coffee solution for 45 hours, brightness decreased significantly, which indicates darker colors, especially in the group without brushing.

| Treatment                  | L*0       | L*1     | L*2       |
|----------------------------|-----------|---------|-----------|
| Without brushing           | 83.38 ± 4.36 | -       | 45.34 ± 4.62 |
| Theodent 4 minutes 40 seconds | 84.70 ± 6.08 | 87.80 ± 3.38 | 72.15 ± 4.39 |
| Pepsodent 4 minutes 40 seconds | 81.23 ± 8.95 | 85.70 ± 4.59 | 66.82 ± 14.57 |
| Theodent 20 minutes         | 83.03 ± 3.65 | 86.20 ± 3.77 | 59.93 ± 8.92 |
| Pepsodent 20 minutes        | 77.28 ± 12.27 | 81.84 ± 9.09 | 55.77 ± 15.75 |

L*0 = Baseline L* value before brushing
L*1 = L* value after brushing
L*2 = L* value after immersion in coffee solution

We calculated the difference in brightness before and after brushing (ΔL*2). Based on the normality test, the values of ΔL*2 showed abnormal distribution. Therefore, the statistical tests performed to determine the significance of correlation between groups was Kruskal Wallis followed by Mann Whitney post hoc test which can be seen in table 4.

| P            | Without Brushing  | Theodent 4 mins 40 secs | Theodent 20 mins | Pepsodent 4 mins 40 secs | Pepsodent 20 mins |
|--------------|------------------|------------------------|------------------|--------------------------|------------------|
| Without Brushing | 1                | 0.002*                 | 0.009*           | 0.002*                   | 0.035*           |
| Theodent 4 mins 40 secs | 0.002*           | 1                      | 0.002*           | 0.654                    | 0.018*           |
| 20 mins      | 0.009*           | 0.002*                 | 1                | 0.406                    | 0.749            |
| Theodent 20 mins | 0.002*           | 0.654                  | 0.406            | 1                        | 0.110            |
| Pepsodent 4 mins 40 secs | 0.035*           | 0.018*                 | 0.749            | 0.110                    | 1                |
| 20 mins      |                  |                        |                  |                          |                  |

*Significance level 0.05.
The * sign showed significant difference

There were significant differences between the no brushing group and all the other groups in the change in brightness before and after brushing (ΔL*2). The Theodent 4 minutes 40 seconds group showed a significant difference compared to the Theodent 20 minutes group and the Pepsodent 20 minutes groups.
The value of a* is defined as the tendency of a color toward red (+) and green (-). In this study, the a* value of bovine incisors was measured before brushing, after brushing, and after immersion in coffee solution. Based on Table 5, a* decreased after brushing, which indicated when toothpaste was used, color changed to a more greenish hue (ranging from 0 – 3). After immersion in coffee solution for 34 hours, a* increased significantly in all groups, with the no brushing group showing the biggest difference and the Theodent 4 minutes 40 seconds group showing the smallest difference. Based on Table 6, significant changes in a* occurred between the Theodent 4 minutes 40 seconds group and all the other groups, with the exception of the Pepsodent 20 minutes group.

Table 5. Mean value of a* before brushing, after brushing, and after immersion in coffee solution.

| Treatment         | a*0        | a*1        | a*2        |
|-------------------|------------|------------|------------|
| Without brushing  | 4.38 ± 4.46| -          | 19.14 ± 2.12|
| Theodent 4 mins 40 secs | 3.75 ± 3.03| 1.21 ± 1.57| 8.18 ± 1.71|
| Pepsodent 4 mins 40 secs | 6.00 ± 4.26| 2.44 ± 2.02| 14.45 ± 7.38|
| Theodent 20 mins  | −0.54 ± 1.32| −0.74 ± 1.59| 14.46 ± 3.67|
| Pepsodent 20 mins | 4.18 ± 4.92| 1.14 ± 3.80| 17.37 ± 6.78|

a0 = Baseline a* value before brushing  
a1 = a* value after brushing  
a2 = a* value after immersion in coffee solution

Table 6. Significance of Δa*2 value with Mann Whitney Test.

| P                  | Without Brushing | Theodent | Pepsodent |  |
|--------------------|------------------|----------|-----------|-----|
|                    | Without Brushing | Theodent | Pepsodent |   |
|                    |                  | 4 mins   | 20 mins  | 4 mins| 20 mins|
|                    |                  | 40 secs  | 40 secs  | 40 secs| 40 secs|
| Without Brushing   | 1                 | 0.002*   | 0.848     | 0.338 | 0.949 |
| Theodent 4 mins 40 secs | 0.002*   | 1        | 0.03*     | 0.220 | 0.002*|
|                    | 20 mins          | 0.848    | 0.03*     | 1    | 0.443 | 0.654|
| Theodent 20 mins   | 0.338            | 0.220    | 0.443     | 1    | 0.124 |
| Pepsodent 4 mins 40 secs | 0.949 | 0.002*   | 0.654     | 0.124 | 1     |
|                    | 20 mins          | 0.949    | 0.002*    | 0.654 | 0.124 |

Significance level 0.05,  
The * sign showed significant difference

The b* value measures the yellow (+) and blue (-) hues. In this study, b* decreased after brushing (Table 7), which indicated toothpaste caused shifts to blue colors. After immersion in coffee solution for 45 hours, b* values increased significantly in each group, with the no brushing group showing the biggest change and the Theodent 4 minutes 40 second group showing the smallest change. Table 8 reveals a significant difference between the no brushing group and the Theodent 20 minutes, Theodent 4 minutes 40 seconds group, and Pepsodent 4 minutes 40 seconds group.
Table 7. Mean value of \( b^* \) before brushing, after brushing, and after immersion in coffee solution

| Treatment                  | \( b_0 \)       | \( b_1 \)       | \( b_2 \)       |
|---------------------------|-----------------|-----------------|-----------------|
| Without brushing          | 35.74 ± 3.67    | -               | 32.45 ± 4.33    |
| Theodent 4 mins 40 secs   | 35.12 ± 2.57    | 32.57 ± 3.27    | 38.84 ± 1.58    |
| Pepsodent 4 mins 40 secs  | 36.15 ± 4.07    | 33.84 ± 4.26    | 34.47 ± 3.33    |
| Theodent 20 mins          | 30.97 ± 3.36    | 29.14 ± 2.14    | 36.70 ± 4.87    |
| Pepsodent 20 mins         | 32.98 ± 5.42    | 31.37 ± 5.58    | 32.30 ± 5.35    |

\( b_0 = \) Baseline \( b^* \) value before brushing  
\( b_1 = \) \( b^* \) value after brushing  
\( b_2 = \) \( b^* \) value after immersion in coffee solution

Table 8. Significance of \( \Delta b_2^* \) value with Mann Whitney Test.

|          | Without Brushing | Theodent 4 mins 40 secs | Theodent 20 mins | Pepsodent 4 mins 40 secs | Pepsodent 20 mins |
|----------|------------------|-------------------------|------------------|-------------------------|------------------|
| **P**    |                  |                         |                  |                         |                  |
| Without Brushing | 1                | 0.002*                  | 0.025*           | 0.009*                  | 0.749            |
| Theodent 4 mins 40 secs | 0.002*          | 1                       | 0.085            | 0.013*                  | 0.655            |
| 20 mins   | 0.025*           | 0.085                   | 1                | 0.025*                  | 0.406            |
| Pepsodent 4 mins 40 secs | 0.009*          | 0.013*                  | 0.025*           | 1                       | 0.655            |
| 20 mins   | 0.749            | 0.655                   | 0.406            | 0.655                   | 1                |

*Significance level 0.05.*  
The * sign showed significant changes

4. Discussion

We performed an *in vitro* study with bovine incisors to determine if theobromine could protect teeth from discoloration due to staining from coffee. Bovine teeth are easier to obtain and are bigger in size compared to human incisors, making it easier to achieve flat surfaces during measurements with a spectrophotometer. In addition, previous studies have shown no significant differences in abrasive effects between human enamel and bovine enamel [11]. However, because the baseline color of bovine teeth is not the same as human teeth, we measured tooth color again after brushing and after immersion in coffee solution.

We showed there was a significant brightening of bovine teeth after brushing with toothpaste, and this also was true after immersion in coffee solution for 45 hours for all the groups. After brushing, a slight change in \( \Delta E^* \) color was seen in the groups that brushed with toothpaste. On CIE Lab measurement, \( \Delta E^* \) value can be detected by the eyes if the value exceeds 1.5. Therefore, \( \Delta E^* \) value occurred within 4 – 6 in these brushing groups can be detected by the eyes. Even though the type of Pepsodent® used in this study did not have a whitening agent, the changes in \( \Delta E^* \) value occurred because of abrasive materials contained in each toothpaste. This may be due to the citric acid and hydrated silica in Theodent® toothpaste and calcium carbonate, sodium silicate, and hydrated silica in Pepsodent. All of these chemicals can remove stains on tooth surfaces. There were more abrasive agents in Pepsodent compared to Theodent; therefore the mean value of \( \Delta L^* \) in Pepsodent groups tended to be higher than the Theodent groups, as shown in Table 5.
According to the CIE L*a*b* calculation, significant changes in ∆E* occurred in all the groups after immersion in coffee because of the significant increases in L* and a* values. The mean values of ∆L*2 were negative, indicating there was a decrease in brightness in all the groups. This was caused by brown-colored chromogenic substances contained in coffee, such as tannin and melanoidin. Meanwhile, the mean values of a* and b* in all the groups increased to become positive, indicating teeth discolored by coffee had a color tendency to turn more red and yellow. A previous study showed red, anthocyanin pigments are contained in coffee beans, potentially explaining this color shift. Tannin in the water often has a yellow to black color, which may also explain the yellowish discoloration in teeth stained with coffee [6].

Furthermore, chlorogenate acid and other types of acids in the coffee can create an acidic environment. Such an environment can cause mineral loss on the hydroxyapatite surface, which reduces crystal size. Reduced crystal size will cause more pores on enamel tissues to allow greater absorption of chromogenic substances into the enamel [12].

Changes in color (∆E*) among the five groups showed there was a significant difference between the brushing group and no brushing group (p < 0.05). This was consistent with previous studies that demonstrated remineralization agents can be used to prevent discoloration [10]. Theodent® toothpaste contains theobromine, calcium acetate, and sodium hydrogen phosphate, while regular Pepsodent® contains micro calcium and pro-fluoride complex. Although it is known the chemicals in Pepsodent can remineralize enamel the mechanism by which theobromine remineralizes enamel is unknown [9,10]. Based on the results of this study, there was no significant difference between toothpastes containing theobromine and toothpastes containing fluoride in preventing discoloration in bovine teeth (p > 0.05). These results showed theobromine and fluoride had similar effects in preventing discoloration.

Enamel color is also affected by enamel structure. Therefore, abrasions to enamel surfaces can affect enamel resistance to discoloration. Other than abrasive substances, abrasion to the tooth can also be caused by the frequency of use of spinning electric toothbrushes. Our data show the groups brushing for 4 minutes 40 seconds tended to have smaller ∆E* changes compared to groups brushing for 20 minutes, even though the differences were not statistically significant (p > 0.05). This was consistent with a study conducted by Wiegand et al. (2009) that revealed a 200 g load and greater brush rotation will cause rougher enamel surfaces, thus enabling chromogen substances to adhere more easily to enamel surfaces and cause greater discoloration [13].

5. Conclusion
In conclusion, brushing with toothpastes containing theobromine and fluoride can minimize discoloration after bovine teeth are immersed in coffee. Moreover, the color changes are reduced when teeth are brushed for 4 minutes 40 seconds compared to 20 minutes.

6. References
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