INTRODUCTION

The change in color of the teeth is commonly referred to as tooth discoloration. Tooth discoloration in enamel or dentin anterior teeth may cause discomfort. Several factors can cause tooth discoloration, one of which is the continued consumption of drinks that can change teeth' color, like tea. This habit will eventually cause an aesthetic disorder for the patients. Tea is enriched with bioactive substances and has a chromogenic effect that contributes to tooth discoloration. Tannin compounds found in tea can lead to the deposition of brown stains on the teeth' surface. The stain level that tea provides on the teeth surface is more than coffee's stain level due to the lower acidity (pH). The low acidity (pH) causes changes in the external teeth' color, unlike the neutral and high acidity. The degree of acidity (pH) can also induce surface roughness on the teeth surface, allowing food stains to penetrate the tooth surface and cause significant extrinsic discoloration.

Natural ingredients such as fruit can be used as an alternative treatment to overcome tooth discoloration because it does not cause side effects and is more affordable. Apples are a fruit that grows in many subtropical regions, especially in Indonesia. Various kinds of apples frequently found in Indonesia include Rome Beauty Apples, Anna Apples, and Manalagi Apples (Malus sylvestris), which are economical and enriched with high nutritional substances. Apples also contain malic acid, which is vital in producing mitochondrial ATP. This substance is also an effective chelating agent, capable of binding and deactivating some toxic metals. Malic acid is a carboxylic acid group that can whiten the teeth by oxidizing the enamel to become neutral and has a whitening effect. A similar study was previously carried out by Nuzulya Puspasari in 2012 using apple juice as a tooth whitening agent after immersing the tooth sample in coffee solution. No study has analyzed Manalagi Apple extract as an alternative tooth whitening agent in tea-induced tooth discoloration. Tea is one of Indonesian people's most frequently consumed drinks that can cause teeth to stain more than coffee. Therefore, the authors were interested in researching this matter.

METHOD

This study was an experimental study with a pre-and post-test control group design. We conducted this study in the Integrated Laboratory of the University of Diponegoro, Semarang, from January...
2019 to January 2020 and was approved by the Ethics Committee of the Faculty of Medicine of the University of Diponegoro No. 472/EC/KEPK/FK UNDIP/XI/2019.

The study samples were teeth obtained from several specialist dental clinics in Semarang with consent from each owner. We obtained twenty-seven teeth samples for this study. The teeth samples included in this study were from 16-25 years old subjects who met the inclusion criteria: post-extraction first premolars, first premolars without caries, and first premolars without hypoplasia.

Twenty-seven samples were immersed in tea solution for two weeks, and then we divided samples into three groups: Control groups (K), P1, and P2. Each group underwent different treatments after being immersed in tea solution for two weeks. P1 group was immersed in 50% of *Malalagi* Apple extract three times a day for one week, each for ten minutes. Meanwhile, the samples of the P2 group were immersed in 50% of *Malalagi* Apple extract three times a day for two weeks, each for ten minutes. The samples of group K served as a control and was not given any treatment.

Discoloration levels were measured before and after the immersion in 50% of *Malalagi* Apple extract utilizing a Colorimeter (Konica Minolta, CR-400). This tool measured the discoloration level with hunter L, a, and b color scales. L’s value was lightness, while the values of a and b were the chromaticity coordination between 0 (black) to 100 (white). The color difference value was obtained by using the formula of \( \Delta E(Lab) = [(L)_2^2 + (a)_2^2 + (b)_2^2]^{\frac{1}{2}} \). The “L” symbol showed the brightness level based on the white color, the “a” symbol indicated redness or greenish levels, and the “b” symbol specified yellowish or bluish levels.

**Staining with Tea Solution**

All of the tooth samples in this study were covered with clear nail polish with an apical part so that the tea solution did not penetrate the dentinal tubes through the roots. The tooth samples were then placed in a container filled with a tea solution and let soak for two weeks.

**Table 1. The differences in tooth discoloration after immersing *Malalagi* Apple extract.**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Number of Samples</th>
<th>Mean ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>9</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>9</td>
<td>6.56 ± 0.34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P2</td>
<td>9</td>
<td>7.31 ± 0.25</td>
<td></td>
</tr>
</tbody>
</table>

*ANOVA test*

**Table 2. The Results of Post Hoc LSD Test in All Treatment Groups.**

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>K1</th>
<th>P1</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment Groups</td>
<td>K</td>
<td>P1</td>
<td>P2</td>
</tr>
<tr>
<td>K</td>
<td>-</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P1</td>
<td>&lt;0.001</td>
<td>-</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P2</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>-</td>
</tr>
</tbody>
</table>

*the Post Hoc LSD test showed significant result if the p-value was <0.05*

**Manalagi Apple (*Malus sylvestris*) Extract**

*Manalagi* Apple (*Malus sylvestris*) extract was made by washing 3 kg of apples, then cut into pieces and crushed in a blender until it became powder. Add 70% ethanol to the powder, then stir for 30 minutes with a magnetic stirrer and let stand for 24 hours. The maceration results were filtered three times with a Buchner funnel covered with filter paper and accommodated in an Erlenmeyer. The filtered filtrate was evaporated with a vacuum rotary evaporator and heated in a water bath at 70°C to obtain dry apple extract. Furthermore, dilution was carried out with distilled water to reach a concentration of 50% (50g/ml), namely the calculation of 50 g of apple extract per 100 ml of distilled water.\(^{10}\)

**Immersion Teeth in *Manalagi* Apple (*Malus sylvestris*) Extract**

Premolar teeth from P1 and P2 groups were immersed in *Manalagi* Apple (*Malus sylvestris*). Extract in 10 ml plastic containers for two weeks with time three times a day for 10 minutes.

**Statistical Analysis**

Statistical analysis was performed employing IBM SPSS Statistic version 16 (IBM Corp., Armonk, NY, USA), and the differences were considered significant at p < 0.05. We used a One-Way ANOVA parametric test to analyze tooth discoloration differences after immersing the samples of the three groups in 50% of *Manalagi* Apple extract, followed by a Post Hoc LSD test to determine the differences between groups.

**RESULT**

The differences in tooth discoloration after immersing in *Manalagi* Apple extract are presented in **Table 1**. Based on the discoloration (ΔE) above, the mean value of tooth discoloration after immersion in *Manalagi* Apple extract in group P1, group P2, and group K were 6.56 ± 0.34, 7.31 ± 0.25, and ΔE 0, respectively.

The One-Way ANOVA test obtained a p-value of <0.001, meaning significant differences between the three treatment groups. Furthermore, we used the Post Hoc LSD test to determine the value difference between one treatment and the others.

Based on the Post Hoc LSD test results in **Table 2**, there were significant differences after immersion in *Manalagi* Apple extract between the K-P1, K-P2, and P1-P2 groups, with a p-value of <0.001.

**DISCUSSIONS**

Immersioning teeth samples in tea solution for two weeks was proven to cause discoloration of teeth. Tea has a chromogenic effect which, if consumed continuously, causes staining on teeth.\(^{3}\) Tannins in tea are the cause of teeth
discoloration. The discoloration of the tooth surface is also caused by a low pH content in tea. This study revealed that immersion in 50% of Manalagi Apple extracts for one week and two weeks caused tooth discoloration. The most prominent color change (ΔE) was 7.63 in group P2, and the smallest ΔE was 6.01 in group P1. The ΔE of group K was 0. Furthermore, the comparison results between the K-P1 group and the K-P2 group showed that the biggest ΔE (7.63) was from comparing the K-P2 group. In other words, immersion in Manalagi Apple extracts for two weeks on a tea-induced discolored tooth was more effective in whitening the tooth than immersion for one week.

This study result similar with Puspasari et al, 2012.[11] The study discovered that immersing the teeth in 50% apple extract for two weeks would make it whiter than immersing in 50% apple extract for one week. The whitening effect of Manalagi Apple (Malus sylvestris) extract is probably due to malic acid content. The extraction process for Manalagi Apple can increase the malic acid concentration. Malic acid has a very low molecular weight so that it can diffuse into enamel and dentin. The pH factor of Manalagi Apple also influences tooth discoloration. Based on this study’s results, Manalagi Apple had a pH of 4.27. Erosion will occur in tooth enamel when it reaches a critical pH of lower than 5.5. This pH is a critical pH that can cause enamel solubility and tooth erosion. The lower the pH, the more acidic it gets. Therefore, teeth whitening material will be absorbed into the dentinal tubules. This process will eventually make the teeth whiter. Another factor that affects tooth discoloration is subject age. This study used teeth from people with an average age of 16-25 years. The tooth enamel has the nature of translucency. The older the enamel layer gets, the thinner it becomes. This process is due to the abrasion process of the teeth, in which the dentin will thicken due to the formation of secondary dentin. Increased dentin thickness accompanied by the enamel layer thinning will cause the teeth to appear more yellow.

CONCLUSIONS
Color changes occurred after immersing the teeth in Manalagi Apple extract for one and two weeks. Immersing the teeth for two weeks caused whiter teeth compared to immersing the teeth for one week.

LIMITATIONS OF THE STUDY
After performing the measurement, we knew that we could not measure all tooth surfaces using a Colorimeter device. This result was due to the Colorimeter’s flat detector shape that did not match the tooth surface’s shape. Further research is required utilizing other tools such as Micro Dental Spectro Shade, CCD Spectrophotometer, which can measure the entire surface of tooth.

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CONFLICT OF INTEREST
There is no conflict of interest.

AUTHOR CONTRIBUTION
All authors contributed equally in the writing of this article.

ETHICS APPROVAL
The study was conducted after obtaining ethical clearance from the Health Research Ethics Commission of the Faculty of Medicine, Diponegoro University / Dr. Kariadi General Hospital Semarang with number 472/EC/KEPK/ FK UNDIP/ XI/2019.

REFERENCES