An Analysis of Dental Enamel after Bleaching using 35% Hydrogen Peroxide with Energy-dispersive X-ray Spectroscopy

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ABSTRACT

Introduction: Hydrogen peroxide (H₂O₂) is an effective bleaching agent of tooth whitening, but its use causes changes in the chemical composition of the elements that configure tooth enamel. The purpose of this study is to determine whether there are changes in the composition of the elements that configure the tooth enamel after bleaching using 35% H₂O₂.

Materials and methods: This study was performed in vitro, which is an experimental research laboratory with controlled time series design. The samples used in this study were six permanent incisors postextraction and are divided into treatment and control groups. The control group was soaked in saline and treatment groups applied with H₂O₂ 35%, with time series of 1, 1.5, 2, 2.5, and 3 hours. The elemental composition of enamel was observed using energy-dispersive X-ray spectroscopy analysis; results can be obtained both qualitatively and quantitatively. This study was analyzed using non-parametric test (Kruskal–Wallis test) to determine changes in enamel composition after bleaching, whether there is a change in enamel composition or not.

Results: The results of this study showed a p-value of 0.406 (p < 0.05; significant). The result shows a change in the composition of dental enamel after bleaching using 35% H₂O₂.

Conclusion: Application of 35% H₂O₂ as the bleaching agent does not have a significant influence on changes in the composition of the elements contained in the enamel.

Keywords: 35% hydrogen peroxide, Bleaching, Composition of dental enamel.


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INTRODUCTION

Enamel is the hardest tissue of teeth, which is the outer layer of the tooth crown. The thickness and density affect the surface of the tooth crown. The composition of elements of tooth enamel is composed of cells, organic matrix, and inorganic matrix; these cells control the initial production of tissue minerals. Tooth enamel consist of consist of 96% inorganic material, 4% water, organic matter, and fibrous tissue. Inorganic materials consisting of crystalline hydroxyapatite has the chemical formula CA₁₀(PO₄)₆(OH)₂. The content of the elements contained within tooth enamel is carbonate (4%), sodium (0.6%), magnesium (1.2%), chloride (0.2%), and a small amount of fluoride (0.01%). The salts comprise of materials such as keratin (pseudokeratin), collagen, peptone, glycoproteins, polysaccharides, fats, and acids amino.

Enamel consists of the outer and inner enamel, which are enamel surface and subsurface enamel. Surface enamel contains more fluorine and a little carbonate so that the surface enamel is harder, less porous, insoluble, and more radiopaque.

Tooth discoloration is an esthetic complaint because it can affect a person's appearance. Tooth discoloration is influenced by a combination of intrinsic and extrinsic factors. The color change is extrinsic to the teeth caused by chromogens derived from the intake of food, tobacco, mouthwash, or plaque on the tooth surfaces, while the tooth discoloration is intrinsically caused by the stain deeper in the tooth enamel, which can be systemic factors, metabolism, genetic, and local.

Tooth whitening (bleaching) is a method to remove stains on teeth. Bleaching is using for tooth whitening in the internal as well as external and for nonvital and vital teeth. External tooth whitening done on vital teeth changes color as a whole. Tooth whitening (bleaching) can be done in the clinic by the dentist directly or performed at home by the patients themselves or by monitoring of the dentist. External bleaching agent that is often used is H₂O₂ and carbamide peroxide. Both of these materials contain H₂O₂, which decomposes into H₂O and O₂. Materials used for teeth whitening carried out directly by the dentist contain peroxide with a high concentration (30–40%), while active ingredients applied for use at home by the patient using materials that are sold freely or under the supervision of a dentist have a lower concentration of carbamide peroxide (10–20%) and H₂O₂ (3–7.5%).
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Hydrogen peroxide is known as dihydrogen dioxide, hydrogen dioxide, oxydol, and peroxide, the chemical formula is $\text{H}_2\text{O}_2$, pH 4.5, which is a clear, colorless, and odorless liquid, with a molecular weight of 34.0147 gm/mol and is more viscous than water.\textsuperscript{10} Hydrogen peroxide as a bleaching agent is effective in tooth whitening, but there are some concerns on the use of this material, which has been reported in some studies; complications after tooth whitening (postbleaching) include changes in the surface morphology of the enamel and dentin, changes in chemical composition, increased permeability, and mechanical changes. Hydrogen peroxide as a bleaching agent of tooth whitening is safe to use when used in a controlled concentration, usage time is not too long (when used in high concentration) and within a certain time interval.\textsuperscript{8}

The purpose of this study was to analyze the compound of dental enamel after bleaching using 35% $\text{H}_2\text{O}_2$.

**MATERIALS AND METHODS**

This study is an experimental research with controlled time series design. This study was conducted at the Laboratory of Oral Biology, Faculty of Dentistry, Hasanuddin University and at the Laboratory of Microstructure Physics, State University of Makassar. This study uses $\text{H}_2\text{O}_2$ for bleaching and insissus permanent with This study uses $\text{H}_2\text{O}_2$ for permanent incisors. Six samples were used in this study. The control group consisted of one sample and treatment group consisted of five samples. Permanent caries-free incisor tooth was stain cleaned and stored in saline solution (0.9% NaCl). Teeth were cut ½ length of the roots and crowns were clipped sagittal with a size of 3 mm using a carborundum disc. Five samples treated by time series at the same time was applied with bleaching material $\text{H}_2\text{O}_2$. In the control group, samples were stored in saline solution and the treated samples were applied with 35% $\text{H}_2\text{O}_2$ for 1, 1.5, 2, 2.5, and 3 hours. After application of each sample, it is cleaned using distilled water and then dried. After drying, the sample was analyzed using energy-dispersive X-ray spectroscopy (EDS).\textsuperscript{11}

**RESULTS**

Observations of dental enamel using EDS show results in the form of graphs. The graphs consists of control charts, treatment charts with 35% $\text{H}_2\text{O}_2$ bleaching for 1 hour, 1 hour 30 minutes, 2 hours, 2 hour 30 minutes and 3 hours (Graphs 1 to 6).

The analysis of nonparametric test (Kruskal–Wallis test) gives a p-value of 4 (the null hypothesis), which means there is no difference. This means that there is no significant difference in the composition of the enamel between the control group and the group that received treatment with 35% $\text{H}_2\text{O}_2$.

**Table 1:** Composition of dental enamel with EDS

<table>
<thead>
<tr>
<th>Enamel composition</th>
<th>EDS</th>
<th>Normal test</th>
<th>Comparative test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>Oxygen</td>
<td>14.0483 ± 1.01873</td>
<td>0.167*</td>
<td>0.406</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.3450 ± 0.11845</td>
<td>0.676*</td>
<td>0</td>
</tr>
<tr>
<td>Calcium</td>
<td>3.2533 ± 0.21172</td>
<td>0.702*</td>
<td>0</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1.3850 ± 0.26599</td>
<td>0.054*</td>
<td>0</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.1433 ± 0.01366</td>
<td>0.093*</td>
<td>0</td>
</tr>
<tr>
<td>Chlorine</td>
<td>0.1417 ± 0.01169</td>
<td>0.033</td>
<td>0</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.1317 ± 0.04070</td>
<td>0.295*</td>
<td>0</td>
</tr>
</tbody>
</table>

*Shapiro–Wilk test: p > 0.05; normal distribution, **Kruskal–Wallis test: p < 0.05; significant; SD: Standard deviation

Graph 1: X-ray diffraction pattern of the enamel (control) soaked with a solution of saline for 24 hours

Graph 2: X-ray diffraction pattern of the tooth enamel bleached using 35% $\text{H}_2\text{O}_2$ for 1 hour
0.3450, the average composition of calcium from each treatment was 3.2533, the average phosphorus composition of each treatment was 1.8850, the average composition of potassium from each treatment was 0.1433, the average composition of the chlorine from each treatment was 0.1417, the average composition of magnesium of any treatment was 0.1317. Statistical test results obtained the value of \( p = 0.406 > 0.05 \), which means \( H_0 \) (the null hypothesis) is received, which means there is no effect. This means that “the treatment of \( \text{H}_2\text{O}_2 \) as a dental bleaching agent does not have a significant influence on the enamel surface of the tooth”.

Table 2 shows analysis of non parametric test (Kruskal–Wallis test), which appears that the value of \( p = 0.406 > 0.05 \) which means \( H_0 \) (the null hypothesis) is received, which means there is no effect. This means that “there is no significant difference in the composition of the enamel between the control group and the group receiving treatment with \( \text{H}_2\text{O}_2 \).

**DISCUSSION**

The purpose of this study was to analyze the content of the elements contained in the enamel after bleaching using 35% \( \text{H}_2\text{O}_2 \). This research was carried out *in vitro*; there is a difference between *in vitro* and oral cavity situation. There is saliva in the oral cavity that can inhibit the demineralization because it contains calcium and phosphate. Saliva also has the effect to neutralize the oral pH (buffer capacity of saliva).12

In this study, control and treatment samples were soaked with saline solution. Saline solution has a neutral pH, which is expected to minimize the effect of the...
results of the study. The results of this study show that the elements can be detected, which are oxygen (O), sodium (Na), magnesium (Mg), phosphorus (P), calcium (Ca), potassium (K), and chlorine (Cl), contained in dental enamel. This shows that research using EDS can be used to analyze qualitatively. The EDS is an ideal system to present all the data of X-ray of a specimen and qualitative analysis could be done both qualitatively and quantitatively.

Calcium is a major component in the structure of the tooth, and enamel demineralization occurs due to release of calcium ions from the tooth enamel; the effect of acid on tooth enamel is a decomposition reaction. The duration or length of the teeth in contact with the material bleaching affects the demineralization process; the number of elements of tooth enamel is not only influenced by the degree of acidity (pH) and the concentration of the bleaching but the length of time or duration of H₂O₂ in contact with the teeth can also affect the levels of dissolution of the content mineral of dental enamel.

The EDS analysis results showed that the average oxygen composition of each treatment was 14.0483, the average composition of sodium from each treatment was 0.22, the average composition of calcium from each treatment was 3.49, the average composition of phosphorus from each treatment was 2.02, the average composition of potassium from each treatment was 0.13, the average composition of chlorine from each treatment was 0.15, the average composition of magnesium from each treatment was 0.11, the average composition of sodium from each treatment was 0.1317, the average composition of potassium from each treatment was 0.1417, the average composition of magnesium of any treatment was 0.1433, the average composition of chlorine from each treatment was 0.14, the average composition of phosphorus from each treatment was 1.97, the average composition of magnesium from each treatment was 0.14, the average composition of calcium from each treatment was 1.88, the average composition of potassium of any treatment was 0.14, the average composition of sodium of any treatment was 0.14, the average composition of chlorine of any treatment was 0.12, the average composition of magnesium of any treatment was 0.11, the average composition of chlorine from each treatment was 0.14, the average composition of phosphorus from each treatment was 1.88, the average composition of potassium from each treatment was 0.14, the average composition of sodium from each treatment was 0.14, the average composition of magnesium from each treatment was 0.11, the average composition of chlorine from each treatment was 0.12, the average composition of magnesium from each treatment was 0.11.

**Table 2: Differences in the composition of the enamel between the control group and the group receiving treatment with H₂O₂**

<table>
<thead>
<tr>
<th>Enamel composition</th>
<th>Oxygen</th>
<th>Sodium</th>
<th>Calcium</th>
<th>Phosphorus</th>
<th>Potassium</th>
<th>Chlorine</th>
<th>Magnesium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.11</td>
<td>0.22</td>
<td>3.49</td>
<td>2.02</td>
<td>0.13</td>
<td>0.15</td>
<td>0.11</td>
</tr>
<tr>
<td>H₂O₂ 1 hour</td>
<td>14.52</td>
<td>0.35</td>
<td>3.35</td>
<td>2.13</td>
<td>0.13</td>
<td>0.15</td>
<td>0.16</td>
</tr>
<tr>
<td>H₂O₂ 1.5 hours</td>
<td>13.95</td>
<td>0.53</td>
<td>3.13</td>
<td>1.94</td>
<td>0.16</td>
<td>0.15</td>
<td>0.20</td>
</tr>
<tr>
<td>H₂O₂ 2 hours</td>
<td>14.09</td>
<td>0.43</td>
<td>3.26</td>
<td>1.88</td>
<td>0.14</td>
<td>0.14</td>
<td>0.11</td>
</tr>
<tr>
<td>H₂O₂ 2.5 hours</td>
<td>14.48</td>
<td>0.30</td>
<td>3.39</td>
<td>1.97</td>
<td>0.14</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td>H₂O₂ 3 hours</td>
<td>12.14</td>
<td>0.24</td>
<td>2.90</td>
<td>1.37</td>
<td>0.16</td>
<td>0.12</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*Shapiro–Wilk test; p > 0.05, data distribution normal; **Kruskal–Wallis test: p < 0.05; significant

CONCLUSION

The 35% H₂O₂ as the bleaching agent does not have a significant influence on changes in the composition of the elements contained in the enamel.

REFERENCES