

# Effect of Whitening Mouthrinses on Color Change of Stained Teeth: *In Vitro* Study

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## ABSTRACT

**Objective:** This *in vitro* study aimed to evaluate the effectiveness of three different whitening mouthrinses on the color change and whiteness of stained teeth at different immersion times.

**Methods:** Thirty-two human incisors teeth were stained in black tea solution for seven days and randomly divided into four groups, which were control (artificial saliva) and three whitening mouthrinses (containing tetrapotassium pyrophosphate, hydrogen peroxide, charcoal powder) (n=8). Spectrophotometric measurements were performed at baseline, after staining, after 12 hours, and 120 hours after keeping in whitening mouthrinses. The color changes and whiteness indexes occurring at different immersion times and after teeth staining were calculated.

**Results:** The whitening mouthrinses tested showed similar changes in color change while exhibiting color change above the clinically acceptable threshold. Regarding whiteness index values, the highest value was obtained in the mouthrinse containing hydrogen peroxide with an immersion time of 120 hours ( $P < 0.05$ ). While there was no significant difference in color change values at different immersion times, it was observed that the whiteness index values increased with increasing immersion time.

**Conclusion:** Whitening mouthrinses can provide a clinically noticeable color change in discolored teeth. It can be said that using mouthrinses containing peroxide can provide more effective whitening on stained teeth.

**Keywords:** Activated charcoal, hydrogen peroxide, tooth bleaching, whiteness index, whitening mouthrinse

## INTRODUCTION

Today, as aesthetic appearance becomes increasingly essential, applications for restoring teeth color have developed, and tooth whitening treatments have become a popular treatment that supports improving aesthetic appearance. Teeth whitening treatments encompass a variety of methods, including different types of whitening agents, concentration levels and application protocols. Today, the over-the-counter (OTC) products are one of the most preferred whitening treatments applied to vital teeth.<sup>1</sup> OTC products are freely sold to consumers in many places such as in supermarkets,

on the internet. Unlike professional teeth whitening methods, OTC products are not under the supervision of a physician and can be used at home without needing a prescription or guidance.<sup>2,3</sup> Products such as whitening toothpaste and mouthrinses, whitening strips, dental floss, and chewing gum marketed with various ingredients fall into this category.<sup>4</sup> One of the OTC products, whitening mouthrinse, has recently been introduced to prevent discoloration, combat plaque buildup, and provide a rapid whitening effect. The easy use and low cost of whitening mouthrinses have enabled to become widespread rapidly, resulting in the production of different



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products in terms of concentration, quantity, and content. The most commonly utilized bleaching agent in whitening mouthrinses is hydrogen peroxide. The whitening properties of agent occurs when the oxygen molecules released by its breakdown break down the pigmented structures in the tooth into smaller structures.<sup>5</sup> In the literature is examined, many studies may be found that have been conducted on products containing hydrogen peroxide (cHP), and the ability of hydrogen peroxide to cause color change in teeth has been demonstrated in previous studies.<sup>6,7</sup> There are also whitening mouthrinses with alternative ingredients such as sodium citrate, sodium hexametaphosphate, and tetrasodium pyrophosphate. These agents can chemically remove external colorations and prevent new chromogen adsorption by disrupting the pellicle structure.<sup>8,9</sup> One of the whitening agent ingredients recently introduced to the market is activated charcoal. Manufacturers state that because activated charcoal has a large surface area (>1000 m<sup>2</sup>/g) besides porous structure, it can absorb chromogenic structures that cause tooth discoloration.<sup>10</sup> Although the use of activated charcoal is recommended with the claim that it can whiten teeth effectively, there is insufficient scientific data on its use as a whitening agent. Considering the results that may arise from patient-controlled use of whitening mouthrinses, there is a need to assess the efficacy of different ingredient-containing whitening mouthrinses on the teeth during different periods of use. Based on this, this research aims to evaluate the effects of three whitening mouthrinses on the color change and whiteness of stained teeth at different immersion times. The first null hypothesis tested was that there would be no difference between whitening mouthrinses in their effect on discoloration and whiteness on stained teeth. The second null hypothesis was that immersion time would not affect the color change and whiteness index of whitening mouthrinses.

## MATERIAL AND METHODS

The study was approved by the Erzincan Binali Yıldırım University Non-invasive Clinic Ethics Committee (approval no.: 2023-23/12, date: 28.12.2023) and conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from the patients who owned the extracted teeth used in this study.

### Sample Size Calculation

The power of the sample size was calculated by G\*Power software (G\*Power Ver. 3.1.9.4, Heinrich-Heine Dusseldorf

University, Dusseldorf, Germany) with a 95% confidence interval, an 95% power, and 0.50 effect size values for 32 samples according to one-way analysis of variance (ANOVA)-type power analysis. Eight samples per group were calculated as minimum sample size.

### Preparation of Dental Specimens

In this study, a total of 32 permanent extracted human incisor teeth without cavities or enamel defects for orthodontic or periodontal reasons in last 6 months. Tissue residues remaining on the tooth surface were removed with the help of periodontal curettes and the surfaces were polished using a polishing rubber. Then, the teeth were divided into two parts, crown and root, from the enamel-cement junction with a low-speed diamond disk saw (Isomet, Buehler Ltd., Lake Bluff IL, USA) under water coolant. The crowns of the teeth were embedded in teflon molds (3 cm in diameter) with the help of self-hardening cold acrylic, leaving the root parts outside. The samples were kept in distilled water at room temperature for one day before starting the staining process.

### Measuring Color Change Values ( $\Delta E_{00}$ )

The samples removed from distilled water were dried using an air-water spray. The first color measurements (T0) were made using a digital spectrophotometer (VITA Easys shade V; Vita Zahnfabrik, Bad Sackingen, Germany). The device was calibrated before measurements. In order to standardize the measurements, measurements were made in D65 standards on a white background. Each sample was measured three times, and the mean L\*, a\*, and b\* values were recorded for each measurement. A single operator made all color measurements.

After the first color measurements, the samples were kept in a solution prepared by adding two bags (2 x 2g) of black tea (Yellow Label Tea, Lipton, London and Türkiye) into 300 mL of boiled water in closed containers for seven days in order to color them. The black tea solution was refreshed daily. At the end of the coloring procedure, color measurement (T1) was made for the second time after all samples were washed under running water and dried.

### Experimental Groups

The samples were randomly divided into four groups based on their whitening mouthrinses composition (n=8):

Mouthrinse containing tetrapotassium pyrophosphate (cTP),

Mouthrinse cHP,

Mouthrinse containing charcoal powder (cCP),

Artificial saliva [control group (CG)].

The composition of each mouthrinse and artificial saliva were presented in Table 1. A previous study reported that keeping dental materials in continuous mouthrinse for 12 hours had the same clinical effect as using mouthrinse for one year (twice a day, one minute)<sup>11</sup>; in this study, samples were subjected to a 12-hour and 120-hour mouthrinse immersion procedure. In this way, it was aimed to simulate the effect of whitening mouthrinse for one and 10 years. Solutions were refreshed

### MAIN POINTS

- The most important finding of the study is that discolored teeth can be affected by whitening mouthrinses.
- Whitening mouthrinses can be recommended to patients as a product that supports whitening treatments, which are in increasing demand today.
- The use of a whitening mouthrinse containing hydrogen peroxide will help achieve better results in terms of patient satisfaction.

every 24 hours. Samples removed from the solutions after 12 and 120 hours were washed in distilled water for five min and dried on blotting paper. The third (T2, following the 12 hours) and fourth (T3, following the 120 hours) color measurements were performed, respectively.

The color changes were assessed using the formula of CIEDE2000 ( $\Delta E_{00}$ ), which represents the distance of two colors to the three-dimensional space. While the color change that occurs after initial and staining is considered  $\Delta E_{001}$  (T0-T1), between staining and 12-hour immersion is  $\Delta E_{002}$  (T1-T2), and between staining and 120-hour immersion is  $\Delta E_{003}$  (T1-T3). The following formulation was used to calculate  $\Delta E_{00}$ :

$$\Delta E_{00} = \sqrt{\left(\frac{\Delta L}{K_L S_L}\right)^2 + \left(\frac{\Delta C}{K_C S_C}\right)^2 + \left(\frac{\Delta H}{K_H S_H}\right)^2} + R_T \left(\frac{\Delta C}{K_C S_C}\right) \left(\frac{\Delta H}{K_H S_H}\right)$$

The variations in hue, chroma, and lightness between the two measurements are expressed in the formulas  $\Delta L$ ,  $\Delta C$ , and  $\Delta H$ . The letter "S" represents the hue and chroma weight functions.  $K_L$ ,  $K_C$ , and  $K_H$  were designated as "1" in this investigation.<sup>6</sup>

#### Analysis of Whitening Efficacy

The whitening effectiveness of whitening mouthrinses was evaluated using the Whitening Index for Dentistry ( $WI_D$ ) and was calculated as follows:

$$WI_D = 0.511L^* - 2.324a^* - 1.100b^*$$

The whiteness difference ( $\Delta WI_D$ ) between the two measurements was calculated with the following formula:<sup>12</sup>

$$\Delta WI_D = WI_{D2} - WI_{D1}$$

Whiteness differences were calculated to be the same as the time intervals indicated in the color change.

#### Statistical Analysis

Data were analyzed with IBM SPSS V23 and the JAMOVI program. Compliance with normal distribution was examined using the Shapiro-Wilk Test. Robust Two-Way ANOVA was used in the JAMOVI program to compare  $\Delta E_{00}$  values. The generalized linear model method was used to compare  $WI_D$  values, and multiple comparisons were examined with the Tukey honestly significant difference test. The significance level was taken as  $P < 0.05$ .

#### RESULTS

$\Delta E_{00}$  values of whitening mouthrinses examined at different time intervals are presented in Table 2. In this study, the clinically acceptable limit for color change exceeded  $\Delta E_{00} = 2.25$ , as stated by Ghinea et al.<sup>13</sup> Accordingly, a clinically noticeable color change was seen in all whitening mouthrinse groups at all time intervals. The results showed that the whitening mouthrinse used and the immersion time did not have a statistically significant effect on the  $\Delta E_{00}$  values representing the color change, and the interaction between the mouthrinse and the immersion time was not statistically significant. ( $P > 0.05$ ) In contrast, the whitening mouthrinses tested appeared to increase color change compared to the CG ( $P > 0.05$ ). Regardless of the time, the cTP group showed the most effect among whitening mouthrinses. The highest  $\Delta E_{00}$  value was again seen in the cTP mouthrinse group in the T1-T2 time interval ( $P > 0.05$ ). The lowest  $\Delta E_{00}$  value was calculated in the T1-T3 time interval in the CG. The T1-T2 time interval was observed to have a higher  $\Delta E_{00}$  value than the T1-T3 time interval in the

**Table 1.** The Compositions of Whitening Mouthrinses and Artificial Saliva

Whitening mouthrinse	Composition	Company
cTP	Aqua, alcohol, sorbitol, tetrapotassium pyrophosphate, pentasodium triphosphate, citric acid, Poloxamer 407, sodium benzoate, eucalyptol, thymol, menthol, sodium saccharin, sodium fluoride, tetrasodium pyrophosphate, propylene glycol, sucralose, aroma, disodium phosphate	Johnson& Johnson, Pomezia, Italy
cHP	Aqua, glycerin, propylene glycol, xylitol, Laminaria saccharina extract, PEG-40 Hydrogenated Castor Oil, PVP, aroma, sodium benzoate, benzoic acid, calcium glycerophosphate, sodium saccharine, magnesium chloride, hydrogen peroxide, limonede.	Eurocosmed-Stupino Ltd, 142800, Moscow, Russia
cCP	Aqua, glycerin, propylene glycol, sorbitol, tetrapotassium pyrophosphate, polysorbate 20, tetrasodium pyrophosphate, zinc citrate, PVM/MA Copolymer, aroma, benzyl alcohol, sodium fluoride, sodium saccharin, Bambusa vulgaris shoot extract, charcoal powder	Colgate-Palmolive, Guildford, GU2 8JZ
CG	Sodium chloride (0.4 g/L), potassium chloride (0.4 g/L), calcium chloride-H <sub>2</sub> O (0.795 g/L), sodium dihydrogen phosphate-H <sub>2</sub> O (0.69 g/L), sodium sulfur-9H <sub>2</sub> O (0.005 g/L), and 1000 mL distilled water	-

cTP, mouthrinse containing tetrapotassium pyrophosphate; cHP, mouthrinse containing hydrogen peroxide; cCP, mouthrinse containing charcoal powder; CG, control group (artificial saliva); PEG-40 Hydrogenated Castor Oil, polyethylene glycol-40 hydrogenated castor oil; PVM/MA Copolymer, polyvinyl methyl ether/maleic anhydride copolymer.

mouthrinse groups, except for the cHP group ( $P > 0.05$ ). In the cHP group, the color change value in the T1-T3 time interval was seen to be higher than the color change value in the T1-T2 interval ( $P > 0.05$ ).

The time-dependent average  $WI_D$  values of whitening mouthrinses are presented in Table 3. The analysis showed that the whitening mouthrinse and immersion time had a statistically significant effect on the  $WI_D$  values representing the whiteness index ( $P = 0.046$ ). The highest  $WI_D$  value among whitening mouthrinses was seen in the T1-T3 time interval in the cHP group, and this value was statistically significant compared to all other groups ( $P < 0.05$ ). The lowest  $WI_D$  value was obtained in the T1-T2 time interval in the same group, and there was no statistically significant difference between this value and the CG ( $P > 0.05$ ). In all groups except the cCP group, it was observed that the  $WI_D$  values obtained in the T1-T3 time interval were statistically significantly higher than the  $WI_D$  values obtained in the T1-T2 time interval. ( $P < 0.05$ ) There was no statistically significant difference between the  $WI_D$  values in the T1-T2 and T1-T3 time intervals in the cCP group ( $P > 0.05$ ).

## DISCUSSION

*In vitro* research conducted recently has concentrated on the impacts of bleaching agents other than hydrogen peroxide on restorations, enamel, and dentin. In this his study, the effectiveness of three whitening mouthrinses at different immersion times in improving tooth color on black tea-stained teeth was evaluated. According to the study results, while there was no significant interaction between the whitening mouthrinses in terms of their effects on the color change of the teeth at different immersion times, significant differences were

found in their impact on the whiteness indexes of the teeth. Therefore, both null hypotheses were partially accepted. The human incisor tooth surfaces used in this study were carefully cleaned to mimic the natural structure, but no smoothing process was applied. The smoothing process causes the enamel thickness to decrease with the removal of the aprismatic layer, therefore the whitening agent penetrates more into the tooth structure.<sup>14</sup> Also, this process makes it more susceptible to pigment absorption.<sup>15</sup> As a result, problems might occur in terms of accurate measurement of color. Many studies have shown that black tea is used to create pigments that cause discoloration in teeth.<sup>12,16,17</sup> In this study, dental samples were stored in black tea for seven days. When the literature was examined, it was found that the formula of CIELAB was often used to evaluate the change in color. However, studies have proven that the CIEDE2000 formula is a better fit for the evaluation of color changes compared to the CIELAB formula.<sup>2,18,19</sup> In parallel with the literature, in this study, a digital spectrophotometer was used for color measurements and the CIEDE2000 formula for determining color changes. According to study results,  $\Delta E_{001} > 2.25$  was shown in all tested groups after being stored in black tea. This result is consistent with other studies indicating that black tea causes significant discoloration of teeth.<sup>16,20</sup> When whitening mouthrinses's effect on color change were evaluated over time, the whitening mouthrinse groups, excluding the CG, exhibited a clinically noticeable color change effect on the teeth in two different immersion periods ( $\Delta E_{002}, \Delta E_{003} > 2.25$ ). Based on this, it should be noted that all of the whitening mouthrinses used in the study reduced tooth stains. However, no significant difference was observed between the groups. Many studies support this outcome.<sup>21,22</sup> In this study, the highest and clinically detectable

**Table 2.** Median, Minimum and Maximum Color Change ( $\Delta E_{00}$ ) Values by Mouthrinse and Time

Whitening mouthrinse	Duration time of use		
	$\Delta E_{001}$	$\Delta E_{002}$	$\Delta E_{003}$
	Median (min.-max.)	Median (min.-max.)	Median (min.-max.)
cTP	4.7 (2.1-11.4)	4.4 (2.6-5.7)	4.2 (2.9-5.9)
cHP	3.1 (2.2-4.7)	3.6 (1.2-5.6)	4.3 (3.1-8.2)
cCP	4.4 (1.4-5.9)	3.9 (1.4-7.5)	3.8 (2.0-5.3)
CG	2.7 (0.7-10.3)	1.7 (1.4-6.8)	1.4 (1.1-5.7)

cTP, mouthrinse containing tetrapotassium pyrophosphate; cHP, mouthrinse containing hydrogen peroxide; cCP, mouthrinse containing charcoal powder; CG, control group (artificial saliva).

**Table 3.** Descriptive Statistics of Whiteness Index ( $\Delta WI_D$ ) Values by Mouthrinse and Time

Whitening mouthrinse	Duration time of use		
	$\Delta WI_{D1}$	$\Delta WI_{D2}$	$\Delta WI_{D3}$
cTP	-6.4 (4.2) <sup>D</sup>	6.7 (2.9) <sup>ABC</sup>	11 (3.7) <sup>AB</sup>
cHP	-8.5 (1.7) <sup>D</sup>	5.8 (5.0) <sup>BC</sup>	13.2 (3.5) <sup>A</sup>
cCP	-9.4 (2.9) <sup>D</sup>	7.5 (3.5) <sup>ABC</sup>	10.2 (5) <sup>ABC</sup>
CG	-8.5 (6.2) <sup>D</sup>	3.9 (3.4) <sup>C</sup>	4.3 (3.6) <sup>BC</sup>

F=2,260.  $P = 0.046$  ( $P < 0.05$ ).

<sup>AB,C,D</sup> There is no difference between time and mouthrinse interactions with the same letter.

cTP, mouthrinse containing tetrapotassium pyrophosphate; cHP, mouthrinse containing hydrogen peroxide; cCP, mouthrinse containing charcoal powder; CG, control group (artificial saliva).

color change was seen in the cTP group at 12 hours immersion time. In comparison, the cHP group showed the highest color change at 120 hours of immersion time. Whitening mouthrinses usually contain low concentrations of whitening substances including pyrophosphates, peroxides, sodium citrate, sodium hexametaphosphate, and activated charcoal. These agents function by either whitening or removing the stain.<sup>6</sup> With repeating pyrophosphate subunits, sodium hexametaphosphate is a longer-chain pyrophosphate derivative with reduce adsorption of stain-chromogen and whitening potential.<sup>23</sup> cTP is available on the market as a type of mouthrinse that does not contain hydrogen peroxide and was reported to have a whitening impact thanks to chemical agents such as tetrapotassium pyrophosphate. cTP is thought to provide effective whitening because to the presence of tetrapotassium pyrophosphate. The findings of this research are compatible with previous ones.<sup>24,25</sup> Ethanol, added as a solvent in cTP, is an alcohol derivative. Gürdal et al.<sup>26</sup> reported in their research that alcohol in whitening mouthrinses was effective. In the study, the fact that cTP contains alcohol and its pyrophosphate content may have caused its higher whitening effectiveness. cHP is available as a whitening mouthrinse cHP. Hydrogen peroxide is a widely used whitening agent, professionally and as a self-applied agent.<sup>23</sup> The whitening effectiveness of hydrogen peroxide is supported in the literature.<sup>6,23,27</sup> Harorlı and Barutçigil<sup>6</sup> stated that the mouthrinses content, brand, and duration of waiting in the mouthrinse significantly affect color recovery. Karadas and Hatipoğlu<sup>21</sup> noted that the whiteness of the enamel-dentin samples they colored in tea solution increased due to being kept in whitening mouthrinses. The immersion time was important for whitening. A study found that chemically induced peroxide containing tooth whitening products accumulate in tooth tissue over time, increasing the total contact time.<sup>28</sup> Conversely, according to the research by Ntovas et al.<sup>22</sup>, the effectiveness of mouthrinses decreases after three weeks of use, and there is not always a positive relationship between increasing time and color change. In this study, it is seen that long-term use of cHP causes high color change. Although the long-term values of pyrophosphate-containing mouthrinse decreased, the longterm increased values of hydrogen peroxide-containing mouthrinse may be due to the accumulation of different chemical ingredients on tooth surfaces and the reduction-oxidation mechanism. This study evaluated a new mouthrinse containing activated charcoal as a whitening agent. Activated charcoal is produced by partially oxidizing of various materials as a natural method. Highly porous activated charcoal compounds can exchange ions through nanopores and adhere to tooth enamel and adsorb stains on the tooth surface. It is stated that whitening toothpastes containing activated charcoal have a whitening effect by causing significant changes in tooth color.<sup>9,29</sup> Studies showing the effect of mouthrinses containing activated charcoal on discolored teeth are limited.<sup>30,31</sup> In the research, it was seen that whitening mouthrinse containing activated charcoal caused color change in the teeth after 12-hour and 120-hour immersion times. The whitening effect of mouthrinses containing activated charcoal is suggested to be due to the abrasive nature of the charcoal particles, which are mechanically effective in removing surface stains. Even

while color changes upon immersion in various solutions are frequently assessed using the color difference formula, this method by itself does not yield enough information regarding the change in color coordinates. As a result, it is inadequate to compare whiteness values just using the CIEDE2000 calculation ( $\Delta E_{00}$ ). Therefore, in the study, the  $WI_D$  (whiteness index) formula, developed specifically for dentistry, was used to evaluate the whiteness degrees of the teeth used and the effect of mouthrinses on the change in whiteness degrees. While the negative results of  $\Delta WI_D$  values calculated after the coloring process indicate a decrease in the whiteness of the teeth, a high positive index value indicates an increase in the whiteness of the teeth.<sup>32</sup> In this research, significant differences were found in  $WI_D$  values after coloring with black tea and immersion in different whitening moutrinses. When the study is examined, the negative values seen in  $WI_{D1}$  values, in line with the  $\Delta E_{001}$  values, show that black tea staining causes a clinically visible degree of darkness in the teeth. However, samples exposed to whitening mouthrinses reached positive values at two different time intervals. This result shows that whitening mouthrinses whitens teeth in short and long-term use. In the study, the highest  $WI_D$  value was seen in the cHP group at a 120-hour immersion time. About  $\Delta E_{003}$ , the high whiteness values can be attributed to the hydrogen peroxide content in the cHP mouthrinse structure and the increase in contact time over time. There are insufficient studies in the literature explaining the relationship between tooth color change and  $WI_D$  values and evaluating the materials used in the study or similar materials.

### Study Limitations

Compared to clinical investigations, this research has certain limitations because it was planned and carried out *in vitro*. The *in vitro* conditions of study was carried out are different from the oral environment due to the absence of a pellicle layer and the absence of factors such as nutrients and saliva. These factors constitute the limitation of our study. On the other hand, temperature and pH changes can also affect color stability and whiteness values. These limitations are among the factors that are likely to affect the results of our study. Thus, there is a need for more *in situ* and clinical research.

### CONCLUSION

The results and recommendations reached within the limits of this study are as follows:

- It was observed that a clinically noticeable color change occurred in all whitening mouthrinses evaluated.
- However, there is no difference in color change between the mouthrinses used.
- There is no significant difference in color change between short- and long-term whitening mouthrinse use.
- It can be said that the use of mouthrinse cHP can provide more effective whitening on stained teeth.
- It was observed that whiteness index values increase significantly as the duration of use of whitening mouthrinses increases.



Within the limitations of this study, it should be taken into account that discoloration may occur as a result of exposure of colored teeth to whitening mouthrinses and that the duration of use of mouthrinses is important for the whitening effect, and potential consumers should be informed about the importance of complying with the recommended application frequency and duration.

## Ethics

**Ethics Committee Approval:** The study was approved by the Erzincan Binali Yıldırım University Non-invasive Clinic Ethics Committee (approval no.: 2023-23/12, date: 28.12.2023) and conducted in accordance with the Helsinki Declaration.

**Informed Consent:** Written informed consent was obtained from the patients who owned the extracted teeth used in this study.

## Footnotes

### Author Contributions

Concept - S.G.; Design - S.G.; Supervision - S.G., İ.H.; Resources - S.G., İ.H.; Materials - S.G.; Data Collection and/or Processing - S.G., İ.H.; Analysis and/or Interpretation - S.G., İ.H.; Literature Search - S.G.; Writing - S.G.; Critical Review - S.G., İ.H.

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