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Determination of the Whitening Effect of Toothpastes
on Human Teeth

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Determinación del efecto blanqueador de los dentífricos
en dientes humanos

Numan Aydın DDS, PhD¹; Serpil Karaoğlanoğlu DDS, PhD²; Elif Aybala Oktay DDS, PhD³
Bilge Ersöz DDS, PhD⁴

1. University of Health Sciences, Gulhane Faculty of Dentistry, Department of Restorative Dental Treatment, Ankara, Turkey. <https://orcid.org/0000-0001-8628-4507>

2. University of Health Sciences, Gulhane Faculty of Dentistry, Department of Restorative Dental Treatment, Ankara, Turkey. <https://orcid.org/0000-0003-0601-8028>

3. University of Health Sciences, Gulhane Faculty of Dentistry, Department of Restorative Dental Treatment, Ankara, Turkey. <https://orcid.org/0000-0003-4716-948X>

4. University of Health Sciences, Gulhane Faculty of Dentistry, Department of Restorative Dental Treatment, Ankara, Turkey. <https://orcid.org/0000-0003-1668-0592>

Correspondence to: Dr. Numan Aydın - dt_numan@hotmail.com

ABSTRACT The desire of individuals to have whiter teeth increases the interest in tooth whitening products. Our aim was to in vitro study the whitening effect of hydrogen peroxide, blue covarine and active charcoal containing whitening toothpastes on human teeth. A total of 40 extracted human incisor teeth were used in the study. To measure the whitening effect of toothpastes, the teeth were divided into four subgroups and placed in the phantom tooth jaw model. Then, daily brushing was done with an electric toothbrush. The colors of the teeth were measured initially using the spectrophotometer (single point and bleached shade mode) and at the end of 7th, 14th and 28th days. Whitening effectiveness of toothpastes were studied according to CIEDE2000 formula (ΔE_{00}) and shade guide units (SGU). One-way analysis of variance (ANOVA) and Tukey test were used in the statistical analysis of the data. ($p < 0.05$). Activated charcoal containing toothpaste showed the greatest whitening effect at the end of 7th, 14th and 28th days ($p < 0.05$). There was no statistically significant difference between the whitening effects of hydrogen peroxide and conventional toothpaste ($p > 0.05$). Blue covarine containing toothpaste had statistically the lowest whitening effect ($p < 0.05$). All toothpastes showed a whitening effect on the teeth after 7 days of use. Activated

charcoal containing toothpaste showed more whitening effect after 28 days of use than hydrogen peroxide, blue covarine and traditional toothpaste.

KEYWORDS: Whitening toothpastes; Active charcoal; Blue covarine; Tooth whitening.

RESUMEN: El deseo de los individuos de tener unos dientes más blancos aumenta el interés por los productos de blanqueamiento dental. Nuestro objetivo fue estudiar el efecto blanqueador de las pastas dentales blanqueadoras que contienen peróxido de hidrógeno, covarina azul y carbón activo en dientes humanos in vitro. En el estudio se utilizaron un total de 40 dientes incisivos humanos extraídos. Para medir el efecto blanqueador de los dentífricos, los dientes se dividieron en cuatro subgrupos y se colocaron en el modelo de diente fantasma en mandíbula. A continuación, se realizó un cepillado diario con un cepillo eléctrico. El color de los dientes se midió inicialmente con un espectrofotómetro (modo de punto único y tono blanqueado) y al final de los días 7, 14 y 28. Se estudió la eficacia blanqueadora de los dentífricos según la fórmula CIEDE2000 (ΔE_{00}) y las unidades de guía de color (SGU). En el análisis estadístico de los datos se utilizó el análisis de varianza de una vía (ANOVA) y la prueba de Tukey. ($p < 0.05$). La pasta de dientes con carbón activado mostró el mayor efecto blanqueador al final de los días 7, 14 y 28 ($p < 0,05$). No hubo diferencias estadísticamente significativas entre los efectos blanqueadores del peróxido de hidrógeno y el dentífrico convencional ($p > 0,05$). El dentífrico que contiene covarina azul tuvo estadísticamente el menor efecto blanqueador ($p < 0,05$). Todos los dentífricos mostraron un efecto blanqueador en los dientes después de 7 días de uso. Los dentífricos con carbón activado mostraron un mayor efecto blanqueador tras 28 días de uso que el peróxido de hidrógeno, la covarina azul y el dentífrico tradicional.

PALABRAS CLAVE: Dentífrico blanqueador; Carbón activado; Covarina azul; Blanqueamiento dental.

INTRODUCTION

Changes in tooth color create a wide variety of cosmetic problems. The desire to have whiter teeth by preventing or cleaning extrinsic stains on teeth has increased the interest in tooth whitening products (1). The success of teeth whitening depends on the type of stain present, which can be categorized as extrinsic staining, internal staining, and internalized discoloration (2). Manufacturers have developed new oral hygiene products to

meet the different expectations of individuals and presented them to consumers.

It is known that hydrate silica, calcium carbonate, dicalcium phosphate, calcium pyrophosphate or sodium bicarbonate contained in whitening toothpastes can mechanically remove external stains (2-4). Toothpastes containing low concentration hydrogen peroxide agents are thought to encourage color change as a result of the interaction of hydrogen peroxide with dentin pigments called chromophores

that define the color of the tooth tissue (5,6). Toothpastes containing blue covarine, instead of an abrasive effect, can leave a translucent bluish layer on the tooth surface. As a result of the interaction of this layer with the light coming to the tooth surface, the teeth appear brighter and whiter (7).

Recently, since toothpastes containing activated charcoal have the capacity to adsorb color pigments, it has been stated that they are effective in removing extrinsic tooth stains (8). However, the literature states that the whitening efficiency of charcoal powder and the effect of this product on the enamel surface are not consistent (9,10).

In daily use, it is important that the whitening performance of toothpaste is visibly perceived by individuals. Visual perception under daily conditions reveals the effectiveness of products. As the effectiveness of toothpastes can be measured using instrumental methods with spectrophotometer and colorimeters, (11) they can also be analyzed by visual comparison methods (12). The CIELab, Color difference (ΔE_{ab}) is the value used to evaluate color changes and is calculated by special formula using the differences in L^* , a^* , b^* values. The extent of the color difference that can be detected visually by the human eye is stated as PT (perceptibility threshold), and the extent of the color difference that constitutes the acceptability between restorative materials is stated as AT (acceptability threshold) (13,14).

With the clinical spectrophotometer device (Vita Easy Shade V, Germany), measurements can be made in bleached shade mode as well as in the CIELAB color system. If the bleached shade mode is SGU (Shade Guide Units), the measured color is set according to the VITA Bleachedguide 3D-MASTER. This mode contributes to a better understanding of the whitening performance and limitations of whitening products. Data on the

numerical and visual whitening efficiency of teeth whitening products are presented in the literature (14).

Studies on the whitening effect of active charcoal-containing whitening toothpastes on teeth are limited. In our study, we aimed to examine the effect of whitening toothpastes (hydrogen peroxide, blue covarine and activated charcoal) on time-dependent whitening on teeth *in vitro*. Our null hypothesis is that whitening toothpastes containing different active ingredients will not exhibit different whitening effectiveness on teeth.

MATERIALS AND METHODS

PREPARATION OF SAMPLES

Approval (2020/510) for the study was obtained from the Non-invasive Research Ethics Committee of Gülhane Training and Research Hospital. A total of 40 human incisor teeth extracted within last month previous to the experimental procedures were used in the study. Teeth extracted from patients (40-65 age) due to periodontal disease were kept at +4 oC in artificial saliva (1.491g KCl, 0.015g $MgCl_2 \cdot 6H_2O$, 0.06g $CaCl_2 \cdot 2H_2O$, 0.005g NaF, 0.108g NaH_2PO_4 , 0.124g Na_2HPO_4 and 1.157g NaCl, pH 7.0) after removing biological residues in the crown and root. Decayed and deformed teeth were not used in the study.

SIMULATED TOOTH BRUSHING

Before starting the brushing process, teeth crowns were polished with a polishing paste to standardize all teeth. Then the teeth were kept in artificial saliva for 24 hours at 37 oC and then randomly divided into 4 subgroups and placed in the phantom tooth jaw model (2 incisors for each model) and brushed for 8 seconds every day with an electric toothbrush (Triumph Professional Care,

Oral B Braun GmbH, Germany). All tooth surfaces were brushed daily with toothpastes containing active charcoal, hydrogen peroxide, blue covarine and Complete Protection (control) with the slurry prepared with distilled water (1:1 ratio) (Table 1). The brushing process was done every day at the same time by a single user, disabling the pressure

sensor of the brush. The brushing process was carried out 2 times a day for 2 minutes. In our study, the brushing time of the teeth was determined based on the daily average brushing process for a tooth of 8 seconds. After daily brushing, the samples were rinsed with water for 10 seconds and then kept in artificial saliva solution.

Table 1. Whitening technologies in the evaluated toothpastes.

Toothpaste	Composition	Tooth whitening technology
Colgate Optic White (Expert White)	Glycerin, Calcium Pyrophosphate, Propylene Glycol, PEG/PPG-11666 Copolymer, PEG-12, PVT, Tetrasodium Pyrophosphate, Sodium Laurly Sulfate, Silica, Aroma, Sodium Monophosphate, Sodium Sacharin, Phosphoric Acid, Hydrogen Peroxide, BHT, Limonene.	Hydrogen peroxide
Signal White Now CC	Aqua, Hydrogenated Starch Hydrolysate, Hydrated Silica, PEG-32, Zinc Citrate, Sodium Laurly Sulfate, Aroma, Cellulose Gum, Sodium Fluoride, Sodium Sacharin, PVM/MA Copolymer, Trisodium Phosphate, Sodium Hydroxide, Glycerin, Sodium Laureth Sulfate, Lecithin, Limonene, CI 74160, CI 77891.	Blue Covarine Pigment
Beverly Hills Formula Perfect White	Hydrated Silica, Aqua, Glycerin, Propylene glycol, Sodium Laurly Sulfate, Aroma, Tetrasodium Pyrophosphate, Potassium nitrate, Tricalcium Pyrophosphate, Pentasodium Pyrophosphate, Sodium fluoride, Charcoal powder, Cellulose gum, Citric acid, Sodium Sacharin, Sodium Hydroxide.	Activated charcoal
Parodontax (Complete Protection)	Sodium bicarbonate, Aqua, Glycerin, hydrated silica, Sodium Laurly Sulfate, Aroma, Xanthan Gum, Cocamidopropyl Betaine, Sodium Sacharin, Sodium Fluoride, Titanium dioxide, steviol glycosides, Limonene, CI 77891.	-

COLOR MEASUREMENTS

Measurements of the color change of teeth on the phantom model were performed under D65 lighting conditions, using the same spectrophotometer device (Vita Easy Shade V, Germany) in the first day and after 7, 14 and 28 days of brushing. Before the color measurement of the teeth, they were rinsed thoroughly with water for 10 seconds and dried with drying paper. The color measurements were made using the single point measurement mode (L^* , a^* , b^* values) and bleach shade mode of the spectrophotometer. If the bleach shade mode is SGU (Shade Guide Units), the measured color was set according to the VITA Bleachedguide 3D-MASTER (VITA Product Number B361). For this purpose, the whitening

index measured after the procedure should be subtracted from the bleach index before treatment. The difference was stated to correspond $\Delta SGUBG$ number. Color measurements were made 3 times from the center point of the core surface of each tooth while the teeth were on the phantom model. Color measurements of teeth were obtained from the vestibular surface for three measurements.

The mean value of the measured L^* , a^* and b^* parameters was calculated in the CIEDE2000 formula (ΔE_{00}) to detect color differences (15). Color changes values due to bleaching (ΔE_{00}) are interpreted as not effective (≤ 0.8), moderately effective ($> 0.8, \leq 1.8$), good efficacy ($> 1.8, \leq 3.6$), very good effectiveness ($> 3.6, \leq 5.4$) and excellent effectiveness (> 5.4) (14).

STATISTICAL ANALYSIS

Statistical analysis of the color change data in the study was performed using the SPSS 22.00 Program (SPSS Inc., Chicago, IL, USA). The color change data of toothpastes on the teeth were evaluated by one-way analysis of variance (ANOVA) and Tukey multiple comparison test ($p < 0.05$).

RESULTS

When we examine the whitening effectiveness of whitening toothpastes on the teeth with the CIEDE2000 formula (ΔE_{00}), the toothpaste containing activated charcoal showed statistically higher whitening effect in 7th, 14th and 28th days. There was no statistically significant difference between hydrogen peroxide containing toothpaste and traditional toothpaste ($p > 0.05$). The toothpaste containing blue covarine showed

statistically the least whitening effect ($p < 0.05$), (Table 2).

When we examined the color change of whitening toothpastes on the teeth according to shade guide units ($\Delta SGUBG$), toothpaste containing activated charcoal produced the highest whitening on the 7th, 14th and 28th days, while toothpaste containing blue covarine produced the least whitening ($p < 0.05$). There was no statistically significant difference between hydrogen peroxide containing toothpaste and traditional toothpaste ($p > 0.05$), (Table 3).

All toothpastes had medium whitening effect (according to ΔE_{00} and $\Delta SGUBG$) after 7 days of use. However, after 28 days, activated charcoal toothpaste showed very good whitening effect, while hydrogen peroxide, blue covarine and traditional toothpaste showed good whitening effect (Figure 1).

Table 2. Examination of the whitening effectiveness of toothpastes on teeth with the CIEDE2000 formula (ΔE_{00}).

Toothpaste/Color Change	7 days (ΔE_{00})	14 days (ΔE_{00})	30 days (ΔE_{00})
Colgate Optic White (Expert White)	3.05±0.2 ^A	3.18±0.2 ^A	3.42±0.1 ^A
Signal White Now CC	2.22±0.1 ^B	2.62±0.2 ^B	2.92±0.2 ^B
Beverly Hills Formula Perfect White	3.45±0.1 ^C	3.98±0.2 ^C	4.13±0.3 ^C
Parodontax (Complete Protection)	2.91±0.1 ^A	3.13±0.1 ^A	3.27±0.2 ^A
P	0.000	0.000	0.000

* A-C shows the statistical significance difference between lines ($p < 0.001$).

Table 3. Examination of the whitening effectiveness of toothpastes on teeth according to the shade guide units (SGU).

Toothpaste / Color Change	7 days ($\Delta SGUBG$)	14 days ($\Delta SGUBG$)	30 days ($\Delta SGUBG$)
Colgate Optic White (Expert White)	3.13±0.6 ^A	3.75±0.5 ^A	4.25±0.5 ^A
Signal White Now CC	2.25±0.5 ^B	2.50±0.5 ^B	3.25±0.5 ^B
Beverly Hills Formula Perfect White	4.30±0.5 ^C	5.50±0.5 ^C	6.50±0.5 ^C
Parodontax (Complete Protection)	3.00±0.5 ^A	3.63±0.5 ^A	4.00±0.5 ^A
P	0.000	0.000	0.000

* A-C shows the statistical significance difference between lines ($p < 0.001$).

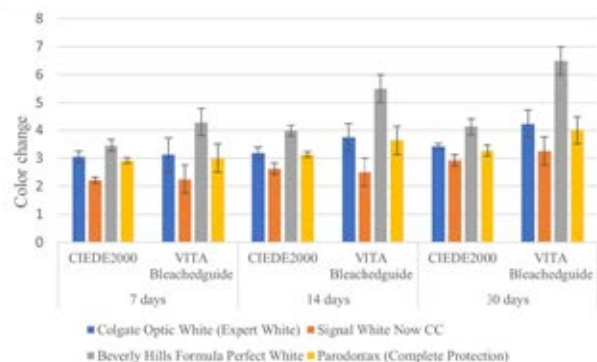


Figure 1. Whitening effectiveness of toothpastes on teeth according to the CIEDE2000 formula and VITA Bleach guide (SGU).

DISCUSSION

In our study, the effect of whitening toothpastes on the color change of teeth was examined for a period of 28 days. Our null hypothesis was rejected as toothpastes had different whitening effects on teeth.

Traditionally, the tooth whitening method is done with two mechanisms: bleaching or removing the external stain. Although whitening of tooth color is accomplished using hydrogen or carbamide peroxides, peroxide-based bleaching can cause adverse biological reactions. Also, peroxide-based bleach needs to be administered or prescribed by a dentist (16). In recent years, different products have been launched, including toothpastes and mouthwashes, which are readily available in pharmacies and supermarkets, to provide a practical, fast, easy and cost-effective whitening effect. Whitening toothpastes used in daily oral hygiene routine that does not require supervision or indication by a dentist, have gained popularity.

Visual and instrumental methods are preferred to measure the color changes in teeth and materials. The spectrophotometer was used in this study because it provides accuracy, repeatability and objective values (17). In 2001, an updated new formula CIEDE2000 (ΔE_{00}) was introduced and proposed by the CIE (15). CIEDE2000 (ΔE_{00})

formula was preferred in our study as Gómez-Polo *et al.* (18) indicated in their study that it is more sensitive in measuring color changes than CIELAB (ΔE_{ab}) formula.

Perceptibility and acceptability threshold values in color changes indicate whether the difference is acceptable (19). For these reasons, two different methods were used to fully explain the whitening effectiveness. A recent study found the perceptibility and acceptability's values of thresholds of 0.8 and 1.8, respectively, by using CIEDE2000 system. Also whitened index PT value was stated as ($\Delta SGUBG$: 1.4) and AT value as ($\Delta SGUBG$: 3.2) (14).

The effectiveness of toothpastes in cleaning is related to the abrasives in them. Abrasive particles help to effectively remove external color pigments and prevent staining (20,21). Despite the abrasives in whitening toothpastes, it is stated that toothbrush/toothpaste should be used together in order for the paste to be effective (22,23). In addition, special abrasives and chemicals can be added to the toothpaste to increase stain removal and speed up the process (24).

The main whitening effect in toothpastes is based on the interaction between abrasive substances and peroxide compounds, surfactants, polyphosphates, and enzymes (25-27). The external stain on the tooth surface can be removed through something abrasive in the toothpaste, while the internal stain can be removed through oxidation based on HP (hydrogen peroxide) or CP (carbamide peroxide) products that help alleviate the internal discoloration of the tooth (28). Basically, the whitening process takes place with the conversion of peroxides to free radicals. In order to take advantage of this feature of hydrogen peroxide, it has been added to some whitening toothpastes in low concentrations (28). According to the literature, the discoloration of teeth can be removed effectively with HP and CP (29). In our

study, toothpaste containing hydrogen peroxide showed a whitening effect on the teeth after 7 days of use.

The whitening effect of toothpastes containing blue covarine instead of abrasive leaves a thin, translucent blue layer on the tooth enamel, changing the perception of yellowish discoloration in the teeth. In the blue spectrum, it creates the visual appearance of whiter and brighter teeth by shifting the network color to white as opposed to yellow. Studies provide conflicting reports on the effectiveness of optical effect pigments in teeth whitening. There are studies stating that this pigment has an effect on color change, (30-32) while some other studies show that it has no effect on color change (33,34). In their clinical study, Schlafer *et al.* (16) stated that toothpaste containing blue covarine did not show the whitening effect on teeth in a single use. In our study, toothpaste containing blue covarine showed less whitening effect than other toothpastes, although it had a whitening effect above the AT value on the teeth after 7 days of use.

The whitening effect of the activated charcoal used in toothpastes is based on its high adsorption capacity of chromophores and color pigments. Activated charcoal is highly porous and has an extremely high surface area (9) and provides effective and gradual cleaning of the tooth structure. Vaz *et al.* (8) conducted a study on the discoloration of whitening toothpastes on cattle incisors and found that there was no difference between toothpastes containing active coal, microparticles and hydrogen peroxide. Palandi *et al.* (35) stated that activated charcoal powder does not increase discoloration when combined with normal and whitening toothpastes. In our

study, unlike the studies in the literature, the best whitening effect on teeth was seen in toothpaste containing activated charcoal. While bovine incisors were used in the studies in the literature, (8,35) human incisors were used in our study. The nature of the sample (human versus animal teeth) might be related with the difference in the results.

In our study, all whitening toothpastes tested *in vitro* had a whitening effect on teeth. Although the whitening efficiency of the toothpaste without bleaching particles was less than the toothpaste containing activated charcoal, it was at the same level as the toothpaste containing hydrogen peroxide and blue covarine.

Toothpastes containing activated charcoal showed better effectiveness in whitening teeth than toothpastes containing blue covarine and hydrogen peroxide. However, it is stated in the literature that toothpastes containing activated charcoal increase the roughness of the enamel surface (35). In addition, individuals consume different beverages in daily life. For this reason, it is considered that it would be beneficial to conduct clinical research in order to fully understand the effectiveness of the toothpastes containing bleaching on teeth.

CONCLUSION

All toothpastes showed a whitening effect on the teeth after one week (7 days) of use while activated charcoal containing whitening toothpaste produced the best whitening effect after 28 days. After 28 days of daily use, the active charcoal containing toothpaste showed “very good effectiveness” regarding whitening the tooth, while hydrogen peroxide, blue covarine and traditional toothpaste showed “good effectiveness”.

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