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BASIC RESEARCH

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Effects of Whitening Dentifrices on the Enamel Color,

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Efecto de los productos de clareamiento sobre el color del esmalte,

la rugosidad de la superficie y la morfología

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ABSTRACT: The aim of this study was to evaluate the whitening and abrasion effects of dentifrices containing different whitening agents on artificially stained and unstained enamel. 160 human dental enamel discs were distributed into four groups according to the type of dentifrices applied on them (n=40): Group I, non-whitening dentifrice (control group); Group II, dentifrice containing charcoal; Group III, dentifrice containing blue covarine; Group IV, dentifrice containing hydrogen peroxide. Half of the specimens in each group were immersed in coffee solution. Color measurements were evaluated from stained and unstained specimens before and after brushing by a spectrophotometer. The surface roughness of each unstained specimen was analyzed using a profilometer after the brushing and bleaching. The surface of one randomly selected specimen from each group was observed using a scanning electron microscope (SEM). Statistical analysis of the color change was performed using the Kruskal-Wallis test. The one-way ANOVA was used to evaluate surface roughness. Group III provided significantly higher recovery on Δ E00 values than other groups on the unstained enamel (p<0.05). Group IV showed significantly the highest Δ E00 values on stained enamel (p<0.05) and also Group IV showed the highest surface roughness values (p<0.05), SEM revealed a more irregular surface in groups III and IV. It can be stated that dentifrice containing blue covarine is both an effective and a safe way to provide whiter teeth with routine home tooth brushing.

KEYWORDS: Whitening dentifrices; Surface roughness; Scanning electron microscope; Tooth whitening; Tooth discoloration.

RESUMEN: El objetivo de este estudio fue evaluar los efectos de blanqueamiento y abrasión de los dentífricos que contienen diferentes agentes blanqueadores en el esmalte teñido y no teñido artificialmente. Se distribuyeron 160 discos de esmalte dental humano en cuatro grupos según el tipo de dentífrico aplicado sobre ellos (n=40): Grupo I, dentífrico no blanqueador (grupo control); Grupo II, dentífrico que contiene carbón vegetal; Grupo III, dentífrico que contiene azul; Grupo IV, dentífrico que contiene peróxido de hidrógeno. La mitad de los especímenes de cada grupo se sumergieron en una solución de café. Las mediciones de color se evaluaron a partir de especímenes teñidos y no teñidos antes y después del cepillado con un espectrofotómetro. La rugosidad de la superficie de cada muestra sin teñir se analizó utilizando un perfilómetro después del cepillado y del clareamiento. La superficie de un espécimen seleccionado al azar de cada grupo se observó utilizando un microscopio electrónico de barrido (MEB). El análisis estadístico del cambio de color se realizó utilizando la prueba de Kruskal-Wallis. Se utilizó el ANOVA para evaluar la rugosidad de la superficie. El grupo III proporcionó una recuperación significativamente mayor en los valores de Δ E00 que otros grupos en el esmalte no teñido (p<0.05). El Grupo IV mostró significativamente los valores más altos de ΔΕ00 en el esmalte teñido (p<0.05) y también el Grupo IV mostró los valores más altos de rugosidad superficial (p<0.05), el análisis en MEB reveló una superficie más irregular en los grupos III y IV. Se puede afirmar que el dentífrico que contiene azul es una forma efectiva y segura de proporcionar dientes más claros con el cepillado de rutina en el hogar.

PALABRAS CLAVE: Dentífricos blanqueadores; Rugosidad de la superficie; Microscopio electrónico de barrido; Blanqueamiento dental; Decoloración dental.

INTRODUCTION

Whiter teeth are considered an excellent esthetic model in contemporary society (1). Thus, bleaching is a common esthetic treatment in dentistry. Dental bleaching is a conservative and effective treatment for patients (2). However, this procedure is costly and should be performed under the supervision of a dentist (3). These circumstances led consumers to add whitening agents to dentifrices which are easier to apply and more affordable. These agents can dissolve surface stains and whiten the teeth (4).

Charcoal is used as a cleaning agent in many different cultures and parts of the world. Nowadays, charcoal-containing dentifrices produced to whiten teeth are widely available in supermarkets and pharmacies (5).

There are dentifrices containing a blue pigment called blue covarine on the market. These dentifrices apply the blue pigment into the enamel structure during daily brushing procedures, which changes the tooth color from yellow to blue. Several studies stated that this change in blue and yellow color makes teeth whiter (6,7,8).

There are studies evaluating the effectiveness of various whitening dentifrices (4-10). However, there are few studies investigating whitening dentifrices' effects on human enamel (2,9,10), which has remained under-researched.

This study aimed to compare the whitening and abrasion effects of dentifrices that contain charcoal, blue covarine, and hydrogen peroxide with a non-whitening dentifrice on stained and unstained enamel. The first hypothesis to be tested was

that whitening dentifrices have more whitening efficiency on stained and unstained enamel than non-whitening dentifrice. The second hypothesis was that brushing with whitening dentifrices increases enamel surface roughness much more than non-whitening dentifrice.

MATERIALS AND METHODS

PREPARATION, STAINING AND BRUSHING OF THE SPECIMENS

The tooth collection and study methodology of this study were reviewed and approved by the ethical research committee of the university (protocol #2021/13). One hundred and sixty enamel discs with a thickness of 2.5mm and a diameter of 5mm were obtained by cutting the buccal surface of human incisors with a watercooled diamond saw. Subsequently, all the specimens were polished using silicon carbide papers to achieve a standard surface. All specimens were placed in acrylic molds and randomly divided into four groups (n=40). Group I, brushing with dentifrice-containing charcoal; group III, brushing

with a dentifrice-containing blue covarine pigment; group IV, brushing with a dentifrice-containing hydrogen peroxide. Information of products, components, and what manufacturers used in the current study, are presented in Table 1.

Half of the specimens in each group (n=20) were immersed in coffee solution (2g coffee powder per 200ml boiled water) for 14 days; the other half were left unstained. This immersion time simulates about two years of daily coffee intake (11). During the staining process, the coffee solutions were stirred and changed daily. After completion of the staining process, the stained specimens were washed and stored in distilled water at 37°C for 24 hours.

In this study, an electric toothbrush (Oral B Genius X 20000, Germany) was used. Individuals brush their teeth twice a day for two minutes (240 seconds) each, so an average of 8 seconds is required for brushing for each tooth per day (12). Therefore, all specimens of groups I, II, III, and IV were brushed for four minutes with slurries prepared with dentifrices and distilled water (1:1) using an electric toothbrush with a standardized force of 2 N to simulate 1-month-brushing (13).

Table 1. The groups, products, manufacturers, and product compositions.

Product	Manufacturer	Composition	Tooth whitening technology
Group I (Control) Colgate Maximum anticaries protection	Palmolive Company, New York, USA	1500 ppm of fluoride, calcium carbonate, sodium lauryl sulfate, sodium saccharin, tetrasodium pyrophosphate, sodium silicate, polyethylene glycol, sorbitol, carboxymethyl cellulose, methylparaben, propylparaben, aromatic composition and water; contains sodium monofluorophosphate	
Group II Colgate Optic White (Charcoal)	Palmolive Company, New York, USA	Aqua, sorbitol, hydrated silica, PEG-12, tetrasodium pyrophosphate, sodium laurly sulfate, aroma, potassium hydroxide, cellulose gum, phosphoric acid, cocamidopropyl betaine, sodium fluoride, sodium sacharin, xanthan gum, charcoal powder, mica, limonene	Activated charcoal
Group III Signal White Now CC	Unilever, UK	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	Blue covarine pigment
Group IV Colgate Optic White (Expert White)	Palmolive Company, New York, USA	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

ROUGHNESS AND COLOR MEASUREMENTS

The surface roughness values (Ra) were evaluated only from the unstained groups before and after the brushing procedure. The data were analyzed using a profilometer (Surftest SJ 201, Mitutoyo Co, Kawasaki, Japan) which had a stylus with a 5µm tip radius and a 90° tip angle. The cut-off value for surface roughness was 0.8mm, and the traversing distance of the stylus was 4.0mm at a constant speed of 0.5µ/s and measuring force of 4µN. Three readings were taken from each specimen's surface in different directions; then, the mean values were obtained.

Color measurements of the specimens were evaluated from all the stained (after immersion with coffee and after brushing with dentifrices) and unstained groups (before and after brushing with dentifrices) by a spectrophotometer (Vita Easyshade, VITA Zahnfabrik, Germany) under standardized lighting conditions (CIE D65 illumination). Before each measurement, the spectrophotometer was calibrated following the manufacturer's instructions.

Three measurements were made in the central areas and the mean values of L*, a*, and b* parameters were applied to the CIEDE2000 formula (Δ E00) to determine the whitening values on the stained and unstained enamel (14).

SCANNING ELECTRON MICROSCOPE (SEM) OBSERVATION

One specimen from each unstained group was selected randomly for SEM analysis to observe

surface morphology after brushing. The selected specimens were dried in a dehumidifier with silica gel for 72 hours, metalized with gold, and observed with a scanning electron microscope (QuantaTM 450 FEG, FEI, Oregon, USA) under ×5000 magnification for qualitative analysis of the surface.

STATISTICAL ANALYSIS

SPSS 19.0 statistics program (SPSS Inc.; Chicago, Illinois) was used for statistical analysis. Shapiro-Wilk test was used for the test of normality. The Kruskal-Wallis test was used to compare the color change values (Δ E00) of the groups because the color evaluation data presented nonparametric distributions. One-way analysis of variance ANOVA test was used for independent group comparisons of surface roughness changes followed by Tukey test for posthoc comparison. All statistical comparisons with a p-value below 0.05 were assumed as statistically significant.

RESULTS

Color change comparison values (Δ E00) are presented in Table 2. Among the unstained groups, group III provided significantly higher recovery value on Δ E00 values (p<0.05). The least improvement occurred with groups I, II and IV. No statistically significant difference was evaluated between them (p>0.05). Among the stained groups' Δ E00 values, no statistically significant difference was evaluated between groups I, II, and III. Besides, these groups' whiteness efficiency was evaluated statistically lower than group IV.

Table 2. Comparison of color change (Δ E00) values of unstained and stained specimens before and after brushing with dentifrices.

Groups	Unstained Specimens (ΔΕ00 ± SD)	Stained Specimens (ΔΕ00 ± SD)
Group I (non-whitening dentifrice)	0.78 ± 0.07^{a}	0.82 ± 0.09^{A}
Group II (Dentifrice containing charcoal)	1.12 ± 0.08^{a}	1.18 ± 0.12^{B}
Group III (Dentifrice containing blue covarine)	1.49 ± 0.14^{b}	1.22 ± 0.17 ^A
Group IV (Dentifrice containing Hydrogen peroxide)	1.18 ± 0.08^{a}	1.7 ± 0.12^{B}

The values are mean \pm SD, SD, standard deviation, Different capital and lower case letters in the same column represent statistical significant difference between groups.

Surface roughness values after brushing are presented in Table 3. Group IV showed the highest

mean values with a significant difference (p<0.05). No statistically significant differences were evaluated between groups I, II and III (p>0.05).

Percentage rates of surface roughness changes are presented in Table 3. The percentage rate of group IV was statistically higher than other groups (p<0.05). The lowest percentage increase was recorded in the group I, but it was not statistically different from groups II and III (p>0.05).

The representative SEM images of each group before and after brushing are shown in Figure 1. A relatively smooth surface and a few fine scratches were observed on the surface of group I, and a similar surface appearance with some fine scratches were observed on the surface of group III. Greater alteration in surface morphology manifested as irregularities in groups II and IV.

Table 3. Comparison of mean and percentage rates of surface roughness of surface roughness (Ra) on unstained groups.

Groups	(Ra ± SD) Before brushing	(Ra ± SD) After brushing	Rate % (before- after brushing) x100
Group I (non-whitening denti- frice)	0.184 ± 0.08	0.207 ± 0.03^{a}	%12,5 ^A
Group II (Dentifrice containing charcoal)	0.193 ± 0.06	0.221 ± 0.07^{a}	%14,5 ^A
Group III (Dentifrice containing blue covarine)	0.198 ± 0.07	0.223 ± 0.03^{a}	%12,6 ^A
Group IV (Dentifrice containing hydrogen peroxide)	0.197 ± 0.04	0.242 ± 0.03^{b}	%22,8 ^B

Different capital and lower case letters in the same column represent statistical significant difference between groups.

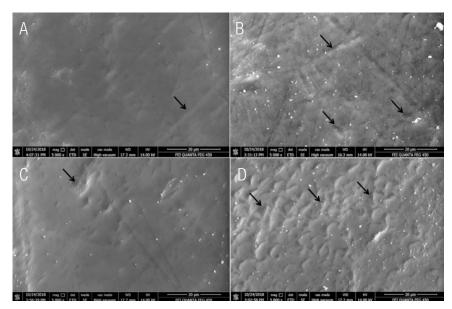


Figure 1. Representative images of enamel surface obtained by SEM after brushing; A (group I, non-whitening toothpaste), B (group II, dentifrice containing charcoal), C (group III; dentifrice containing blue covarine), D (group IV, dentifrice containing hydrogen peroxide) at 5000x magnification. (Black arrows indicate the irregularities on the enamel surface.

DISCUSSION

Four groups were tested in this study: non-whitening dentifrice (control), dentifrice-containing charcoal, dentifrice-containing blue covarine, and dentifrice-containing hydrogen peroxide. Obtained results showed that blue covarine was the most effective on unstained enamels and the hydrogen peroxide was the most effective on stained enamels for color improvement. The findings obtained in this study are consistent with Franco *et al.*'s findings. (13) According to the aforementioned findings, the first hypothesis was accepted.

Blue covarine in whitening dentifrices provides an optical effect that deposits a semitransparent, thin blue layer on the enamel and modifies the perception of yellowish discoloration in teeth. Gerlac *et al.* (15) stated that blue opposes yellow in the color spectrum and shifts the net color towards white, so the teeth appear whiter and brighter (7). This study's findings supported the findings of Tao *et al.* (10) and Bergesch *et al.* (16), who pointed out

the immediate and good efficacy of blue covarine. However, in contrast with our results, and studies by Torres *et al.* (17) and Horn *et al.* (18) reported no whitening effect of blue covarine. Although blue covarine showed better results regarding unstained enamel whitening, it showed similar effects on the control and the dentifrice-containing dentifrice-containing charcoal on the coffee-stained enamel surfaces, consistent with the study of Aydin *et al.* (12).

Extrinsic stains on the enamel surface could be removed by abrasive agents contained in whitening dentifrices, but intrinsic colorations need to be removed through the conversion of hydrogen peroxide or carbamide peroxide to free radicals. Some whitening dentifrices contain various concentrations of hydrogen peroxide to utilize the oxidative function of this agent (19). One of the whitening dentifrices used in the current study contains polyphosphates and hydrogen peroxide (Colgate Optic White). Thus, it was expected that it would be superior to the others in terms of whitening. Accor-

ding to findings obtained in this study, containing dentifrice-containing hydrogen-peroxide showed similar results to non-whitening dentifrice and charcoal containing dentifrice on the unstained enamel. On the contrary, the findings showed that the color improvement of the dentifrice-containing hydrogen peroxide showed better results than the dentifrices containing charcoal, blue covarine and non-whitening on the stained enamel. It was thought that this is due to the abrasive effect of the dentifrice, which outperforms its chemical effects. These findings are consistent with Alshara et al. (20) and Lippert et al. (21), who have pointed out that hydrogen peroxide is unstable in low concentrations (approximately 1% by weight), and the action time is inherently low during brushing.

The whitening efficacy of charcoal is due to its high capacity to retain and adsorb chromophores in the oral environment. This study concluded that charcoal was not effective for whitening stained and unstained enamel. Although limited information is available about clinical studies that may guide the use of charcoal on dental whitening. our results supported the findings of Franco et al. (13), who reported that charcoal was not effective for dental whitening. On the contrary, a study evaluated the whitening efficacy of charcoalcontaining dentifrice using the VITA Classical Shade Guide and demonstrated a small whitening effect (22). The evaluation method used in that study is subjective, and we think that this subjectivity may be why the results are different from the results in our study (23).

The perceptibility threshold (PT) and acceptability threshold (AT) values for color differences are prominent reference factors to evaluating the color stability of materials. Given the parameters established by Paravina *et al.* (24), the acceptability threshold is a Δ E00 of 1.8, and the perceptibility threshold is a Δ E00 of 0.8. According to our results, none of the groups showed statistically detectable color change. On the other hand,

all the dentifrices except the control group used in this study reduced the color change in both stained and unstained enamel below the AT value. Therefore, the color change promoted by all whitening dentifrices used in this study was clinically perceptible, although not statistically detectable.

It was also evaluated whether the abrasion efficiency of the non-whitening dentifrice and the whitening dentifrices containing charcoal, blue covarine, and hydrogen peroxide cause alteration in the enamel surface roughness. The surface structure of charcoal is porous, so it may affect the properties of the enamel surface while providing effective whitening to the teeth (25). However, no statistical difference was found between the non-whitening dentifrice, the dentifrice-containing charcoal and the dentifrice-containing blue covarine's abrasion effects on the enamel in this study. On the other hand, the dentifrice-containing hydrogen peroxide showed significantly higher surface roughness than the other groups. These results consistent with a previous study which demonstrates that dentifrice-containing hydrogen peroxide caused the highest increase in a mean difference of surface roughness than the other whitening dentifrices (9). According to these results, the second hypothesis was also accepted.

A clinical study demonstrated that the surface roughness value of $0.25\text{-}0.5\mu\text{m}$ could be detected by the patient's tongue (26). Our findings showed that none of the dentifrices used in this study created an enamel surface that would disturb the user.

In this study, it would be more meaningful to compare the percentage change in surface roughness since the surface roughness values before the brushing was different. The dentifrice with 1% hydrogen peroxide had the highest increase in percentage rate difference of surface roughness and was evaluated significantly higher than the other groups. This is thought to be because this

dentifrice contains abrasive particles and hydrogen peroxide.

Stout (27) defined that the combination of qualitative data with a microscope and quantitative measurement ensures characterization of the surface. Therefore, in this study, besides the profilometric analyses, SEM analysis was performed from one specimen in each group to observe the morphological changes on the enamel surface caused by whitening dentifrices. The control and blue covarine group revealed a smooth surface, while charcoal and hydrogen peroxide caused few pores and some scratches that became more obvious on the enamel surface. These observations were found similar to the study of Shamel *et al.* (9).

A limitation of this study is that surface hardness was not evaluated after brushing. The effects of whitening dentifrices can be evaluated on the enamel surface hardness in future studies. Another limitation is that the specimens were only immersed with coffee for staining. In addition, contrary to *in-vitro* studies, colorants can be diluted with saliva in the oral environment. Therefore, further *in-vivo* studies are needed to detect the whitening effects of whitening dentifrices on the enamel surface immersed with different colorants.

CONCLUSION

Within the limitations of this study, it can be stated that dentifrice-containing blue covarine is an effective and safe way to provide whiter teeth with routine tooth brushing. Also, the dentifrice-containing hydrogen peroxide showed a promising whitening effect on the stained enamel. We think the findings obtained in this study are useful to guide patients and dental clinicians using and selecting dentifrices that contain whitening agents.

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No financal affiliation releated to this study.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTION STATEMENT

Conceptualization and design: A.H.

Literature review: A.H. and E.H.

Methodology and validation: A.H. and E.H.

Formal analysis: A.H.

Investigation and data collection: A.H. and E.H.

Resources: A.H. and E.H.

Data analysis and interpretation: E.H.

Writing-original draft preparation: E.H.

Writing-review & editing: A.H.

Supervision: A.H.

Project administration: A.H.

Funding acquisition: A.H. and E.H.

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