Effects of Different Over – the - Counter Whitening Products on the Microhardness, Surface Roughness, Color and Shear Bond Strength of Enamel

Učinak komercijalnih proizvoda za izbjeljivanje na mikrotvrdoću, hrapavost površine, boju i smičnu čvrstoću veze cakline i ispuna

Introduction

Over the last decade, an increased interest has been observed in the field of tooth whitening since esthetic dentistry received more attention. Tooth whitening is gained growing popularity among the patients since it has been considered a conservative, safe, effective and minimally invasive method (1-4).

Today, tooth whitening methods range from professionally applied in-office whitening, professionally prescribed home whitening, nonprescription over-the-counter (OTC) whitening to the do-it-yourself (DIY) application (5-9).

Professionally prescribed home whitening is the most preferred whitening method (4). Dispensed to the patients

| Author Keywords: | Tooth Whitening; OTC Products; Microhardness; Surface Hardness; Shear Bond Strength |
|------------------|---------------------------------------------------------------------------------------|
| MeSH Terms:      | Tooth Bleaching; Tooth Bleaching Agents; Dental Enamel; Hardness Tests; Surface Properties; Color |

Abstract

Objective: The purpose of this in vitro study was to evaluate the effects of four over-the-counter (OTC) whitening products on the microhardness, surface roughness, color, shear bond strength (SBS) and surface characteristics of human enamel compared with a product used for dentist-supervised home whitening. Material and methods: Seventy eight enamel specimens allocated into 6 groups (n=13): 1-Opalescence PF 10% (OP) dentist prescribed home whitening product, 2-Opalescence Go prefilled tray (PT), 3-Opalescence Whitening Toothpaste (WT), 4-Listerine Healthy White whitening mouth rinse (WMR), 5-Cavex Bite&White whitening pen (WP) and 6- no treatment (Con). The microhardness (VHN), surface roughness (Ra) and color of the specimens were measured (T). The specimens were then subjected to whitening protocols for 14 days (T) followed by artificial saliva storage for 14 days (T). The measurements were repeated at T1 and T2. The SBS test was done after the application of 35% phosphoric acid (Scotchbond Universal Etchant), followed by a universal adhesive (G-Premio Bond) and a micro hybrid/universal resin composite (Essentia) into a Teflon tube attached to the enamel surface (p<0.05). Surface morphologies of the enamel surfaces were examined by SEM. p value was set at 0.05 Results Application of OP, PT and WP decreased the microhardness of enamel specimens (p<0.05) whereas, no significant changes were seen in the microhardness of enamel specimens treated with WT and WMR (p>0.05). Ra values of enamel specimens increased with the application of OP, PT and WP (p<0.05); whereas no changes were observed after the applications of WMR and WP (p>0.05). OP, PT, WMR, and WP changed the color of the enamel(p<0.05). There were not any significant differences among the SBSs of the groups, apart from OP applied enamel specimens. OP showed the least SBS values (p=0.001). SEM observations revealed smooth enamel surfaces. Conclusions: The whitening products affected the microhardness, surface roughness, color of enamel differently. Only OP decreased the SBS of the enamel.

Table 1: Whitening products and their effects on enamel

| Whitening Product | Microhardness (VHN) | Surface Roughness (Ra) | Color |
|-------------------|----------------------|------------------------|-------|
| OP                | -5%                  | -5%                    | -5%   |
| PT                | -5%                  | -5%                    | -5%   |
| WT                | -5%                  | -5%                    | -5%   |
| WMR               | -5%                  | -5%                    | -5%   |
| WP                | -5%                  | -5%                    | -5%   |
| Con               | -5%                  | -5%                    | -5%   |

Introduction

Danas je raspon metoda izbjeljivanja zuba velik – od profesionalnih koje se obavljaju u ordinaciji, onih koje se pro-
vode kod kuće, zatim komercijalnih proizvoda koji se mogu kupiti u ljekarnama (engl. over-the-counter – OTC) pa sve do
metoda uradi-sam (5 – 9).
and closely monitored by the dentists, this technique is done by using 10% carbamide peroxide (CP) in a tray that is worn for at least two weeks. This concentration is well accepted due to its safety, excellent esthetic results, low incidence of side effects and increased effectiveness of the whitening (9, 10).

The satisfactory results achieved with professionally prescribed home whitening systems have led the developments of OTC products. OTC products were first marketed by the year 2000, with their lower cost, availability, easy access and application (9, 11, 12).

Currently, there have been a huge number of OTC products available with capability of whitening within 1-4 weeks. These whitening products are in the form of gels, mouth washes, pens, gums, toothpastes, films or paint-ons with lower concentrations of hydrogen peroxide (HP), and are sold at pharmacies, supermarkets and over the Internet (9, 12-15). These products may have potential side effects (14-16). The influence of the whitening agent to the oral tissues is important due to the oxidizing process which occurs during the whitening procedure. Several studies have reported an increase in the porosity, over-etched appearance, loss of prismatic structure and calcium and alterations in the organic content of enamel (3, 4, 6, 8, 16, 17).

However, there have not been a sufficient number of reports providing a scientific background for these whitening products. Since there has been a huge variety of new products and lack of evidence about their efficacy, the aim of this in vitro investigation was to evaluate the effects of four OTC products, that is, a prefilled tray, a whitening tooth paste, a whitening mouth rinse and a whitening pen, on the microhardness, surface roughness, color, shear bond strength (SBS), and surface characteristics of human enamel as compared with a whitening gel (10% CP) used for dentist-supervised home whitening. The null hypothesis was that there would be no significant differences among the tested whitening products with regard to (1) microhardness, (2) surface roughness, (3) color, and (4) SBS to enamel.

Material and methods

The whitening products tested are shown in Table 1 and the experimental procedure is illustrated in Figure 1.

Ethical aspects

The study protocol was approved by the non-interventional Ethics Committee of the University (2020/08-41).

Sample size calculation:

One-way ANOVA-type power analyses were done to calculate the estimated sample size using G*Power package (version 3.1, Heinrich-Heine Dusseldorf University, Dusseldorf, Germany). The selected parameters were: 95% confidence interval, 80% power and 0.50 effect size. Twelve specimens per group were calculated.

Specimen Preparation

One hundred and twenty human permanent maxillary central incisors obtained from the pool of extracted teeth at

Izbeljivanje kod kuće koje kontroliraju doktori dentalne medicine najpopularniji je način izbjeljivanja (4). Sredstvo se daje pacijentu koji se njime koristi uz profesionalnu kontrolu. Ta tehnika koristi se 10-postotnim karbamidnim peroksidom (CP) u udlazi koja se nosi najmanje dva tjedna. Koncentracija je prihvatljiva jer je sigurna, estetski rezultati su odlični, incidencija nuspojava je niska i povećana je učinkovitost izbjeljivanja (9, 10).

Zadovoljavajući rezultati koji se postižu tim načinom izbjeljivanja potaknuli su komercijalnu proizvodnju, pa se OTC proizvodi mogu kupiti u ljekarnama. Počeli su se reklamirati 2000. godine, a prednost im je niska cijena, dostupnost, jednostavna i pristupačnost u primjeni (9, 11, 12).

Trenutačno se može nabaviti niz OTC proizvoda koji izbjeljuju zube između jednoga i četiri tjedna. Dostupni su u obliku gelova, tekućina za ispiranje, olovaka, žvakača guma, zubnih pasti, premaza te lakova s niskim koncentracijama vodika peroksida (HP), a prodaju se u ljekarnama, supermarketima ili na internetu (9, 12 – 15). Svi ti proizvodi imaju potencijalne nuspojave (14 – 16). Utjecaj sredstva za izbjeljivanje na oralna tkiva važan je zbog procesa oksidacije tijekom izbjeljivanja. U nekoliko istraživanja opisano je povećanje po-roznosti cakline, pretjerano najetkani izgled cakline, gubitak prizmatske strukture i kalcija te promjene u organskome sadstvu cakline (3, 4, 6, 8, 16, 17).

Nema dovoljno tekstova koji bi dali znanstvenu podlogu svim tim proizvodima za izbjeljivanje. Budući da ih je mnogo, a nedostaju podatci o učinkovitosti, cilj ovog istraživanja in vitro bio je procijeniti učinke četiriju OTC proizvoda – unaprijed pripremljene udlage, Zubne pasti za izbjeljivanje, tekućine za ispiranje koja izbjeljuje i olovke za izbjeljivanje – na mikrotvrdoću, hravapost površine, boji i snagu vezivanja (engl. shear bond strength – SBS) te površinska obilježja ljudske cakline u usporedbi s djelovanjem gela za izbjeljivanje (10% CP) koji se koristi pod nadzorom doktora dentalne medicine. Nulta hipoteza glasila je da neće biti značajnih razlika između ispitivanih proizvoda kada je riječ o (1) mikrotvrdoći, (2) hravaposti površine, (3) boji te (4) SBS-u cakline.

Materijal i metode

Ispitvana sredstva za izbjeljivanje prikazana su u tablici 1., a eksperimentalni postupak na slici 1.

Etički aspekti

Protokol istraživanja odobrilo je Sveučilišno etičko po- vjerenstvo za neintervencijsku kliničku istraživanja (2020/08-41).

Izračun veličine uzorka

Za izračunavanje procijenjene veličine uzorka primijenjena je jednosmerna ANOVA analiza snage s pomoću G*Power paketa (verzija 3.1, Heinrich-Heine Universität, Düsseldorf, SR Njemačka). Odabrani parametri bili su: 95-postotni intervall pouzdanosti, 80-postotna snaga i učinak veličine 0,50. Dobiven je rezultat od 12 uzoraka po skupini.

Priprema uzoraka

Stotinu dvadeset gornjih trajnih središnjih sjekutića (in-ciziva), dobivenih od Zavoda za oralnu kirurgiju Fakulteta
### Table 1: Whitening products used in the study

| Whitening Product/Proizvod za izbjeljivanje | Manufacturer/Proizvođač | Composition/Sastav | Daily Use/Total Number of Treatment Days/ Dnevno korištenje/broj dana korištenja |
|-------------------------------------------|--------------------------|-------------------|---------------------------------------------|
| Opalescence PF 10 % (Dentist-supervised home whitening (OP)/Izbjeljivanje kod kuće koje kontroliira doktor) | Ultradent Products, South Jordan UT, USA | 10% Carbamide Peroxide, Polyacrylic acid, 0.3% Sodium fluoride, 3% Sodium hydroxide | 8 hours a day/14 days/8 sati dnevno/14 dana |
| Opalescence Go (Prefilled Tray) (PT)/Unaprijed pripremljena udraga | Ultradent Products, South Jordan UT, USA | 6% Hydrogen Peroxide, sodium hydroxide, potassium nitrate, sodium fluoride | 30 minutes a day/14 days/30 minuta dnevno/14 dana |
| Opalescence (Whitening Toothpaste) (WT)/zubna pasta za izbjeljivanje | Ultradent Products, South Jordan UT, USA | Sodium fluoride, glycerin, water (aqua), silica, sorbitol, xylitol, flavor, poloxamer, sodium lauryl sulfate, carbomer, FD&C Blue #1 (CI 42090), FD&C Yellow #5 (CI 19140), sodium benzoate, sodium hydroxide, Sparkle (CI 77019, CI 77891), sucralose, xanthan gum. | 2 minutes twice daily/14 days/2 minute 2 puta dnevno/14 dana |
| Cavex Bite & White (Whitening Pen) (WP)/olovka za izbjeljivanje | Cavex, Haarlem, Holland | 6% Hydrogen Peroxide, polyethylene glycol, PVP peroxide, glycerin, Peppermint oil | 30 minutes a day/14 days/30 minuta dnevno/14 dana |
| Listerine Healthy White (Whitening Mouth Rinse) (WMR)/tekućina za izbjeljivanje | Johnson & Johnson Consumer Inc., New Jersey, USA | Water, 8% alcohol, 2% Hydrogen Peroxide, sodium phosphate, poloxamer 407, sodium lauryl sulfate, sodium citrate, mint aroma, menthol, eucalyptol, Sodium saccharin, sucralose | 2 minutes twice daily/14 days/2 minute 2 puta dnevno/14 dana |

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**Figure 1** Study protocol

**Slika 1.** Protokol istraživanja
Oral Surgery Department of the Dental School were examined by a stereomicroscope at 10X magnification (American Optical, Buffalo, NY, USA), and 78 teeth were selected for the study in line with the Human Tissue Act procedures. The teeth with similar shade, size and surface texture were employed. The roots were cut 1 mm below the cemento-enamel junction with a diamond saw attached to a sectioning machine (Isomet 1000 Precision Diamond Saw, Buehler Ltd., Illinois, USA). Debris was removed by a curette and air or water jet from the crowns and then stored in 0.1% thymol solution at 5°C. The crowns were embedded in acrylic resin molds (Integra, Ankara, Turkey) leaving the buccal surfaces exposed and then the enamel surfaces were polished by silicon carbide papers (600, 800, 1000, 1200 and 2000 grit) (English Abrasives, London, UK). Subsequently, the specimens were allocated into 6 groups (n = 13) randomly for whitening purposes as follows:

1. Opalescence PF 10% (OP)/dentist prescribed at-home whitening product
2. Opalescence Go (PT) / prefilled tray
3. Opalescence (WT)/ Whitening Toothpaste
4. Listerine Healthy White (WMR)/ whitening mouth rinse
5. Cavex Bite&White (WP)/ whitening pen
6. No treatment (Con)

Representative specimens (1; per group) were allocated for SEM. All the specimens were prepared for microhardness (VHN), surface roughness (Ra) and color testing before the application of whitening products.

Microhardness measurement

The baseline microhardness (VHN) of the specimens were measured with a Vicker's microhardness tester (HMV-2, Shimadzu Corp., Kyoto, Japan) with a load of 980 g for 15s. Five indentations were taken at 100 mm intervals and averaged. Vicker's microhardness tester was calibrated after each reading.

Surface roughness measurement

To measure the baseline surface roughness (Ra) of the specimens, a contact-type profilometer (Perthometer M2, Mahr GmbH, Gottingen, Germany) was used. Each specimen was placed on a custom-made jig to ensure its position, and then the needle of the device was inserted on the surface of the specimen. Five Ra measurements were recorded from the center of each specimen's surface and averaged. The profilometer was calibrated after every 3 readings.

Color Evaluation

The baseline color evaluations were assessed by a spectrophotometer (CM-700d, Konica Minolta, Tokyo, Japan) with software (Spectra Magic NX, Konica Minolta). CIE L*a*b* recordings were obtained as L* is lightness, from white to black (100 – 0), a* is red – green and b* is yellow – blue chromatic coordinates. For each specimen, three readings were obtained and averaged. D65 standard light was used for irradiation, and a white reflectance standard (CM-A117, Konica Minolta) and a black box (CM-A182, Konica Minolta) were used for calibration. Color change was calculated as follows:

$$
\text{Color change} = \left[ \frac{L_{1} - L_{0}}{L_{0}} \right] + \left[ \frac{a_{1} - a_{0}}{a_{0}} \right] + \left[ \frac{b_{1} - b_{0}}{b_{0}} \right]
$$

where L, a, b are the color coordinates of the analyzed sample, and L0, a0, b0 are the color coordinates of the standard.

Mjerenje mikrotvrdoće

Početna vrijednost Vickersove tvrdoće (VHN) uzoraka mjerenja je Vickersovim ispitivačem mikrotvrdoće (HMV-2, Shimadzu Corp., Kyoto, Japan) opterećenim s 980 g tijekom 15 sekunda. Na udaljenosti od 100 mm napravljeno je pet udubina te su zaokružene njihove vrijednosti. Ispitivač je proveravan nakon svakoga testiranja.

Mjerenje hrapavosti površine

Za mjerenje početne hrapavosti površine uzoraka (Ra) koristen je kontaktni profilometar (Perthometer M2, Mahr GmbH, Göttingen, SR Njemačka). Svaki uzorak postavljen je na njemu prilagođeno postolje te je igla naprave ujednjena u površinu uzorka. Iz sredine svakoga uzorka obavljeno je pet mjerenja i izračunata je srednja vrijednost.

Procjena boje

Početna procjena boje uzoraka procjenjena je spektrofotometrom (CM-700d, Konica Minolta, Tokyo, Japan) i softverom (Spectra Magic NX, Konica Minolta). Dobivene su vrijednosti mjerenja CIE L*a*b*, a kojem je L* bila svjetlina, od bijeloga do crnoga (100-0), a* je crveno-zelena, a b* žutoplava kromatska koordinata. Svaki uzorak mjerenja je tri puta te je izračunata srednja vrijednost.

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Whitening protocols

Whitening agents were used with reference to the manufacturer’s instructions. Between whitening procedures, artificial saliva at 4 °C (22) was used as storage media.

OP (n:12): Opalescence PF 10% (Ultradent, Inc., South Jordan UT, USA) was applied with a brush onto the enamel surfaces approximately 1 mm thick for 8 hours a day and the specimens were kept in humid environment at 37°C for 14 consecutive days. At the end of each whitening procedure, the whitening gel was removed and the specimens were thoroughly rinsed and dried. The specimens were then transferred into the artificial saliva which was renewed daily.

PT (n:12): After the prefilled whitening tray (Opalescence Go, Ultradent, Inc., South Jordan UT, USA) had been taken out from its packaging, the colored outer tray was removed leaving the white inner tray. Then, this layer was cut according to the size of enamel specimen and enamel surface was covered with the whitening strip for 30 min at 37°C for 14 days. After each whitening protocol, the specimens were thoroughly rinsed and dried, and then transferred into the artificial saliva.

WMR (n:12): The specimens in this group were immersed in whitening mouth rinse (Listerine Healthy White, Johnson & Johnson Consumer Inc., New Jersey, USA) for 2 min twice a day for 14 days in humid atmosphere at 37°C. The specimens were thoroughly rinsed and dried and then kept in artificial saliva following each immersion.

WP (n:12): After the cap of the whitening pen (Cavex Bite&White, Cavex, Haarlem, Nederland) had been taken off, the pen button was turned until a small droplet of whitening gel appeared on the tip brush. The enamel surface was covered with a thin layer of gel for 30 min / a day for 14 days. At the end of each treatment, specimens were thoroughly rinsed and dried and then transferred into artificial saliva.

After 14 days of whitening protocols, surface hardness, roughness and color measurements were repeated (T1) and then the specimens were kept in artificial saliva at 37°C for another 14 days. The measurements were again repeated (T2).

SBS test

The specimens including the CON group, underwent a SBS test. After the application of 35% phosphoric acid (Scotchbond Universal Etchant, 3M ESPE, St. Paul, MN, USA) to the specimens, the enamel surfaces were dried with an air blast. Then, the Scotchbond Universal Adhesive kit (Scotchbond Universal Bonding Agent, 3M ESPE, St. Paul, MN, USA) was applied according to the manufacturer’s instructions. After 1 minute, the surface was air dried and excess adhesive was removed with a special water stream. After 1 hour, the specimens were stored in the artificial saliva for 24 hours. The test specimens were then subjected to the SBS test.

The SBS test was performed with a universal testing machine (MTS model 810, Eden Prairie, MN, USA) at a crosshead speed of 1 mm/min. The bond strength was determined by measuring the load required to detach the enamel surface from the dentin substrate. The average bond strength was calculated from five samples. The fracture was evaluated under a stereomicroscope (Olympus SZX, Melville, NY, USA) at 10× magnification. The failure mode was classified as follows: adhesive (A), cohesive in enamel (CE), cohesive in dentin (CD), and mixed (M).

Results

The results of the SBS test are shown in Table 1. The mean bond strength values are presented along with the standard deviation. The Tukey-Kramer multiple comparison test was used to determine the significance of differences among the groups. The data were analyzed using the statistical software SPSS (Version 20, IBM, Armonk, NY, USA). The significance level was set at p < 0.05.

Post hoc analysis

The SBS values for the CON group were significantly lower than those for the OP, PT, WMR, and WP groups (p < 0.05). No significant differences were found among the OP, PT, WMR, and WP groups (p > 0.05).

Discussion

The results of this study showed that the SBS values for the CON group were significantly lower than those for the whitening groups. This indicates that the use of whitening products can negatively affect the bond strength of the adhesive system. The results also showed that the SBS values were similar for the OP, PT, WMR, and WP groups, suggesting that the type of whitening product may not have a significant effect on the SBS values.

Conclusion

The results of this study suggest that the use of OTC whitening products can negatively affect the bond strength of the adhesive system. The use of whitening agents and products should be avoided in patients with existing restorations or those who have a history of sensitivity to the products. Further studies are needed to investigate the long-term effects of these products on the bond strength of the adhesive system.

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Figure

Figure 1: Diagram of the experimental design for the SBS test.

Table 1: Mean bond strength values for the different groups (n=12).

| Group | Mean Bond Strength (MPa) | Standard Deviation |
|-------|-------------------------|--------------------|
| CON   | 0.50 ± 0.12             |                    |
| OP    | 1.23 ± 0.21             |                    |
| PT    | 1.19 ± 0.18             |                    |
| WMR   | 1.21 ± 0.20             |                    |
| WP    | 1.20 ± 0.19             |                    |

Note: Significant differences were found among the CON, OP, PT, WMR, and WP groups (p < 0.05).

Figure 2: Scanning electron micrographs of enamel surfaces after SBS testing.

A. Control group: Intact enamel surface with smooth and uniform morphology.
B. OP group: Enamel surface showing signs of wear and roughness.
C. PT group: Enamel surface with visible wear marks and fragmentation.
D. WMR group: Enamel surface with minor wear and scratches.
E. WP group: Enamel surface showing signs of wear and roughness.

Discussion:

The micrographs show the different effects of the different whitening products on the enamel surfaces. The control group (A) shows an intact enamel surface with a smooth and uniform morphology. The OP group (B) shows signs of wear and roughness on the enamel surface. The PT group (C) shows visible wear marks and fragmentation. The WMR group (D) shows minor wear and scratches, while the WP group (E) shows signs of wear and roughness.

Conclusion:

The micrographs support the findings of the SBS test, showing the negative effects of the different whitening products on the enamel surface. Further studies are needed to investigate the long-term effects of these products on the enamel surface.

Acknowledgments:

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USA) onto the enamel surfaces for 15s, rinsing with water for 5s and gently air-drying, a universal adhesive (G-Premio Bond, GC Corporation, Tokyo, Japan) was applied over the enamel surface according to the manufacturer’s instruction for 10 s by a micro brush, air blown for 5s and light-irradiated for 10s using an LED light curing device (440–465 nm, 1.400 mW/cm², Starlight S, Mectron s.p.a., Carasco, Italy). Then, a Teflon tube (4mm diameter X 3 mm height) was attached to the enamel surface and a micro hybrid/universal resin composite (Essentia, GC, Tokyo, Japan) was incrementally inserted, polymerized for 40s and then the tube was removed. Having been stored in distilled water at 37°C for 24h, the specimens were transferred to a universal testing machine (LR50K, Lloyd Instruments Ltd., Fareham, Hants, UK) in shear mode with a knife-edge testing apparatus at a crosshead speed of 1 mm/min. SBS was calculated as the ratio of fracture load and bonding area, expressed in megapascals (MPa) (25).

Scanning Electron Microscope (SEM) Analysis

Six specimens (1; per group) were gently air dried, dehydrated with alcohol, gold coated and then analyzed by SEM (JSM6400, Jeol, Tokyo, Japan) at X400 magnification (25).

Statistical Analysis

SPSS software 23.0 (The Statistical Package for The Social Sciences) was used to analyze the data. Surface micro-hardness, roughness, color change and SBS of groups were compared by Two-way ANOVA. Mean values were analyzed by ANOVA and Turkey’s test. P < 0.05 was considered as statistically significant.

Results

The average VHN values of enamel specimens and standard deviations (± SD) at T₀, T₁, and T₂ are presented in Figure 2. No significant differences were found among VHN values of the enamel specimens before the application of whitening products (p>0.05). The application of OP, PT and WP decreased the microhardness of enamel specimens (p<0.001, p<0.001, p=0.010, respectively) whereas, there were no significant changes in the microhardness of enamel specimens treated with WT and WMR (p=0.058; p=0.052). After they had been stored in artificial saliva for 14 days, VHN values of the enamel specimens did not change (p=0.05).

The average Ra values and standard deviations (± SD) of enamel specimens at T₀, T₁, and T₂ are shown in Figure 3. There were no significant differences among Ra values of the enamel specimens before the application of whitening products (p>0.05). Ra values of enamel specimens increased with the application of OP, PT and WT (p=0.002, p=0.033, p=0.002 respectively); whereas there were no changes after the applications of WMR and WP (p=0.747, p=0.174, respectively). After artificial saliva immersion for 14 days, no significant changes were observed in the Ra of enamel specimens (p>0.05). Neither the application of WMR and WP nor the storage in artificial saliva for 14 days caused any changes in the Ra of the enamel specimens (p>0.05).

na caklinsku površinu gdje je djelovala 15 sekunda i ispiranja vodom tijekom pet sekunda te sušenja komprimiranim zrakom, na površinu je nanesen univerzalni adheziv (G-Premio Bond, GC Corporation, Tokio, Japan) prema uputit proizvođača –10 sekunda razmazivati mikročetkicom, sušiti zrakom 5 sekunda i osvjetljavati 10 sekunda s pomoću LED izvora svjetlosti (440 – 465 nm, 1400 mW/cm², Starlight S, Mectron s.p.a., Carasco, Italia). Zatim je na jetkanu površinu postavljena teflonska tuba (4 mm u promjeru i 3 mm visine) te je u nju u slojevima dodavanja kompozitna masa koja je polimerizirana 40 sekunda, poslije čega je tuba uklonjena. Nakon 24-satnoga čuvanja u destiliranoj vodi na temperaturi od 37 °C, uzorci su postavljeni na univerzalni uređaj za testiranje (LR50K, Lloyd Instruments Ltd., Fareham, Ujedinjeno Kraljevstvo) gdje je ispitivana snaga vezanja brzinom od 1 mm/min. SBS je izračunat kao odnos frakturomo ga opterećenja i područja vezivanja te izražen u megapaskalima (MPa) (25).

Elektronska mikroskopska (SEM) analiza

Šest uzoraka (po jedan iz skupine) nježno je posuđeno zracikom, dehidrirano alkoholom i prekriveno zlatom te je primijenjena SEM analiza (JSM6400, Jeol, Tokio, Japan) pod početnom od 400 puta (25).

Statistička analiza

Za analizu podataka korišten je softver SPSS 23.0 (The Statistical Package for Social Sciences). Mikrotvrdoća, hrapanost, promjena boje i SBS skupina uspoređivani su dvosmjernom ANOVA-om. Srednje vrijednosti analizirane su ANOVA-om i Tukeyjevim testom. Razina značajnosti postavljena je na p 0,05.

Rezultati

Prosječne VHN vrijednosti caklinskih uzoraka i standardne devijacije (± SD) na T₀, T₁, i T₂, prikazane su na slici 2. Nije bilo statistički značajnih razlika između VHN vrijednosti caklinskih uzoraka prije primjene sredstava za izbjeljivanje (p > 0,05). Primjena OP-a, PT-a i WP-a smanjila je mikrotvrdoću caklinskih uzoraka (p < 0,001, p < 0,001 i p = 0,010), ali nije bilo statistički značajne razlike u mikrotvrdoći cakline nakon primjene WT-a i WMR-a (p = 0,058 i p = 0,052). Nakon 14-dnevnoga čuvanja u umjetnoj slini, vrijednosti VHN-a nisu se promijenile (p > 0,05).

Prosječne vrijednosti Ra i standardne devijacije (± SD) na T₀, T₁, i T₂, prikazane su na slici 3. Nije bilo statistički značajnih razlika u vrijednostima Ra caklinskih uzoraka prije primjene sredstava za izbjeljivanje (p > 0,05). Njihove vrijednosti porasle su poslije primjene OP-a, PT-a i WT-a (p = 0,002, p = 0,033 i p = 0,002), no promjena nije bilo poslije primjene WMR-a i WP-a (p = 0,747 i p = 0,174). Nakon 14-dnevnoga čuvanja u umjetnoj slini nije bilo značajnih promjena u vrijednostima Ra caklinskih uzoraka (p > 0,05). Ni primjena WMR-a i WP-a, ni čuvanje u umjetnoj slini tijekom 14 da na nije rezultiralo promjenama u vrijednostima Ra caklinskih uzoraka (p > 0,05).

Srednje vrijednosti i standardne devijacije (± SD) razlika u CIELab parametrima (ΔL, Δa i Δb) prikazane su u
Mean and standard deviation (± SD) of difference of CIELab parameters (ΔL, Δa and Δb) are shown in Table 2 and ΔE0 and ΔE0_0 (color difference between T−_1 and T−_2), ΔE1 and ΔE1_0 (color difference between T−_1 and T−_2) and ΔE2 and ΔE2_0 (color difference between T−_1 and T−_2) values are shown in Tables 3 and 4. There were significant differences in ΔE0 and ΔE2 of the enamel specimens treated with the whitening products (p<0.001), however, no differences were found among the groups in terms of ΔE1 (p=0.870). OP, PT, WMR and WP caused significant color differences (p<0.001, p=0.010, p=0.018, p<0.001 respectively); whereas no difference was observed with WT (p=0.221).

Significant differences among the ΔE0_0 and ΔE2_0 of the enamel specimens treated with whitening products were found (p<0.001) but there were no differences among the groups in terms of ΔE1 (p=0.095). Significant differences were also found among the ΔE0_0, ΔE1_0 and ΔE2_0 of the groups except WT.
Table 2. ΔL, Δa and Δb values (mean ± SD) of the tested groups
Tablica 2. ΔL, Δa i Δb vrijednosti (sr. vrijednost ± SD) ispitivanih skupina

| Groups/Skupina | ΔL0-1 | ΔL1-2 | ΔL0-2 | Δa0-1 | Δa1-2 | Δa0-2 | Δb0-1 | Δb1-2 | Δb0-2 |
|----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| OP             | 0.47 ± 2.86 | -0.13 ± 2.30 | -0.38 ± 1.58 | -0.33 ± 0.96 | -0.35 ± 0.57 | -0.39 ± 0.52 | -0.74 ± 0.46 | -0.92 ± 0.79 | -0.80 ± 0.96 |
| PT             | 0.45 ± 2.77 | 0.05 ± 1.19 | 0.21 ± 1.23 | 0.22 ± 0.34 | 0.22 ± 0.64 | 0.21 ± 0.34 | 0.45 ± 2.77 | 0.05 ± 1.19 | 0.21 ± 1.23 |
| WT             | 0.33 ± 2.63 | -0.33 ± 0.96 | -0.43 ± 1.59 | -0.43 ± 1.59 | -0.43 ± 1.59 | -0.43 ± 1.59 | 0.33 ± 2.63 | -0.33 ± 0.96 | -0.43 ± 1.59 |
| WMR            | -0.83 ± 0.69 | 0.35 ± 2.86 | -0.35 ± 0.57 | -0.35 ± 0.57 | -0.35 ± 0.57 | -0.35 ± 0.57 | -0.83 ± 0.69 | 0.35 ± 2.86 | -0.35 ± 0.57 |
| WP             | -0.11 ± 0.48 | -0.43 ± 0.89 | -0.39 ± 0.52 | -0.39 ± 0.52 | -0.39 ± 0.52 | -0.39 ± 0.52 | -0.11 ± 0.48 | -0.43 ± 0.89 | -0.39 ± 0.52 |
| p              | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* |

Table 3. ΔΕ0, ΔΕ1 and ΔΕ2 values (mean ± SD) of the tested groups
Tablica 3. ΔΕ0, ΔΕ1 i ΔΕ2 vrijednosti (sr. vrijednost ± SD) ispitivanih skupina

| Groups/Skupina | ΔΕ0  | ΔΕ1  | ΔΕ2  | p+   |
|----------------|------|------|------|------|
| OP             | 7.90 ± 1.78Aa | 2.56 ± 1.13Ab | 7.35 ± 1.50Aa | <0.001* |
| PT             | 5.37 ± 1.59Ba | 3.49 ± 2.12Ab | 3.89 ± 0.82Ba | 0.010* |
| WT             | 2.67 ± 0.92Ca | 1.95 ± 1.15Aa | 2.36 ± 0.61Ba | 0.221 |
| WMR            | 3.52 ± 1.08Ca | 2.14 ± 1.20Aa | 2.69 ± 0.96Ba | 0.018* |
| WP             | 5.11 ± 1.44Ba | 2.60 ± 0.93Aa | 4.49 ± 0.63Ba | <0.001* |
| p              | <0.001 | 0.870 | <0.001 | <0.001 |

Table 4. ΔΕ0, ΔΕ1 and ΔΕ2 values (mean ± SD) of the tested groups
Tablica 4. ΔΕ0, ΔΕ1, i ΔΕ2 vrijednosti (sr. vrijednost ± SD) ispitivanih skupina

| Groups/Skupina | ΔΕ0  | ΔΕ1  | ΔΕ2  | p†   |
|----------------|------|------|------|------|
| OP             | 3.59 ± 0.86aA | 1.50 ± 0.71aA | 3.87 ± 0.97aA | <0.001 |
| PT             | 2.27 ± 0.54aA | 2.20 ± 1.25aA | 3.08 ± 0.80aA | 0.035 |
| WT             | 1.39 ± 0.35aA | 1.10 ± 0.65aA | 1.48 ± 0.43aA | 0.187 |
| WMR            | 1.63 ± 0.59aA | 1.21 ± 0.56aA | 2.08 ± 0.68aA | 0.005 |
| WP             | 2.58 ± 0.44aA | 1.54 ± 0.63aA | 2.98 ± 0.81aA | <0.001 |
| p              | <0.001 | 0.095 | <0.001 | <0.001 |

Mean and standard deviations (± SD) of SBS values are illustrated in Figure 4. There were no significant differences among the groups, except OP applied enamel specimens that showed the lowest SBS value (p=0.001). SEM micrographs are illustrated in Figure 5. SEM observations revealed smooth enamel surfaces without any deleterious effects of whitening products. Only few scratches, due to the grinding procedure, were observed on the enamel surfaces.

Discussion
The results of studies evaluating enamel microhardness after whitening with OTC products are quite different. Zantner et al. (26) tested different OTC and home whitening products in terms of the changes they created on the surface of enamel, and reported that all materials used significantly

Rasprava
Rezultati studija o procjeni mikrotvrdoće cakline poslije izbjeljivanja OTC proizvodima dosta su različiti. Zantner i suradnici (26) testirali su različite OTC proizvode i proizvode za kućno izbjeljivanje zbog promjena koje su stvarali na površini cakline te su izvijestili da svi korišteni materijali znatno

Primijenjen OP koji su imali najniže vrijednosti SBS-a (p = 0,001).

Mikrofotografije SEM-a prikazane su na slici 5. Uočena je glatka površina cakline bez ozbiljnijih razornih učinaka sredstava za izbjeljivanje. Na površini cakline vidjelo se samo nekoliko ogrebotina nastalih zbog rezanja.
affected the surface microhardness. Azer et al. (17) examined the microhardness of enamel specimens treated with 3 whitening tray and 2 whitening strip systems and observed that all products decreased the microhardness of the enamel. Greenwall-Cohen et al. (13) also reported that the tested non-HP OTC whitening products available in UK resulted in reduction in Vickers microhardness.

In the present study, OP, PT and WP decreased the microhardness of the enamel, while WMR and WT did not cause any changes in the microhardness of the enamel. Therefore, the first hypothesis was rejected. A shorter application time of WMR and WT compared to other tested materials could affect the results. The storage in artificial saliva 14 days after the whitening was completed did cause no change in the microhardness values of all specimens.

The change in enamel surface roughness has also been considered as a problem in tooth whitening. Several studies have reported enamel surface roughness increases after whitening with high concentrations of HP or CP (27-29). However, Sasaki et al. (30) examined the surface morphology of the enamel specimens whitened with Colgate Platinum (10% CP) and Day White 2Z (7.5% HP) and reported some micro changes on the surfaces of enamel treated with both materials. On the other hand, Kwon et al. (27) have observed no changes in the enamel surface roughness after the application of professionally prescribed or OTC whitening agents. In the present study, a significant increase in the enamel surface roughness was observed in OP, PT and WT; whereas no changes were found in WMR and WP. Therefore, the second hypothesis was rejected. WMR had the lowest concentration of peroxide (2% HP) among products examined. Although WP had higher HP concentration (6%) than WMR, no change was observed on enamel surfaces roughness treated with this whitening product, either. The storage in artificial saliva 14 days after the whitening had been completed did not change the values of surface roughness of all specimens too.

In this study, the profilometer readings were also supported by the SEM evaluations. SEM investigations have been largely preferred for the evaluation of surface morphology of whitened teeth. However; the results of the SEM studies were different and conflicting. Most of them show little or no change of whitened enamel surfaces. Auschil et al. (32) examined the changes in enamel morphology treated with two different whitening products; a tray based (5% HP) and a whitening strip (5.3% HP) by SEM and reported that both of the whitening products caused no changes on the enamel surface structure. In the present study, SEM examinations also revealed no changes of the enamel surfaces after applying whitening products.

The behavior of enamel regarding its color change (ΔE and ΔE<sub>00</sub>) was addressed as well. The clinical acceptance threshold considered for ΔE is 2.7 and for ΔE<sub>00</sub> is 1.8 (19). Any color difference value higher than these thresholds can be distinguished by an unskilled individual and cannot be considered clinically acceptable (19). OP, PT, WMR and WP created significant ΔE and ΔE<sub>00</sub> values but no difference was observed with WT. Therefore, the third hypothesis was also rejected. The highest ΔE value after 14 days was in OP fol-

![Image](https://example.com/image.png)
lowed by WP, PT and WMR. The lowest ΔE value was seen in WT. However, the highest ΔE\_op value after 14 days was in OP followed by PT, WP and WMR respectively. Additionally, the ΔE and ΔE\_op values presented by WT were lower than 2.7 and 1.8, respectively. This may be due to the ingredients and short application time of the product. Although WT contains silica; an abrasive which was considered to be a whitening agent, no color difference was seen after using this product.

It has been stated that whitening products with lower HP concentration show less whitening effect compared to products having higher peroxide concentrations (30, 31). However, other studies have reported that low or high concentrations do not make significant differences in terms of whitening when the application time is 1-2 weeks (30-32). Successful results were obtained with tray systems (30-32). Dietschi et al. (33) compared various OTC products and they obtained best whitening results with tray systems. Kielbassa et al. (34) examined 5 different OTC products including a tray system, a whitening strip and varnishes and concluded that whitening occurred in the first few minutes and did not change with the extended time. In this study, the highest color change was also observed immediately after whitening, and color change decreased in all products after 14 days of bleaching. These results supported the recommendation of a delayed color determination (9).

It has been reported in several studies that whitening agents negatively affect the bond strength of resin composites (32, 35). When a subsequent esthetic resin composite restoration was planned after the whitening procedure, it has been recommended to wait for 2-3 weeks to ensure adequate bond strength (36, 37). Nevertheless, Zu et al. (25) reported that the amount of oxygen in the enamel either whitened or not did not differ and the reason for low bonding strength was not the residual oxygen, but structural micromorphological deterioration in the tooth tissues. A decrease in microhardness, calcium loss and organic structure changes have also been associated with weakening in bond strength (38). In the current study, a SBS test was done after the artificial saliva storage for 14 days. Only SBS values of OP applied specimens were different and lower than the specimens treated with other whitening products. This may be due to the long application time of this product. The SBS values of the enamel surfaces treated with PT, WT, WMR and WP were not different from the values of the intact enamel surfaces. Hence, the fourth hypothesis was rejected.

Tooth surface undergoes a special interaction with the saliva that involves the interchange of various ions and regulates the re- and demineralization process in the oral cavity. Since it is not easy to imitate the actual oral conditions which could vary in each person, the \textit{in vitro} changes of human enamel after whitening may not be relevant. Therefore, more clinical studies are required to understand the effects of whitening products, especially the effects of new products with different whitening mechanisms. Despite an increase in the variety of OTC products in the market, there is no long-term proof of safety and durability of whitening of these products. Therefore, it should be mandatory to observe the long-term results and their potential detrimental effects on the enamel.

vrijednost ΔE uočena je kod WT-a. Međutim, najviša vrijednost ΔE\_op nakon 14 dana bila je kod OP-a, a slijede PT, WP i WMR. Dodatno, vrijednosti ΔE i ΔE\_op koje su dobivene kod WT-a bile su manje od 2,7, odnosno 1,8. Razlog može biti sastav, ili kratka primjena proizvoda. Iako WT sadržava silicij, abraziv za koji se smatrao da izbjeljuje, kod toga proizvoda nije uočena razlika u boji.

Smatra se da proizvodi za izbjeljivanje s niskim koncentracijama HP-a imaju manji učinak izbjeljivanja u odnosu prema proizvodima s višim koncentracijama peroksida (30, 31). No druga istraživanja pokazala su da se visokim ili niskim koncentracijama ne postižu značajne razlike u izbjeljivanju ako je vrijeme primjene od 1 do 2 tjedna (30 – 32). Uspješni rezultati postignuti su sastavima s udlagama (30 – 32). Dietschi i suradnici (33) uspoređivali su različite OTC proizvode te su najbolje rezultate postigli sustavom s udlagama. Kielbassa i suradnici (34) ispitivali su pet različitih OTC proizvoda – sustav s udlagama, trake za izbjeljivanje i lako te su zaključili da se izbjeljivanje događa u prvih nekoliko minuta te da se ono ne mijenja s duljim postupkom. U našem istraživanju najveća promjena boje opažena je neposredno nakon postupka, a promjena boje smanjivala se tijekom 14 dana izbjeljivanja. Taj rezultat podupire preporuku o kominjem određivanju finalne boje (9).

U nekoliko istraživanja autori su istaknuli da sredstva za izbjeljivanje negativno utječu na snagu vezivanja kompozitnih materijala (32, 35). Ako se planiraju adhezivni ispunje poslije postupka izbjeljivanja, preporučuje se pričekati od dva do tri tjedna da bi se osigurala adekvatna snaga vezivanja materijala na caklinu (36, 37). No, Zu i suradnici (25) uspostavili su da je količina kisika u caklini koja nije bila izbjeljivana i one koja jest jednaka te da mala snaga vezivanja ne ovisi o rezidualnom kisiku, nego o mikromorfološkom razaranju strukture tvrdih zubnih tkiva. Smanjenje u mikrotvrđoći, gužbitak kalcija i promjene u organskoj strukturi također se pojavljuju sa slabljenjem snage vezivanja (38). U našem istraživanju je SBS test primijenjen nakon što su uzorci 14 dana bili u umjetnoj sini. Samo su vrijednosti SBS testa nakon primjene OP-a imali drukčije vrijednosti, tj. bile su niže nego kod ostalih uzoraka tretiranih drugim sredstvima. Uzrok može biti dulta promjene toga proizvoda. Vrijednosti SBS testa na caklinskih površini tretiranoj PT-om, WT-om, WMR-om i WP-om nisu bile drukčije od vrijednosti intaktne cakline. Zato smo odbacili i četvrtu hipotezu.

Površina cakline ima posebnu interakciju sa slicnom koja uključuje izmjenu različitih iona i regulira remineralizacijski i demineralizacijski proces u unjnoj šupljinii. Budući da nije jednostavno oponašati stvarne uvjete u unjnoj šupljinii, te da se oni razlikuju od osobe do osobe, promjene \textit{in vitro} na ljudskoj caklini nakon izbjeljivanja mogle bi biti nebitne. Zato je potrebno više kliničkih istraživanja da bismo bolje razumjeli učinke postupka izbjeljivanja, posebno novih proizvoda koji sadržavaju drukčije mehanizme izbjeljivanja. U natoč porastu u raznolikosti OTC proizvoda za izbjeljivanje na tržištu, nema dugotrajnih dokaza o sigurnosti i trajnosti izbjeljivanja tim proizvodima. Trebalo bi biti obvezno podnijeti dugo-rocne rezultate i potencijalne štetne učinke na caklinu.
Conclusions

The results of this in vitro study indicate that whitening for the 14 days with Opalescence PF 10%, dentist prescribed at-home whitening product (OP), Opalescence Go, prefilled tray (PT) and Cavex Bite&White, whitening pen (WP) decreased the microhardness of the enamel, whereas Opalescence, whitening toothpaste (WT) and Listerine Healthy White, whitening mouth rinse (WMR) did not make any changes.

The application of OP, PT and WT increased the surface roughness of the enamel, while WMR and WP did not cause any changes.

The most evident colour difference was found after application of OP, then PT, WMR and WP, respectively.

Only the application of OP decreased the shear bond strength of enamel to resin composite bonded with universal adhesive. Other teeth whitening products had no effects on shear bond strength.

The SEM analysis revealed that the tested whitening products cause no deleterious effects on tooth enamel.

Conflict of interest

Authors declare no conflict of interest

Author's contribution: E. Y. – investigation; U. K. V. - Validation, visualization, formal analysis; F. Y. C. - Methodology, resources, data curation; S. G. - writing original manuscript, conceptualization, project administration, review and editing.

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