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Which Whitening Mouthwash With Different Ingredients Is More Effective on Color and Bond Strength of Enamel?

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ABSTRACT

Objective: To examine the effects of six whitening mouthwashes on tooth color and immediate bond strength to the enamel.

Materials and Methods: Human incisors were divided into seven groups ($n = 10$) according to mouthwashes (R.O.C.S Black Edition White, Splat White Plus, Colgate Plax White Charcoal, Signal White Now, Listerine Advanced White, Colgate Optic White, and distilled water). After the initial color measurements, the teeth were exposed to mouthwash for 4 weeks. Then, the color measurements were repeated. Then, cylindrical composite resin blocks were immediately applied to the enamel surfaces and subjected to shear bond strength tests. Data were analyzed using Kruskal-Wallis and Bonferroni tests ($\alpha = 0.05$).

Results: Δb , ΔL , and ΔE_{00} values did not present significant differences among the groups. Significant differences among the groups were determined for Δa and ΔWI_D values ($p < 0.05$). R.O.C.S Black Edition White and Splat White Plus produced clinically acceptable color changes. Signal White Now, Splat White Plus, and Listerine Advanced White created acceptable whiteness changes. The mouthwashes did not statistically affect the bond strength compared to the distilled water ($p > 0.05$).

Conclusions: Whitening mouthwash containing blue covarine revealed more acceptable color and whitening changes. Mouthwash containing charcoal led to the lowest enamel bond strength values.

Clinical Significance: The content of whitening mouthwashes affected the degree of tooth whitening and shear bond strength to enamel.

1 | Introduction

Discoloration can occur in natural teeth over time due to various external and internal factors. Teeth whitening treatment has become a popular aesthetic dental procedure because it can improve the color and appearance of teeth. This treatment can eliminate internal and external staining without using restorative materials [1, 2]. Vital teeth whitening treatments are generally categorized as at-home (dentist-supervised nightguard whitening), in-office or power whitening (professionally applied), and over-the-counter (OTC) or mass-market products [3]. Access to OTC whitening products has become widespread due

to their convenience and affordability. Such products include mouthwashes, toothpaste, dental floss, whitening strips, and gummies [2]. These products often contain lower levels of hydrogen peroxide, carbamide peroxide, or alternative bleaching agents [4–6].

One common OTC product often recommended by physicians is mouthwash. Mouthwashes are typically suggested for controlling plaque and maintaining gum health. Recently, whitening mouthwashes have entered the market, incorporating whitening solutions containing varying amounts of hydrogen peroxide or other chemicals [7]. Whitening mouthwashes aim

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to quickly whiten teeth by preventing tooth stains, becoming popular due to their easy use by patients and low cost [8]. However, not all whitening mouthwashes contain hydrogen or carbamide peroxide as active ingredients [9]. Some studies have mainly focused on whitening mouthwashes containing hydrogen peroxide, but their effects are notably lower than professional whitening treatments [2, 10]. Mouthwashes containing hydrogen peroxide can pose a potential risk due to their low pH and uncontrolled oral application. In response, whitening mouthwashes without hydrogen peroxide have been developed, utilizing alternative substances such as sodium hexametaphosphate, tetrasodium pyrophosphate, and phthalimido-peroxycaproic acid [4, 11, 12]. Additionally, whitening mouthwashes containing blue covarine, charcoal, and bromelain (a natural ingredient derived from pineapple) are also available [13–16]. Although research on these products is limited, the manufacturers offer intriguing possibilities for effective teeth whitening.

The effectiveness of whitening mouthwashes on tooth color has generally been studied on artificially stained teeth, leading to controversial conclusions [2, 17, 18]. In these studies, only the surface stain removal properties of whitening mouthwashes can be evaluated, and their actual whitening effects cannot be accurately assessed [4]. On the other hand, numerous studies have shown that teeth whitening can lead to a decrease in bonding strength of adhesive agent to enamel due to the increase in free oxygen radicals on the enamel surface [19–23]. Additionally, it has been associated with a decrease in microhardness in enamel, calcium loss, and changes in the organic structure, all of which contribute to a decrease in bonding strength [24–26]. A few studies have explored the effects of whitening mouthwashes on the bonding strength to enamel, so this topic has yet to be thoroughly researched.

Recently, many whitening mouthwashes with different ingredients have appeared, and their effectiveness is still open to debate. This in vitro study was designed to evaluate the effects of six whitening mouthwashes with different whitening ingredients on enamel color and shear bond strength (SBS). The study hypotheses to be tested were as follows: (1) the whitening mouthwashes would cause clinically acceptable color change on the teeth, (2) color change would differ among whitening mouthwashes, and (3) the whitening mouthwashes would affect the SBS to enamel.

2 | Materials And Methods

2.1 | Study Approval

This in vitro study was approved by Recep Tayyip Erdogan University Non-invasive Clinical Research Ethics Committee (Approval no: 2024/77).

2.2 | Specimen Preparation

Seventy human upper incisor teeth extracted during routine treatments were collected. The teeth were cleaned to remove

residual tissues and debris using a hand-scaler. Teeth with fractures, cracks, caries, developmental anomalies, and extrinsic and intrinsic stains were discarded from the study after being examined under a stereomicroscope (Zeiss Stemi 305; Gottingen, Germany). The collected teeth were then stored in a 0.1% thymol solution for 1 week at room temperature. Afterward, the roots were embedded in acrylic resin up to the cemento-enamel junction. A diamond blade (Impact PC10; Equilam Lab Equip, Diadema, SP, Brazil) was used to obtain enamel-dentin blocks (size, 6×6 mm; thickness, 3 mm) by cutting vertically and horizontally under water-cooling. Each enamel-dentin sample was embedded in auto-polymerized acrylic resin (Integra, BG Dental; Ankara, Turkey) to expose the enamel surface without any pre-treatment application. The samples were stored in distilled water until tested and then randomly divided into seven groups according to the whitening mouthwashes tested ($n = 10$) as follows: R.O.C.S Black Edition White (RBEW), Splat White Plus (SWP), Colgate Plax White+ Charcoal (CPWC), Signal White Now (SWN), Listerine Advanced White (LAW), Colgate Optic White (COW), and distilled water. Specimens stored in distilled water were used as the control group in the bond strength test. The chemical compositions and manufacturers of the materials used in this study are shown in Table 1.

2.3 | Color Measurements

Color values of each sample were measured using a spectrophotometer (VITA Easyshade Advance, Zahnfabrik, Bad Säckingen, Germany) based on the Commission Internationale de l'Eclairage (CIE) $L^*a^*b^*$ system. The spectrophotometer was calibrated using a calibration plate according to the manufacturer's instructions before each measurement. CIE $L^*a^*b^*$ records were obtained as L^* white to black (100–0) luminance, a^* red–green, and b^* yellow–blue chromatic coordinates. Color change (ΔE_{00}) and whiteness change (WI_D) of the samples were calculated based on CIEDE2000 (ΔE_{00}) [27] and whiteness index (WI_D) [28] systems according to the following equations:

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

$$WI_D = 0.55 L^* - 2.32 a^* - 1.100 b^*$$

$$\Delta WI_D: WI_{DT1} - WI_{DT0}$$

$\Delta L'$, $\Delta C'$, and $\Delta H'$ in the CIEDE2000 (ΔE_{00}) equation are the differences between the lightness, color intensity (chroma), and hue measurements of the samples before and after the process, respectively. S_L , S_C , and S_H are weighting functions added to the formula to eliminate the irregularities observed in the CIELAB system. The parametric factors K_L , K_C , and K_H are correction terms for errors due to experimental conditions. RT (rotation function) is the function that indicates the interaction between color intensity and color tone changes in the blue region.

ΔE_{00} values for acceptance (AT) and perception (PT) limits (50%:50%) were 1.8 (AT) and 0.8 (PT) units, respectively [27, 29]. ΔWI_D for AT and PT limits (50%:50%) were 2.6 (AT) and 0.7 (PT)

TABLE 1 | The chemical compositions and manufacturers of mouthwashes and restorative materials used in the study.

Material	Lot number	Manufacturer	Composition
Listerine Advanced White mouthwash	736,360	Johnson & Johnson; Skillman, NJ, USA.	Water, alcohol, sorbitol, tetra potassium pyrophosphate, penta sodium triphosphate, citric acid, poloxamer 407, flavors, sodium saccharin, sucralose, sodium fluoride, sodium benzoate, tetrasodium pyrophosphate, menthol, eucalyptol, thymol, aroma, propylene glycol, disodium phosphate.
Colgate Plax White and Charcoal mouthwash	L0207956	Colgate-Palmolive; New York, USA	Aqua, glycerin, propylene glycol, sorbitol, tetrapotassium pyrophosphate, polysorbate 20, tetrasodium pyrophosphate, zinc citrate, PVM/MA copolymer, aroma, benzyl alcohol, sodium fluoride, sodium saccharin, <i>Bambusa vulgaris</i> shoot extract, charcoal powder, CI 15510, CI 17200, CI 19140, CI 42051.
Colgate Optic White mouthwash	L1131	Colgate-Palmolive; New York, USA	Water, glycerin, sorbitol, propylene glycol, PVM/MA copolymer, tetrapotassium pyrophosphate, polysorbate 20, sodium fluoride, sodium saccharin, CI 42051.
Signal White Now mouthwash	5CYA	COSMINT SPA Company; Olgiate Comasco, Italy	Water, sorbitol, PEG-40, trisodium phosphate, PVM/MA copolymer, sodium lauryl sulfate, aroma, benzyl alcohol, phenoxyethanol, sodium fluoride, sodium saccharin, lestin, glycerin, lemon, CI 74160.
R.O.C.S Black Edition Whitening mouthwash	L326	EUROCOSMED-Stupino; Moscow, Russia	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, limonene
Splat White Plus mouthwash	316,682	SPLAT Global; Russia	Aqua, hydrogenated starch hydrolysate, PVP, polyglyceryl-4 laurate/sebacate, polyglyceryl-6 caprylate/caprate, sodium coco-sulfate, aroma, cyclodextrin, zinc gluconate, citrus limon peel oil, ananas sativus (pineapple) fruit extract, maltodextrin, thymus serpyllum oil, glycyrrhiza glabra root extract, stevia rebaudiana leaf extract, glycerin, pentylene glycol, bifida ferment lysate, phthalimidoperoxycaproic acid, potassium thiocyanate, lactoferrin, lactoperoxidase, glucose oxidase, glucose pentaacetate, sodium benzoate, potassium sorbate, benzyl alcohol, citric acid, limonene, citral, linalool.
Clearfil Majesty Flow Composite	430,094	Kuraray Noritake Dental; Okayama, Japan	Silanated barium glass filler, silanated colloidal silica, triethylene glycol dimethacrylate, hydrophobic aromatic dimethacrylate, di-camphorquinone, filler (81%w)
Clearfil S3 Bond Universal	6L0063	Kuraray Noritake Dental; Okayama, Japan	10-Methacryloyloxydecyl dihydrogen-phosphate, bisphenol A-glycidyl methacrylate, 2-hydroxyethyl methacrylate, hydrophobic dimethacrylates, dicamphoroquinone, ethanol, water, and silanated colloidal silica
K-Etchant Syringe	680,218	Kuraray Noritake Dental; Okayama, Japan	Phosphoric acid (35%), water, colloidal silica, pigment

units, respectively [28, 29]. Also, in the whiteness index (WI_D) equation for dentistry, higher WI_D values indicate whiter samples (whiteness), whereas lower WI_D values (including negative values) indicate darker samples (darkness). Three readings were obtained for each sample and averaged. Measurements were taken at baseline (T0) and after the 4-week (T1) mouthwash cycle procedure.

2.4 | Mouthwash Cycle Procedure

The samples were prepared for gargling cycles after the initial color measurements (T0). Each group of teeth was immersed in 25 mL of relevant mouthwash and shaken for 60s, then rinsed with running water for 30s. This process was repeated with fresh mouthwash. This gargling procedure represented the use of mouthwash twice a day [30]. This daily routine was repeated seven times, simulating 1 week of use. The teeth were then stored in distilled water for a day. The same process was repeated four times to simulate 4 weeks of use. Samples were kept in distilled water until the next experimental cycle. After completing this phase, color measurements were conducted again (T1).

2.5 | SBS Test and Failure Modes

After the final color measurement (T1), the samples were washed under running water and prepared for the SBS test without waiting. Phosphoric acid (35%, K-etchant; Kuraray Noritake Dental, Okayama, Japan) was applied to the enamel surfaces for 15s, rinsed with water, and gently air dried. A universal bond (Clearfil S3 Bond Universal; Kuraray Noritake Dental, Okayama, Japan) was applied to the enamel surface using a microbrush for 10s, following the manufacturer's instructions. Subsequently, it was air-dried for 5s and then cured for 10s using an LED light device (VALO Cordless LED, Ultradent; South Jordan, UT, USA). A Teflon tube (2.5mm internal diameter and 3 mm high) was mounted onto the bonded enamel surface. Then, 2-mm-thick flowable composite resin (Clearfil Majesty Flow Composite; Kuraray Noritake Dental, Okayama, Japan) was applied to the enamel surface and polymerized using an LED light device for 20s.

Samples were stored in distilled water at 37°C for 24h and then examined at a 1 mm/min speed on a universal testing machine (Model 3344; Instron Corporation, MA, USA). SBS was expressed in megapascals (MPa), the ratio of fracture load to bond area. After the SBS test, all samples were examined under a stereomicroscope at 25× magnification for failure mode distributions. Failure modes were categorized as adhesive, mixed, cohesive in enamel, or cohesive in composite resin [31].

2.6 | Scanning Electron Microscope (SEM) Analysis

One example of each failure mode was dehydrated with alcohol and coated with gold. Then, failure zones were examined and analyzed in detail using an SEM (Jeol JSM-6610, Jeol Ltd.; Sydney, Australia) at 18× magnification.

2.7 | Statistical Analysis

The sample size ($n=10$) of each group was determined based on previous studies [32, 33] and checked using G×Power software (Heinrich Heine University, Germany). The sample size was found to be sufficient for this study (power $(1-\beta)=0.86$, effect size = 0.30). Data analysis was performed using IBM SPSS Statistics software (version 25). The Kolmogorov-Smirnov test indicated that color and SBS data were not normally distributed ($p<0.05$). Consequently, non-parametric Kruskal-Wallis test with adjusted Bonferroni tests were conducted to assess the differences among groups. The statistical significance level was determined as $p<0.05$.

3 | Results

Δa , Δb , ΔL , ΔE_{00} , and ΔWI_D values of the groups are given in Table 2. Statistically significant differences were determined between the groups for Δa ($p<0.001$) and ΔWI_D ($p=0.004$) values. The SWN group showed the highest Δa values, with a statistical difference compared with the SWP ($p=0.007$), CPWC ($p=0.037$), LAW ($p=0.004$), and COW ($p=0.010$) groups. For ΔWI_D , the SWN group showed significantly higher values than the RBEW ($p=0.047$), CPWC ($p=0.044$), and COW ($p=0.028$) groups. There was a significant difference between the groups regarding ΔL , Δb , and ΔE_{00} values ($p>0.05$). All mouthwashes produced clinically perceptible color change ($\Delta E_{00}>0.8$) and whiteness change ($\Delta WI_D>0.7$). However, clinically acceptable color change ($\Delta E_{00}>1.8$) was observed in the RBEW and SWN groups, and acceptable whiteness change ($\Delta WI_D>2.6$) was observed in the SWP, SWN, and LAW groups.

The distribution of the SBS values of the groups is listed in Table 3. SBS values showed significant differences between the groups ($p=0.007$). All mouthwashes did not cause any significant difference in bond strength compared to the control group (distilled water). A significant difference was detected between the CPWC and the COW groups ($p=0.038$). The CPWC group showed the lowest SBS values. The failure modes distribution of the samples is shown in Figure 1. The most common mode of failure was adhesive, and the least common was cohesive in the enamel. Representative SEM images of the failure modes are shown in Figure 2 (18× magnification).

4 | Discussion

The CIE-Lab color system, established by CIE in 1976, is commonly used for numerical color evaluation [27, 29]. Recently, CIE introduced a new formula called CIEDE2000 to calculate color differences, utilizing chroma, lightness, and hue values. Some studies have found that this new formula provides better results than CIE-Lab in assessing color differences [6, 34]. In this study, color differences were calculated using the CIEDE2000 formula. Among the tested whitening mouthwashes, only the RBEW and SWN groups produced clinically acceptable median color changes ($\Delta E_{00}>1.8$). Thus, the first null hypothesis was rejected. RBEW contains hydrogen peroxide, which spreads throughout the organic matrix of the tooth, creating free radicals that cause teeth to whiten.

TABLE 2 | Minimum-maximum, mean (\pm SD), median values for color (Δa , Δb , ΔL , ΔE_{00} and ΔWI_D) parameters of the groups.

	Groups	Min.–maks.	Mean \pm SD	Median	Test statistics	<i>p</i>
ΔL	RBEW	0.01–37.21	8.51 \pm 11.75	3.61 ^a	3.10	0.685
	SWP	0.01–46.24	8.61 \pm 14.66	1.69 ^a		
	CPWC	0.36–65.61	10.11 \pm 19.78	3.87 ^a		
	SWN	0.01–28.09	8.67 \pm 9.77	4.21 ^a		
	LAW	0.01–32.49	8.02 \pm 12.40	1.63 ^a		
	COW	0.01–44.89	5.69 \pm 13.85	1.17 ^a		
Δa	RBEW	0.16–2.56	1.04 \pm 1.05	0.36 ^{ab}	23.04	0.000*
	SWP	0.00–1.00	0.46 \pm 0.37	0.43 ^a		
	CPWC	0.04–4.84	1.22 \pm 1.76	0.36 ^a		
	SWN	2.25–18.49	5.44 \pm 4.85	3.83 ^b		
	LAW	0.04–1.69	0.45 \pm 0.51	0.25 ^a		
	COW	0.01–1.69	0.54 \pm 0.54	0.43 ^a		
Δb	RBEW	0.00–26.01	7.72 \pm 9.71	3.19 ^a	8.79	0.117
	SWP	0.09–40.96	8.43 \pm 12.59	2.25 ^a		
	CPWC	0.00–102.01	14.68 \pm 31.19	3.25 ^a		
	SWN	1.00–50.41	15.70 \pm 14.17	14.07 ^a		
	LAW	0.01–30.25	7.84 \pm 10.54	1.71 ^a		
	COW	0.00–24.01	3.97 \pm 7.64	0.57 ^a		
ΔE_{00}	RBEW	0.54–5.09	2.07 \pm 1.56	1.84 ^a	11.07	0.050
	SWP	0.91–5.91	2.06 \pm 1.53	1.49 ^a		
	CPWC	0.74–7.13	2.15 \pm 1.88	1.64 ^a		
	SWN	1.86–4.83	3.31 \pm 1.09	3.25 ^a		
	LAW	0.61–4.37	1.94 \pm 1.45	1.21 ^a		
	COW	0.30–5.41	1.53 \pm 1.44	1.22 ^a		
ΔWI_D	RBEW	–1.99–6.82	2.66 \pm 3.08	2.29 ^a	17.35	0.004*
	SWP	–1.17–5.89	2.91 \pm 2.08	2.75 ^{ab}		
	CPWC	–7.83–12.08	2.85 \pm 5.28	2.12 ^a		
	SWN	4.18–13.69	7.81 \pm 2.74	7.68 ^b		
	LAW	0.93–6.16	3.10 \pm 1.74	2.85 ^{ab}		
	COW	0.16–6.17	2.64 \pm 1.83	2.38 ^a		

Note: According to the Kruskal Wallis test, different superscript letters indicate a significant difference ($p < 0.05$).

*indicates significant differences among groups.

These potent low-molecular-weight molecules penetrate inter-prismatic spaces, breaking down complex chromophore molecules to achieve successful whitening [15]. A previous study determined that a mouthwash containing hydrogen peroxide produced better color change after 45 days of use [4]. SWN contains blue covarine, which deposits onto the tooth surface and causes the teeth to shift from yellow to blue [6]. Although it exhibited relatively higher Δb (tendency towards blue) values, this was not statistically significant, likely due to the higher standard deviation. It has been reported that brushing with a whitening toothpaste containing blue covarine improved the

whiteness of teeth [35]. However, there is no information in the literature regarding the effectiveness of mouthwashes containing blue covarine on teeth.

ΔE_{ab} and ΔE_{00} formulas are used to analyze color differences after teeth whitening, but they do not provide enough information about the whiteness change in teeth [6, 28, 34]. In this study, ΔWI_D based on CIELAB coordinates was used to evaluate whiteness change. The SWP, SWN, and LAW groups gave ΔWI_D values above 2.6, which is the clinically accepted value [28]. The ΔWI_D value of the SWN group was statistically

TABLE 3 | Distribution and comparison of SBS values and \pm SD (MPa) of the groups.

Groups	Min.–maks.	Mean \pm SD	Median	Test statistics	<i>p</i>
RBEW	9.16–15.75	12.43 \pm 2.11	11.91 ^{ab}	17.89	0.007*
SWP	5.97–13.70	9.99 \pm 2.16	10.31 ^{ab}		
CPWC	6.93–12.16	8.89 \pm 0.85	8.15 ^a		
SWN	8.08–15.64	11.61 \pm 2.26	11.74 ^{ab}		
LAW	6.93–16.63	10.26 \pm 2.96	10.02 ^{ab}		
COW	8.06–16.89	12.42 \pm 2.58	12.14 ^b		
Distilled water	7.77–16.50	12.23 \pm 2.91	11.06 ^{ab}		

Note: According to the Kruskal Wallis test, different superscript letters indicate a significant difference ($p < 0.05$).
*indicates significant differences among groups.

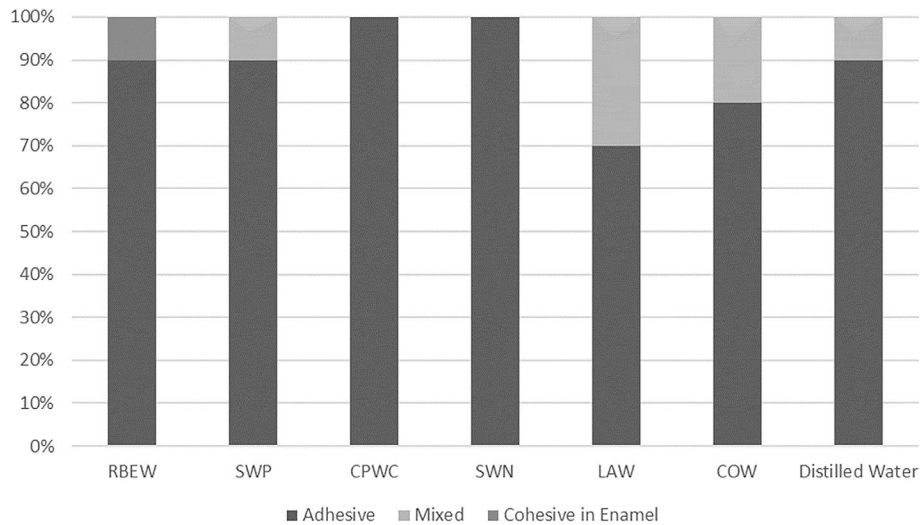


FIGURE 1 | Distribution frequency (%) of failure modes for the groups.

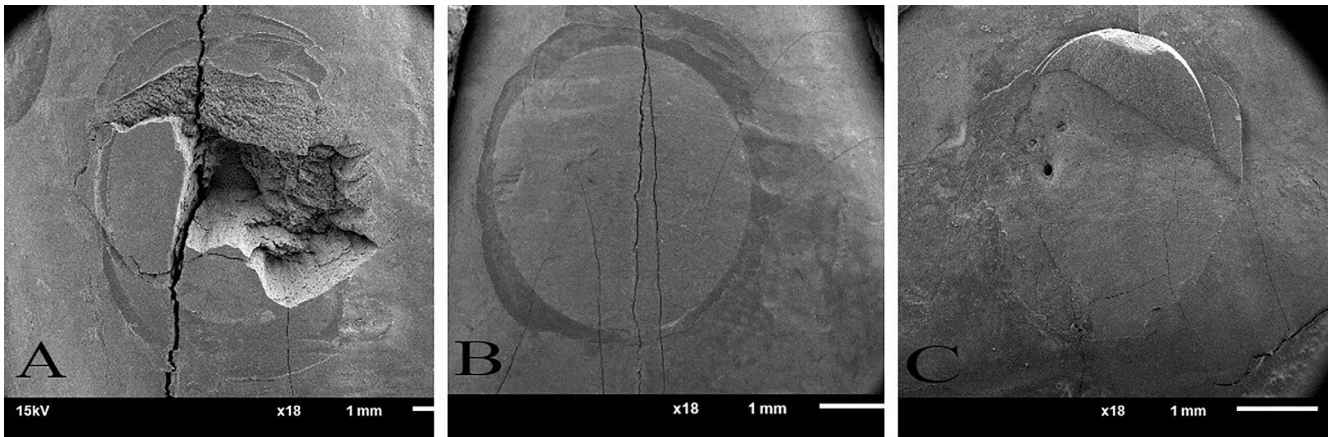


FIGURE 2 | Micrographs of enamel surfaces of failure modes at 18 \times magnification (A) Cohesive in enamel, (B) Adhesive, (C) Mixed.

superior to the other groups, which may be explained by the presence of blue covarine. The whiteness effect of the SWP group may be attributed to the inclusion of bromelain, a proteolytic enzyme from pineapple extract. A study has suggested that bromelain breaks down stains on the tooth surface into very small pieces, allowing light to reflect off the tooth surface more easily and providing a brighter smile appearance

[36]. The whiteness change in the LAW group may be due to tetrasodium pyrophosphate, which removes stains by etching them from the surface [13].

In the present study, although SWN mouthwash showed the highest ΔL , Δa , and Δb values, no significant difference was found among the mouthwashes on color change (ΔE_{00}). Thus,

the second null study hypothesis was accepted. Although whitening mouthwashes contain various chemicals such as sodium citrate, bromelain, activated charcoal, blue covarine, sodium hexametaphosphate, peroxides, enzymes, and pyrophosphates [4, 13, 14], hydrogen peroxide is a powerful oxidizing agent. However, this agent can be added to whitening mouthwashes at concentrations as low as 1%–2% for safety reasons [4]. In the formula of the whitening mouthwashes used in the present study, whitening was provided with hydrogen peroxide in RBEW, bromelain in SWP, activated charcoal in CPWC, blue covarin (color index 74,160) in SWN, tetrasodium pyrophosphate in LAW and Patent Blue V (color index 42,051) in COW, which provides optical properties. A previous study reported that color changes (ΔE) of the enamel specimens showed no significant difference between whitening mouthwashes with and without hydrogen peroxide [3], which supports our findings. Another study stated that the experimental mouth rinse with bromelain and papain had no whitening effect on stained tooth specimens compared to whitening mouthwashes [10], which conflicts with the results of this study. In this study, SWP containing bromelain showed clinically acceptable whiteness change. These different results can be explained by different experimental designs and different contents of the mouthwashes.

CPWC with activated charcoal showed no acceptable median color and whitening effect. Previous studies reported conflicting results regarding the effect of charcoal-based powder on color change (ΔE_{00}) [37, 38]. However, there are no studies determining whitening mouthwash containing activated charcoal. COW did also not show acceptable color and whiteness changes. The same outcome has been stated in a previous study, which used COW toothpaste with 12 weeks of brushing [39].

In this study, mouthwashes did not cause a significant difference in enamel bond strength compared to the control group. Thus, the third null hypothesis was rejected. Numerous studies have reported decreased bond strength immediately following teeth whitening procedures. This decrease has been attributed to the presence of peroxide within the bleached substrate, which can inhibit resin polymerization and diminish the formation and penetration of resin tags into bleached enamel [22, 23]. Therefore, waiting 2–3 weeks is recommended to ensure sufficient bond strength [26]. In this study, the use of a mouthwash (RBEW) containing hydrogen peroxide did not produce a significant difference in bond strength. The efficacy of a bleaching agent and the amount of free radicals released from bleaching agents mainly depend on the peroxide concentration and application time [22]. The mouthwash used in this study contains very low levels of hydrogen peroxide ($\approx 1.5\%$) and was applied for a very short time (1 min). Also, it has been stated that the release of free radicals and structural changes primarily occur at the surface [19]. Before adhesive application, surface conditioning with phosphoric acid might eliminate low-concentration free radicals on the surface [19], resulting in bond strength values comparable to those of the control specimens. A previous study reported that the use of a mouthwash containing natural hydrogen peroxide (3%) for 30 s caused reduced SBS to the enamel [32]. Another study stated that the use of dentifrice with carbamide and hydrogen peroxides can decrease micro-shear bonding strength in enamel samples exposed to dentifrice suspension by brushing for 15 min per day [25]. These findings contradict our

results, which may be explained by differences in the content of the materials and the way they are used.

On the other hand, charcoal-based whitening mouthwash (CPWC) led to the lowest SBS values but was statistically similar to the control group. To our knowledge, no studies evaluated the effects of charcoal-based mouthwashes on enamel bonding strength. Therefore, the findings in this study could not be directly compared with other studies. A previous study determined that a charcoal-based powder significantly reduced the microhardness of enamel samples exposed to brushing for 3 min three times a day for 14 days [38], associated with wear caused by abrasive particles. Also, it has been reported that the low pH of some mouthwashes can promote some enamel demineralization and softening, causing a reduction of the chemical bond strength of 10-methacryloyloxydecyl dihydrogen-phosphate-based adhesives [40, 41].

In our study, the mouthwash cycle procedure was applied in accordance with the manufacturers' recommendations. It was rinsed continuously in 25 mL of mouthwash for 60 s to simulate daily mouthwash care. Then, specimens were rinsed under running water for 30 s and rinsed again for 60 s. Manufacturers' instructions for use should inform consumers about the importance of complying with the products' recommended frequency and application time because consumers may want to use teeth whitening products more frequently and for extended periods. Long-term use of mouthwash may lead to possible adverse effects such as significant enamel damage [9], which is why dentists should prescribe teeth whitening products.

This in vitro study has several limitations. First, because the teeth were selected randomly, tooth ages and previous interventions applied to the teeth are unknown. Additionally, studies cannot fully simulate oral conditions. However, the results emerging from research can help design clinical trials. Further research under clinical conditions is recommended to better understand the effectiveness of whitening mouthwashes and evaluate the stability of whitening. Additionally, as consumers' interest in teeth whitening increases, new whitening technologies will be developed. It is recommended that future research addresses these situations.

5 | Conclusions

Within the limitations of this in vitro study, it was determined that:

1. R.O.C.S Black Edition White and SWN produced more acceptable median color change ($\Delta E_{00} > 1.8$).
2. The whitening mouthwashes tested caused a similar color change in enamel.
3. Signal White Now produced the highest color and whiteness changes.
4. The use of mouthwashes did not significantly affect bond strength compared to the control group. However, Colgate Plax White mouthwash containing charcoal led to the lowest mean bond strength values.

Author Contributions

Elif Varli Tekingur, Fatih Bedir, Muhammet Karadas, and Rahime Zeynep Erdem contributed to the study design, analysis, and interpretation and drafted, critically revised the manuscript, and approved the final version of the manuscript.

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Disclosure

The authors have nothing to report.

Ethics Statement

This study was approved by the Ethics Committee of RTE University to exempt the request for written informed consent (approval no. 2024/77). This study was conducted in accordance with all relevant dictates of the Declaration of Helsinki of the World Medical Association.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. A. Joiner, "The Bleaching of Teeth: A Review of the Literature," *Journal of Dentistry* 34, no. 7 (2006): 412–419.
2. M. Karadas and Z. Y. Duymus, "In Vitro Evaluation of the Efficacy of Different Over-the-Counter Products on Tooth Whitening," *Brazilian Dental Journal* 26, no. 4 (2015): 373–377.
3. J. C. Favaro, O. Geha, R. D. Guiraldo, M. B. Lopes, A. M. F. Aranha, and S. B. Berger, "Evaluation of the Effects of Whitening Mouth Rinses Combined With Conventional Tooth Bleaching Treatments," *Restorative Dentistry & Endodontics* 44, no. 1 (2019): e6.
4. F. G. Lima, T. A. Rotta, S. Penso, S. S. Meireles, and F. F. Demarco, "In Vitro Evaluation of the Whitening Effect of Mouth Rinses Containing Hydrogen Peroxide," *Brazilian Oral Research* 26, no. 3 (2012): 269–274.
5. F. F. Demarco, S. S. Meireles, and A. S. Masotti, "Over-the-Counter Whitening Agents: A Concise Review," *Brazilian Oral Research* 23, no. Suppl 1 (2009): 64–70.
6. S. S. Meireles, J. P. de Sousa, R. B. E. Lins, and F. C. Sampaio, "Efficacy of Whitening Toothpaste Containing Blue Covarine: A Double-Blind Controlled Randomized Clinical Trial," *Journal of Esthetic and Restorative Dentistry* 33, no. 2 (2021): 341–350.
7. A. Miranda Dde, C. E. Bertoldo, F. H. Aguiar, D. A. Lima, and J. R. Lovadino, "Effects of Mouthwashes on Knoop Hardness and Surface Roughness of Dental Composites After Different Immersion Times," *Brazilian Oral Research* 25, no. 2 (2011): 168–173.
8. R. W. Gerlach, H. L. Tucker, M. K. Anastasia, and M. L. Barker, "Clinical Trial Comparing 2 Hydrogen Peroxide Tooth Whitening Systems: Strips vs Pre-Rinse," *Compendium of Continuing Education in Dentistry* 26, no. 12 (2005): 874–878.
9. M. Karadas, "Efficacy of Whitening Oral Rinses and Dentifrices on Color Stability of Bleached Teeth," *Acta Biomaterialia Odontologica Scandinavica* 1, no. 1 (2015): 29–34.
10. J. Oliveira, R. S. Sarlo, E. Bresciani, and T. Caneppele, "Whitening Efficacy of Whitening Mouth Rinses Used Alone or in Conjunction With Carbamide Peroxide Home Whitening," *Operative Dentistry* 42, no. 3 (2017): 319–326.
11. A. Consolaro, "Mouthwashes With Hydrogen Peroxide Are Carcinogenic, But Are Freely Indicated on the Internet: Warn Your Patients!," *Dental Press Journal of Orthodontics* 18, no. 6 (2013): 5–12.
12. F. Gasparri, B. R. Schemehorn, and A. Zanardi, "Efficacy of Teeth Whitening With a Mouthwash: In Vitro and In Vivo Approaches," *Journal of Clinical Dentistry* 29, no. 1 (2018): 13–17.
13. O. T. Harorli and C. Barutcgil, "Color Recovery Effect of Commercial Mouth Rinses on a Discolored Composite," *Journal of Esthetic and Restorative Dentistry* 26, no. 4 (2014): 256–263.
14. H. K. Yazdi, N. Nasoohi, and M. Benvidi, "In Vitro Efficacy of Listerine Whitening Mouthwash for Color Recovery of Two Discolored Composite Resins," *Frontiers in Dentistry* 16, no. 3 (2019): 181–186.
15. H. Eimar, R. Siciliano, M. N. Abdallah, et al., "Hydrogen Peroxide Whitens Teeth by Oxidizing the Organic Structure," *Journal of Dentistry* 40, no. Suppl 2 (2012): e25–e33.
16. C. J. Tredwin, S. Naik, N. J. Lewis, and C. Scully, "Hydrogen Peroxide Tooth-Whitening (Bleaching) Products: Review of Adverse Effects and Safety Issues," *British Dental Journal* 200, no. 7 (2006): 371–376.
17. C. R. Torres, L. C. Perote, N. C. Gutierrez, C. R. Pucci, and A. B. Borges, "Efficacy of Mouth Rinses and Toothpaste on Tooth Whitening," *Operative Dentistry* 38, no. 1 (2013): 57–62.
18. I. M. Jaime, F. M. Franca, R. T. Basting, C. P. Turssi, and F. L. Amaral, "Efficacy of Hydrogen-Peroxide-Based Mouthwash in Altering Enamel Color," *American Journal of Dentistry* 27, no. 1 (2014): 47–50.
19. Y. L. Cheng, J. Musonda, H. Cheng, T. Attin, M. Zheng, and H. Yu, "Effect of Surface Removal Following Bleaching on the Bond Strength of Enamel," *BMC Oral Health* 19, no. 1 (2019): 50.
20. S. Vidhya, S. Srinivasulu, M. Sujatha, and S. Mahalaxmi, "Effect of Grape Seed Extract on the Bond Strength of Bleached Enamel," *Operative Dentistry* 36, no. 4 (2011): 433–438.
21. G. E. Kunt, N. Yilmaz, S. Sen, and D. O. Dede, "Effect of Antioxidant Treatment on the Shear Bond Strength of Composite Resin to Bleached Enamel," *Acta Odontologica Scandinavica* 69, no. 5 (2011): 287–291.
22. M. Karadas and S. Demirbuga, "Influence of a Short-Time Antioxidant Application on the Dentin Bond Strength After Intracoronary Bleaching," *Microscopy Research and Technique* 82, no. 10 (2019): 1720–1727.
23. A. L. Briso, R. M. Toseto, V. Rahal, P. H. dos Santos, and G. M. Ambrosano, "Effect of Sodium Ascorbate on Tag Formation in Bleached Enamel," *Journal of Adhesive Dentistry* 14, no. 1 (2012): 19–23.
24. A. Braun, S. Jepsen, and F. Krause, "Spectrophotometric and Visual Evaluation of Vital Tooth Bleaching Employing Different Carbamide Peroxide Concentrations," *Dental Materials* 23, no. 2 (2007): 165–169.
25. A. L. Briso, R. M. Toseto, A. M. de Arruda, P. R. Tolentino, R. S. de Alexandre, and P. H. dos Santos, "Evaluating the Bonding of Two Adhesive Systems to Enamel Submitted to Whitening Dentifrices," *Acta Odontologica Latinoamericana* 23, no. 2 (2010): 111–116.
26. S. Gurgan, T. Alpaslan, A. Kiremitci, F. Y. Cakir, E. Yazici, and J. Gorucu, "Effect of Different Adhesive Systems and Laser Treatment on the Shear Bond Strength of Bleached Enamel," *Journal of Dentistry* 37, no. 7 (2009): 527–534.

27. R. D. Paravina, R. Ghinea, L. J. Herrera, et al., "Color Difference Thresholds in Dentistry," *Journal of Esthetic and Restorative Dentistry* 27, no. Suppl 1 (2015): S1–S9.
28. M. M. Perez, L. J. Herrera, F. Carrillo, et al., "Whiteness Difference Thresholds in Dentistry," *Dental Materials* 35, no. 2 (2019): 292–297.
29. R. D. Paravina, M. M. Perez, and R. Ghinea, "Acceptability and Perceptibility Thresholds in Dentistry: A Comprehensive Review of Clinical and Research Applications," *Journal of Esthetic and Restorative Dentistry* 31, no. 2 (2019): 103–112.
30. P. Ntovas, K. Masouras, and P. Lagouvardos, "Efficacy of Non-Hydrogen Peroxide Mouthrinses on Tooth Whitening: An In Vitro Study," *Journal of Esthetic and Restorative Dentistry* 33, no. 7 (2021): 1059–1065.
31. C. Wang, Y. Ou, L. Zhang, et al., "Effects of Regional Enamel and Prism Orientations on Bovine Enamel Bond Strength and Cohesive Strength," *European Journal of Oral Sciences* 126, no. 4 (2018): 334–342.
32. Z. C. Ozduman, B. Oglakci, M. Dogan, C. Deger, and D. E. Eliguzeloglu, "How Does Antiseptic Mouthwashes Against SARS-COV-2 Affect the Bond Strength of Universal Adhesive to Enamel?," *Microscopy Research and Technique* 85, no. 3 (2022): 1199–1208.
33. K. Sai, T. Takamizawa, A. Imai, et al., "Influence of Application Time and Etching Mode of Universal Adhesives on Enamel Adhesion," *Journal of Adhesive Dentistry* 20, no. 1 (2018): 65–77.
34. C. Gomez-Polo, M. Portillo Munoz, M. C. Lorenzo Luengo, P. Vicente, P. Galindo, and A. M. Martin Casado, "Comparison of the CIELab and CIEDE2000 Color Difference Formulas," *Journal of Prosthetic Dentistry* 115, no. 1 (2016): 65–70.
35. L. Z. Collins, M. Naeeni, and S. M. Platten, "Instant Tooth Whitening From a Silica Toothpaste Containing Blue Covarine," *Journal of Dentistry* 36, no. Suppl 1 (2008): S21–S25.
36. E. A. Munchow, H. J. Hamann, M. T. Carvajal, R. Pinal, and M. C. Bottino, "Stain Removal Effect of Novel Papain- and Bromelain-Containing Gels Applied to Enamel," *Clinical Oral Investigations* 20, no. 8 (2016): 2315–2320.
37. M. C. Franco, J. Uehara, B. M. Meroni, G. S. Zuttion, and M. S. Cenci, "The Effect of a Charcoal-Based Powder for Enamel Dental Bleaching," *Operative Dentistry* 45, no. 6 (2020): 618–623.
38. A. G. Emidio, V. Silva, E. P. Ribeiro, et al., "In Vitro Assessment of Activated Charcoal-Based Dental Products," *Journal of Esthetic and Restorative Dentistry* 35, no. 2 (2023): 423–430.
39. U. Koc Vural, Z. Bagdatli, A. E. Yilmaz, F. Yalcin Cakir, E. Altundasar, and S. Gurgan, "Effects of Charcoal-Based Whitening Toothpastes on Human Enamel in Terms of Color, Surface Roughness, and Microhardness: An In Vitro Study," *Clinical Oral Investigations* 25, no. 10 (2021): 5977–5985.
40. W. F. Vieira-Junior, L. N. Ferraz, M. Giorgi, G. Ambrosano, F. Aguiar, and D. Lima, "Effect of Mouth Rinse Treatments on Bleached Enamel Properties, Surface Morphology, and Tooth Color," *Operative Dentistry* 44, no. 2 (2019): 178–187.
41. J. Fehrenbach, C. P. Isolan, and E. A. Munchow, "Is the Presence of 10-MDP Associated to Higher Bonding Performance for Self-Etching Adhesive Systems? A Meta-Analysis of In Vitro Studies," *Dental Materials* 37, no. 10 (2021): 1463–1485.