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The effects of the use of whitening mouthwash after home bleaching on the color stability and surface hardness of enamel

Iklima Mehmethanoglu[1*], Ipek Cubukcu[1], Neslihan Celik[1] and Pinar Gul[1]

Abstract

Background To evaluate the effectiveness of whitening mouthwashes, both with and without hydrogen peroxide (HP), after at-home teeth whitening in preserving the achieved whiteness and assessing their impact on enamel surface hardness.

Methods One hundred extracted human premolar teeth were divided into two groups, and home bleaching agents (Philips Zoom NiteWhite 22% Carbamide Peroxide, Ultradent Opalescence PF 16% Carbamide Peroxide) were applied to the groups. The teeth subjected to home bleaching were further divided into five subgroups, and each subgroup underwent a four-week cycle of application of a coloring agent and whitening mouthwash (Colgate Optic White (COW)(%2 HP), Rocs Black Edition (Rocs) (%1 HP), SPLAT Professional Bioactive gargle white Plus (SPLAT) (Ananas sativus fruit extract containing), Listerine Advanced White (Listerine) (Pyrophsphate containing), and distilled water (DW) (control)). Color and microhardness measurements were recorded at baseline, after home bleaching, and after treatment with whitening mouthwashes.

Results Compared with those treated with Opalescence PF, the samples treated with Zoom achieved a significantly greater degree of whitening ($p < 0.001$). When the effects of postwhitening mouthwash were evaluated (ΔE_{002}), Listerine presented the lowest ΔE_{002} value in both home bleaching groups, whereas the control group presented the highest ΔE_{002} value. Both home-bleaching agents caused a significant increase in the initial WI_D values ($p < 0.05$). After home-bleaching, the increased WI_D values (WI_{D2}) significantly decreased in all mouthwash groups following exposure to the staining and whitening mouthwash cycle (WI_{D3}) ($p < 0.05$). The impact of home bleaching agents on microhardness was not significantly different ($p = 0.151$). When we examined the impact of whitening mouthwashes on microhardness, in the Zoom group, no statistically significant difference was observed in surface hardness ($p > 0.05$). However, in the Opalescence PF group, only the Listerine group showed a statistically significant increase ($p < 0.05$).

Conclusions Higher concentrations of carbamide peroxide provide faster and more effective whitening. Whitening mouthwash containing HP and pyrophosphate is effective in maintaining tooth whiteness after home bleaching; however, its impact on enamel microhardness depends on the formulation. Notably, only Listerine in the Opalescence PF group significantly increased surface hardness. Given the drawbacks of HP, pyrophosphate-based mouthwash may serve as a safer alternative.

*Correspondence:
Iklima Mehmethanoglu
gndgdiklima@gmail.com

Keywords Home bleaching, Hydrogen peroxide, Pyrophosphate, Pineapple extract, Whitening mouthwash, Color stability, Enamel surface hardness

Introduction

With increasing interest in esthetics in recent years, patients desire a perfect smile [1]. This has led to an increased demand for tooth whitening [2, 3]. Today, the most conservative way to change the color of teeth is through tooth whitening procedures [4]. Teeth whitening can be achieved through techniques performed under the supervision of dentists and by patients individually using over-the-counter (OTC) products available in supermarkets or pharmacies without the need for a dentist's prescription [5, 6].

Dentists employ two primary techniques: the 'in-office whitening' technique, which uses high concentrations of whitening agents (30–35% hydrogen peroxide (HP)), and the 'at-home whitening' technique, which uses low concentrations of whitening agents (10–20% carbamide peroxide (CP)). The technique most commonly applied by dentists for vital teeth is the at-home whitening technique [7, 8].

OTC products, also known as 'over-the-counter' products in the literature, include dental trays, strips, whitening products, toothpaste, and mouthwash [9]. Mouth whitening has gained significant popularity among OTC products because of its ease of use and cost-effectiveness [10]. The primary active ingredient in these products is often HP [11]. However, given the potential risks associated with peroxide-based whitening treatments, such as their low pH and uncontrolled application in the oral cavity, which can lead to local side effects such as oral mucosal irritation, pulp sensitivity, pulpitis, or alterations in the enamel surface, there has been a quest to develop whitening mouthwashes containing alternative agents to HP [12–16]. Pyrophosphates are among the alternative agents. They adhere to hydroxyapatite in the tooth structure, hindering mineral accumulation at calcium sites and reducing the formation of dental calculus, which leads to tooth staining [17]. Tetrasodium pyrophosphate chemically removes external stains on teeth and inhibits the absorption of new chromogens into teeth, thereby preventing changes in tooth color [18]. With the growing interest in natural agents in recent years, the use of natural enzymes obtained from plants for this purpose has garnered increasing attention. Natural proteolytic enzymes derived from plants, such as bromelain, have been incorporated into toothpaste and mouthwash for research into their effectiveness in tooth whitening [19, 20]. Bromelain, a proteolytic enzyme obtained from pineapples (Ananas comosus), prevents the buildup of oral microorganisms and stains the tooth surface by hydrolyzing the pellicle [20, 21].

The whiteness achieved after a tooth whitening procedure may fade over time [22]. OTC products can assist in maintaining color after whitening procedures because of their effectiveness in removing stains [11, 23]. However, the American Dental Association (ADA) Council on Scientific Affairs has expressed concerns about the long-term safety of unsupervised whitening procedures due to potential undiagnosed or underlying oral health issues [24–26]. New products enter the market every day. It is important to evaluate not only the impact of these products on postwhitening color retention but also their effects on dental tissues.

This study aims to evaluate the color stability and surface hardness of teeth after home bleaching using whitening mouthwashes containing hydrogen peroxide (Colgate Optic White (COW)(2% HP), Rocs Black Edition (Rocs)(1% HP)) and not containing hydrogen peroxide (SPLAT® Professional Bio-active mouthwash White Plus (SPLAT)(Ananas sativus fruit extract containing), Listerine Advanced White (Listerine)(Pyrophsphate containing)). Our null hypotheses are as follows:

1. There is no significant difference was observed in the effect of CP agents at different concentrations on enamel color.
2. The use of different whitening mouthwashes does not create a significant difference in maintaining the achieved whiteness after the home bleaching procedure.
3. Different concentrations of CP agents do not have different effects on enamel surface hardness.
4. The use of different whitening mouthwashes after a home whitening procedure does not cause different effects on enamel surface hardness.

Materials and methods

Experimental design

This study was approved by the Atatürk University Faculty of Dentistry Ethics Committee (decision no. 68, dated 22.06.2022). In this study, 100 extracted human premolar teeth were utilized. Two different home bleaching agents, 22% CP (Philips Zoom NiteWhite 22% CP, DiscusDental, USA) and 16% CP (Opalescence PF 16% CP, Ultradent Products Inc., South Jordan, UT, USA), were applied to the teeth. The teeth treated with home bleaching underwent a cycle of staining agent and

whitening mouthwash application. Four distinct whitening mouthwashes, namely, COW(%2 HP), Rocs (%1 HP), SPLAT (Ananas sativus fruit extract containing), and Listerine (Pyrophsphate containing), were employed. The experimental design is presented in Fig. 1.

Specimen preparation

The power analysis, conducted via G*Power 3.1.9.4 software from Heinrich Heine University Düsseldorf, indicated that a minimum of 10 samples per experimental group should be included in this study, with a test power of 96. Human premolar teeth were extracted and then cut approximately 2 mm below the enamel–cementum junction via a precision electric cutter (Isomet 1000, Buehler, Lake Bluff, IL, USA) to separate the crowns from the roots. The crown portions were bisected in a buccolingual direction to utilize flatter enamel surfaces. The obtained enamel surfaces were embedded in acrylic resin via cylindrical silicone molds measuring 5 mm in height and 10 mm in diameter. This ensured that the enamel surfaces were centrally aligned and parallel to the ground. To achieve a uniform enamel surface, the prepared samples were sequentially polished with silicone carbide papers with grit sizes of 600, 800, and 1200, each for 20 s. Finally, the samples were stored in distilled water at 37 °C.

Microhardness measurement

Microhardness measurements of the samples were taken at three different time points: initially (Measurement 1, M1), after home bleaching (Measurement 2, M2), and following exposure to the staining and whitening mouthwash cycle (Measurement 3, M3). These measurements were conducted via a microhardness testing machine (Vickers-Fm800, Tokyo, Japan). A standard force of 100 Newtons was applied to the samples for 15 s. Indentations formed on the samples were identified under 40× magnification, and the lengths of the horizontal and vertical lines of each indentation were marked for measurement. The machine, which uses a program that employs these measurements as data, calculates the microhardness values. Three measurements were taken at different points on each sample, and the arithmetic average of these measurements was considered the microhardness value (VHN) for the sample. The initial surface microhardness

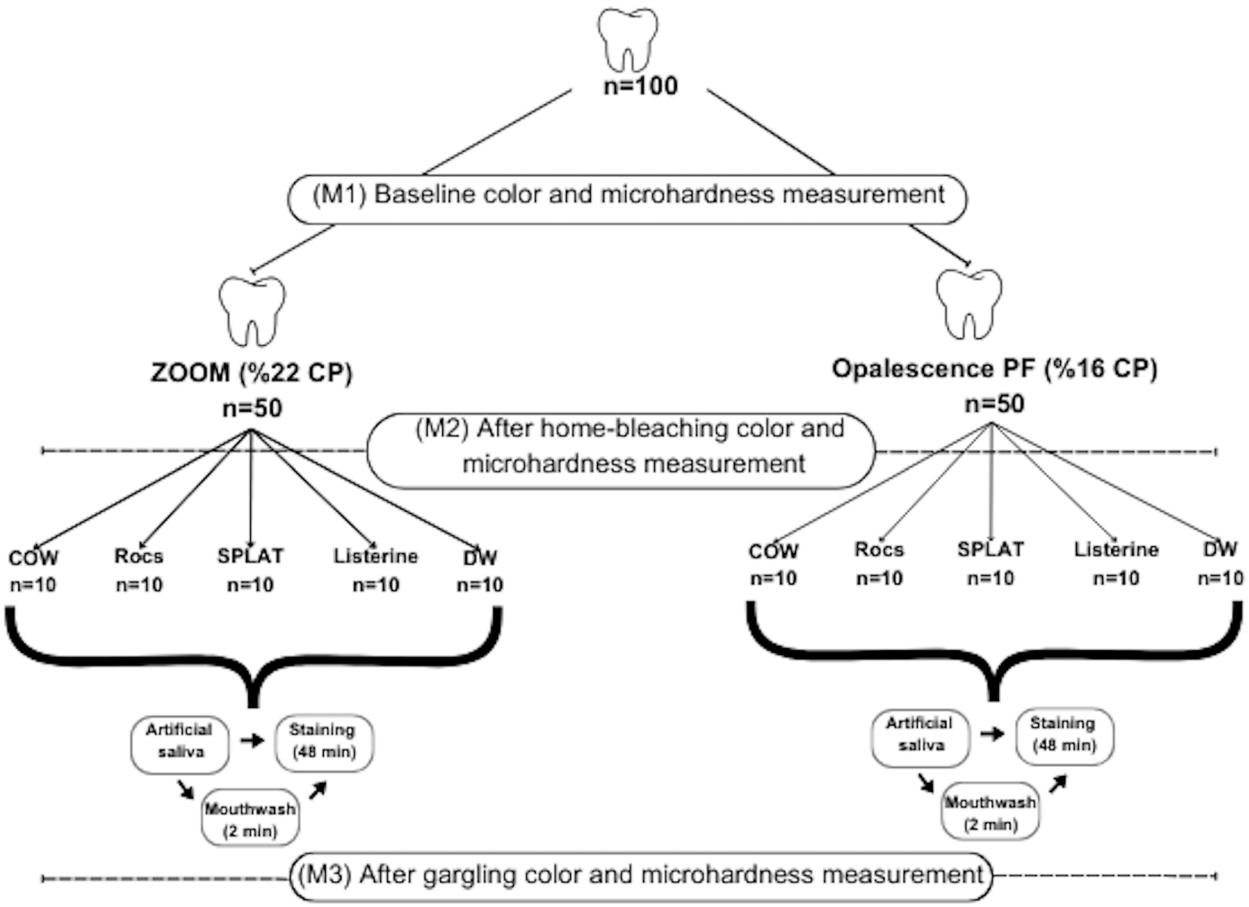

Fig. 1 Experimental design

values were statistically compared among the groups, and no significant difference was found ($p > 0.05$); therefore, the specimens were randomly distributed.

Color measurement

Three measurements were taken for each sample at three different times: initially (M1), after home bleaching (M2), and following exposure to the staining and whitening mouthwash cycle (M3). The initial color measurements were conducted 24 h after sample preparation. The color measurements were repeated three times for each sample. All color measurements were performed according to the CIEDE2000 color coordinates relative to the D65 standard illumination against a standard white background (L: 94.6, a: 0.2, b: −0.8) via a clinical spectrophotometer (SpectroShade MicroTM, Verona, İtalya). The color change values were calculated via CIEDE2000 (ΔE_{00}) between M1 and M2, between M2 and M3, and between M1 and M3. For CIEDE2000, the perceptibility threshold is defined as $\Delta E_{00} > 0.8$ units, and the acceptability threshold is defined as $\Delta E_{00} \leq 1.8$ units at a 50%:50% ratio [27, 28].

In addition, the Whiteness index (WI_D) of the samples was measured at baseline (M1), after home bleaching (M2), and after exposure to staining and whitening mouthwash cycle (M3). The equation used to calculate the Whiteness index WI_D was:

$$WID = 0.511L * -2.324a * -1.100b *$$

WI_D is a simple linear formulation obtained from the values of the three CIELAB chromatic coordinates and has a clear interpretation: high positive values of the WID index indicate whiter height values of the sample, while low (even negative) values indicate lower whiteness values of the sample [27, 29, 30].

Whitening procedure

In accordance with the manufacturer's instructions, a 22% CP whitening agent (Philips Zoom NiteWhite 22% Carbamide Peroxide, DiscusDental, USA) was applied for 2 h a day for 7 days, and a 16% CP (Opalescence PF 16% Carbamide Peroxide, Ultradent Products Inc., South Jordan, UT, USA) application agent was used for 4 h a day for 14 days (Table 1).

Staining procedure

The average daily coffee consumption was 3.2 cups, with each cup taking 15 min to consume [31]. Consequently, the daily coffee consumption time was 48 min. The staining solution was prepared by dissolving 2 g of soluble instant coffee (Nescafe Classic, Nestle SA, Vevey, Switzerland) in 200 ml of boiling water, following the manufacturer's recommendation, at room temperature. As specified in the experimental design, the samples were exposed to coffee for 48 min every day for four weeks. After staining, the samples were rinsed in deionized water and cleaned ultrasonically for 2 min.

Mouthwash exposure procedure

As specified in the experimental design, the samples were exposed to a 2-min gargling routine every day for 4 weeks. The contents of the mouthwashes used are listed in Table 2.

Statistical analysis

The obtained data were analyzed via the SPSS 20 (IBM, Chicago, IL, USA) statistical package program. First, the distribution of the data and the homogeneity of variance were analyzed via the Kolmogorov–Smirnov and Levene tests. Repeated-measures ANOVA was used to compare the ΔE_{00}, VHN and WI_D values values obtained as a result of bleaching and mouth rinse solution application to teeth. One-way ANOVA and Tukey post hoc tests were used to compare the mouth rinse solutions for each bleaching group in each period. In addition, one-way ANOVA and Tukey post hoc tests were used for the general comparison of mouth rinses, and the independent samples t test was used for the general comparison of bleaching methods. The statistical significance level was set at $p < 0.05$.

Results

Color results

The results of the repeated-measures ANOVA (Table 3) indicated a significant effect of the period, period-bleaching, and period-mouth rinses interactions on color change (ΔE_{00}) ($p < 0.05$.)

The ΔE_{00} values for all groups at different time intervals are provided in Table 4. In the table, the color measurement difference between the baseline and posthome

Table 1 Home bleaching agents used in the study

Products	Manufacturer	Components	LOT no
Philips Zoom NiteWhite 22% Carbamide Peroxide	Discus Dental, ABD	%22 Carbamide Peroxide	19,207,021
Opalescence PF 16% Carbamide Peroxide	Ultradent Products Inc., South Jordan, UT, USA	%16 Carbamide Peroxide, NaF	BGGL7

Table 2 Mouth whitening

Products	Manufacturer	Components
Colgate Optic White (COW)	Colgate-Palmolive Company NY, USA	Ingredients Water, Glycerin, Propylene Glycol, Sorbitol, Hydrogen Peroxide (%2), Polysorbate 20, Sodium Acrylates/Methacryloylethyl Phosphate Copolymer, Phosphoric Acid, Citric Acid, Flavor, PVM/MA Copolymer, Sodium Saccharin
Rocs Black Edition (Rocs)	R.O.C.S., Tallınn, Estonia	Aqua, Glycerin, PEG-40 Hydrogenated Castor Oil, Hydrogen Peroxide (1%), Citric Acid, Aroma, Sodium Hydroxide, Sodium Saccharin
SPLAT® Professional Bıo-actıve gargara Whıte Plus (SPLAT)	STS Holding Group LTD, Gabrovo, Bulgaria	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 Fruit Extract, Maltodextrin, Thymus Serpillum 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
Listerine Advanced White (Listerine)	Johnson & Johnson Healthcare Products, Skillman, NJ, USA	Aqua, Alcohol, Sorbitol, Tetrapotassium Pyrophosphate, Tetrasodium Pyrophosphate, Pentasodium Triphosphate, CitricAcid, Poloxamer 407, Sodium Benzoate, Eucalyptol, Thymol, Menthol, Sodium Saccharin, Sodium Fluoride, Propylene Glycol, Sucralose, Aroma, DisodiumPhosphate
Distilled water (DW)	-	-

Table 3 Results of repeated-measures ANOVA for the ΔE_{00} values

Source	SS	df	MS	F	p
Period	73.315	2.000	36.657	56.194	< 0.001*
Period* Bleaching	7.922	2.000	3.961	6.072	0.003*
Period* Mouthrinses	114.972	8.000	14.371	22.031	< 0.001*
Period* Bleaching * Mouthrinses	6.652	8.000	0.832	1.275	0.259

SS Sum of squares, *MS* mean of squares

*$p < 0.05$ significant

bleaching data is shown as ΔE_{001}, the difference between the posthome bleaching and postwhitening mouth rinsing cycles is shown as ΔE_{002}, and the difference in color between the baseline and postwhitening mouth rinsing cycles is shown as ΔE_{003}.

After home bleaching, both groups presented noticeable color changes ($\Delta E_{00} > 0.8$). Compared with those treated with Opalescent PF, the samples treated with Zoom home-bleaching agents whitened more, and this difference was statistically significant ($p < 0.001$). When

Table 4 ΔE_{00} (mean ± standard deviation) values of the groups and statistical comparison results

BLEACHING	MOUTHRINSE	ΔE_{001} (M1-M2)	ΔE_{002} (M2-M3)	ΔE_{003} (M1-M3)
	COW	3.89 ± 0.53^{aA}	1.72 ± 0.99^{bA}	2.84 ± 0.60^{cBC}
	Rocs	4.03 ± 1.39^{aA}	2.48 ± 0.93^{bAB}	1.83 ± 0.63^{cAB}
Zoom (% 22CP)	SPLAT	3.39 ± 0.73^{aA}	3.25 ± 0.64^{aBC}	0.99 ± 0.45^{bA}
	Listerine	3.83 ± 0.80^{aA}	1.21 ± 0.74^{bA}	3.02 ± 0.96^{cC}
	DW	3.60 ± 064^{aA}	4.37 ± 2.00^{aC}	2.35 ± 1.56^{bBC}
		$p = 0.509$	$p < 0.001$	$p < 0.001$
	COW	2.68 ± 0.66^{aAB}	1.32 ± 1.09^{bA}	1.77 ± 0.78^{bA}
	Rocs	2.54 ± 0.81^{aAB}	1.41 ± 0.73^{bA}	1.52 ± 0.89^{bA}
Opalescence PF (% 16 CP)	SPLAT	3.26 ± 0.63^{aB}	3.10 ± 1.23^{aB}	1.52 ± 0.83^{bA}
	Listerine	2.15 ± 0.62^{aA}	0.91 ± 0.46^{bA}	1.90 ± 0.87^{aA}
	DW	2.42 ± 0.47^{aA}	3.87 ± 1.76^{bB}	2.23 ± 1.26^{aA}
		$p = 0.007$	$p < 0.001$	$p = 0.414$

$p < 0.05$: Statistically significant. Lowercase letters in the same row show the results of the comparison of ΔE values between periods for each group (repeated-measures ANOVA). Capital letters in the same column show the comparison results of mouth rinse solutions for each bleaching group (one-way ANOVA). Different letters in the same column or row indicate statistical significance

the effect of mouth rinses after whitening (ΔE_{002}) was evaluated, in both home bleaching groups, the smallest ΔE_{002} value was observed with Listerine, whereas the largest ΔE_{002} value was observed in the DW (control) group. The ΔE_{002} ranking in both home bleaching groups was as follows: Listerine < COW < Rocs < SPLAT < DW.

Compared with the DW (control) group, the COW, Roc, and Listerine groups presented lower ΔE_{002} values, and the differences among them were statistically significant ($p < 0.05$). On the other hand, the SPLAT group statistically resembled the DW (control) group ($p > 0.05$). COW and listerine in both the home bleaching group and Rocs in the Zoom group presented ΔE_{002} values below the clinically acceptable threshold ($\Delta E_{00} < 1.8$), indicating their effectiveness in maintaining tooth whiteness through home bleaching. The SPEAT, in both home bleaching groups, presented ΔE_{002} values above the clinically acceptable threshold, indicating a similar effect to that in the DW group in preserving the whiteness of the achieved teeth.

An examination of the ΔE_{003} values revealed noticeable color changes ($\Delta E_{00} > 0.8$) in all the subgroups. In the Zoom group, the COW, Rocs, Listerine, and DW subgroups presented ΔE_{003} values below the clinically acceptable threshold. In the Opalescence PF group, the COW, Roc, and SPLAT subgroups presented values below this threshold, whereas the Listerine and DW groups presented values above it. Considering the previous ΔE_{00} values, COW, Rocs, and listerine significantly whitened the initial enamel color, while it darkened for DW.

The results of ANOVA analysis for repeated measures (Table 5) showed that there was a significant effect of period, period-whitening and period-mouthwash interactions on the Whiteness index (WI_D) ($p < 0.05$).

The statistical comparison results of the mean WI_D values of the groups are presented in Tables 6. Both home-bleaching agents caused a significant increase in the initial WI_D values ($p < 0.05$). However, there was no significant difference between the two home-bleaching agents ($p > 0.05$).

After home-bleaching, the increased WI_D values (WI_{D2}) significantly decreased in all mouthwash groups following exposure to the staining and whitening mouthwash cycle (WI_{D3}) ($p < 0.05$). However, COW and Listerine remained significantly higher than the initial WI_D

Table 5 Results of Repeated Measures ANOVA for WI_D values

Source	SS	df	MS	F	p
Period	2999.077	2.000	1499.539	304.023	< 0.001*
Period* Bleaching	150.752	2.000	75.376	15.282	< 0.001*
Period* Mouthrinses	105.549	8.000	13.194	2.675	0.008*
Period* Bleaching * Mouthrinses	30.554	8.000	3.819	0.774	0.626

SS Sum of squares, *MS* mean of squares

*$p < 0.05$ significant

Table 6 WI_D (mean ± standard deviation) values of the groups and statistical comparison results

BLEACHING	MOUTHRINSE	WI_{D1} (M1)	WI_{D2} (M2)	WI_{D3} (M3)
	COW	−21.84 ± 11.59 ^{aAB}	−13.48 ± 11.81 ^{bABC}	−17.44 ± 12.18 ^{cAB}
Zoom (% 22CP)	Rocs	−17.09 ± 4.72 ^{aAB}	−7.65 ± 3.54 ^{bBC}	−14.04 ± 5.70 ^{cAB}
	SPLAT	−28.67 ± 9.89 ^{aA}	−19.86 ± 10.94 ^{bA}	−26.79 ± 11.69 ^{aA}
	Listerine	−28.49 ± 11.32 ^{aA}	−18.34 ± 10.66 ^{bAB}	−22.74 ± 11.18 ^{cAB}
	DW	−16.63 ± 6.15 ^{aB}	−6.50 ± 6.53^{bC}	−13.36 ± 8.02 ^{cB}
		$p = 0.006$*	$p = 0.005$*	$p = 0.019$*
	COW	−18.27 ± 5.54 ^{aA}	−13.03 ± 6.77^{bA}	−15.34 ± 6.29 ^{cA}
	Rocs	−23.31 ± 9.22 ^{aA}	−17.60 ± 10.32 ^{bA}	−21.46 ± 9.56 ^{aA}
Opalescence PF (% 16 CP)	SPLAT	−16.97 ± 6.55 ^{aA}	−9.16 ± 7.23^{bA}	−15.06 ± 7.17 ^{aA}
	Listerine	−19.75 ± 9.60 ^{aA}	−13.41 ± 9.78^{bA}	−15.14 ± 10.40 ^{cA}
	DW	−23.38 ± 9.82 ^{aA}	−18.21 ± 10.08 ^{bA}	−20.30 ± 8.95^{bA}
		$p = 0.314$	$p = 0.165$	$p = 0.270$

* $p < 0.05$: Statistically significant. Lowercase letters in the same row show the results of comparison of WI_D values between periods for each group (Repeated Measures ANOVA). Capital letters in the same column show comparison results of mouthrinse solutions for each bleaching group (One-way ANOVA). Different letters in the same column or row indicate statistical significance

Table 7 Results of repeated-measures ANOVA for VHN values

Source	SS	df	MS	F	p
Period	83,896.417	2.000	41,948.208	24.714	< 0.001*
Period* Bleaching	9020.920	2.000	4510.460	2.657	0.073
Period* Mouthrinses	11,060.989	8.000	1382.624	0.815	0.591
Period* Bleaching * Mouthrinses	26,342.608	8.000	3292.826	1.940	0.057

SS Sum of squares, *MS* mean of squares

*$p < 0.05$ significant

Table 8 VHN (mean ± standard deviation) values of the groups and statistical comparison results

BLEACHING	MOUTHRINSE	M1	M2	M3
	COW	264.99 ± 40.06 [a]	282.11 ± 49.90 [a]	263.42 ± 35.45 [a]
Zoom (% 22 CP)	Rocs	245.97 ± 32.79 [a]	245.23 ± 64.36[a]	273.06 ± 56.86 [a]
	SPLAT	220.60 ± 38.15 [a]	260.54 ± 68.36[a]	238.37 ± 64.65 [a]
	Listerine	230.37 ± 44.68 [a]	281.45 ± 27.44[b]	287.19 ± 45.22 [b]
	DW	228.73 ± 24.71 [a]	238.26 ± 45.27 [ab]	262.56 ± 34.83 [b]
		$p = 0.074$	$p = 0.235$	$p = 0.271$
	COW	220.84 ± 26.92 [a]	240.06 ± 52.21 [ab]	270.23 ± 52.09 [b]
	Rocs	206.57 ± 40.13 [a]	284.94 ± 35.77 [b]	279.37 ± 40.95 [b]
Opalescence PF (% 16 CP)	SPLAT	204.41 ± 35.23 [a]	240.37 ± 46.54 [ab]	251.46 ± 27.67 [b]
	Listerine	236.02 ± 58.56 [a]	236.79 ± 67.14 [a]	282.77 ± 32.27 [b]
	DW	201.29 ± 43.81 [a]	227.47 ± 51.90 [ab]	251.04 ± 47.73 [b]
		$p = 0.334$	$p = 0.130$	$p = 0.270$

Lowercase letters in the same row show the results of the comparison of VHN values between periods for each group (repeated-measures ANOVA). Different letters in the same row indicate statistical significance ($p < 0.05$)

values (WI_{D1}) in both home-bleaching protocols, while SPLAT showed values similar to the initial ones. Rocs maintained a higher value than the initial WI_D value in the Zoom group, while Opalescence PF showed values similar to the initial ones.

In the Zoom group, after the staining and whitening mouthwash cycle, the SPLAT group had significantly lower WI_D values compared to the control group ($p = 0.019$), while the other groups showed WI_D values similar to the control group. In the Opalescence PF group, all mouthwash groups had similar WI_D values to the control group ($p = 0.270$).

Microhardness test results

The results of the repeated-measures ANOVA (Table 7) indicated a significant effect of period on surface hardness (VHN) ($p < 0.001$).

The hardness test results are presented in Table 8. Both home-bleaching agents generally increased the surface hardness. However, this increase was not typically statistically significant ($p > 0.05$). When the effects of home bleaching agents on microhardness were compared, the difference among them was not statistically significant ($p = 0.151$).

When whitening mouth rinses were applied to teeth subjected to home bleaching, in the Zoom group, none of the mouth rinses caused a statistically significant difference in surface hardness ($p > 0.05$). In the Opalescence PF group, only listerine resulted in a statistically significant increase in surface hardness ($p < 0.05$).

Discussion

In this study, the effects of two different home bleaching agents, one containing 22% CP and the other containing 16% CP, on the color and surface hardness of tooth enamel were compared. In addition, the effectiveness of whitening mouthwashes containing HP and alternative whitening agents to HP in maintaining the whiteness obtained after home bleaching application and their effects on the surface hardness of enamel were investigated. These results partially supported the first, second, and fourth hypotheses, while the third hypothesis was fully supported.

Coffee, one of the most common teeth-staining agents to which teeth are exposed daily, was used to standardize the discoloration procedure during the mouth rinse cycle. The average daily coffee consumption was 3.2 cups, and the duration of consuming one cup of coffee was

approximately 15 min [31]. Accordingly, in this study, samples were exposed to the coffee solution for 48 min, which represented the daily duration of coffee consumption. Significant color changes were observed in the control group exposed to coffee after both home bleaching applications, but no mouth rinses were applied.

Based on the obtained data, the application of 22% CP, despite a shorter treatment period (2 h per day for 7 days), resulted in a significant increase in ΔE_{00} values, while the increase in WI_D values was statistically similar to that of the 16% CP application (4 h per day for 14 days), partially supporting the first hypothesis. Our results suggest that, contrary to Farawati et al. [32], a higher concentration of CP may enhance color change in a shorter period. Similarly, Kıhn et al. [33], in their in vivo studies, reported that 35% CP provided significantly higher ΔE_{00} values than 16% CP**,** which aligns with the results of the present study.

The application of COW and Listerine in both home-bleaching groups, and Rocs in the Zoom group, resulted in ΔE_{002} values ($\Delta E_{00} < 1.8$) below the clinically acceptable threshold and WI_D values higher than the baseline values, indicating that whitening mouthwashes containing HP (COW, Rocs) and pyrophosphate (Listerine) effectively maintained the whitening effect achieved after home-bleaching. Although the pyrophosphate-containing mouthwash had the lowest ΔE_{002} values, it was found to be significantly more effective compared to HP-containing whitening mouthwashes. There was no statistically significant difference between the 2% and 1% HP-containing whitening mouthwashes. The whitening mouthwash containing pineapple extract (SPLAT) showed ΔE_{002} values above the clinically acceptable threshold in both home-bleaching groups, WI_D values similar to baseline levels, and significantly lower WI_D values compared to the control group in the Zoom group. This indicates that this mouthwash is ineffective in maintaining the whitening effect after home whitening and is statistically similar to the control group. These results partially support the second hypothesis. Our results regarding HP appeared to be consistent with the results of other studies in the literature [3, 34, 35]. Unlike our results, Ntovas et al. [17] reported that pyrophosphate mouth rinses do not provide significant whitening. In the present study, unlike that of Ntovas et al., the enamel sample surface was abraded, which may have led to the removal of the more resistant aprismatic layer in the outer layer and a decrease in enamel thickness, allowing whitening agents to penetrate the tooth structure more effectively.

HP, the most commonly used agent for tooth whitening, has raised safety concerns because of its association with various adverse effects, despite its proven effectiveness. Some of these complications include oral mucosa irritation, dryness, loss of taste, and mucosal whitening. HP has also been linked to DNA damage [17, 36]. Despite containing low levels of HP, whitening mouth rinses may increase the risk of these potential complications with frequent use. In this context, the use of natural substances in tooth whitening has gained attention, primarily because of their low potential for side effects. Pineapple is a natural alternative. Pineapple contains enzymes such as bromelain, catalase, and polyphenol peroxidase. Bromelain, a proteolytic enzyme, is considered an effective oxidizing agent and has been suggested to play a significant role in tooth whitening by removing or breaking down the protein part of the pellicle layer adhering to the tooth surface [37, 38]. Vejai Vekaash et al. [38] reported that pineapple extract, when used in combination with HP, resulted in significantly greater tooth whitening than HP alone. However, in line with the results of the current study, Oliveira et al. [19] experimentally prepared a bromelain-containing mouth rinse and reported that it did not have a significant whitening effect. While pineapple extract, with its proteolytic enzymes, is considered a potential natural alternative for tooth whitening, there is no consensus in the literature on this matter.

When the microhardness data were examined, both home bleaching agents increased the surface hardness of the enamel; however, this increase was generally not statistically significant. Additionally, no statistically significant difference was observed between the home bleaching agents, thus leading to the acceptance of the third hypothesis. Preserving enamel microhardness is vital for maintaining the integrity of tooth structure and ensuring dental functions. A review of the literature concerning the effect of CP on enamel surface hardness in teeth whitening reveals a lack of consensus [39, 40]. In their in situ studies, Soares et al. reported a significant reduction in enamel microhardness when 10% and 16% CP were used. Conversely, Smidt et al. [41] evaluated the impact of three different CP agents on enamel microhardness in situ and reported no significant changes. Alterations in enamel microhardness are considered indicative of changes in enamel mineral content [42]. The hardness of enamel postwhitening is influenced by the composition of the whitening agent [43]. One of the home bleaching agents used in our study, 16% CP, contained sodium fluoride (NaF). NaF is a commonly used remineralization agent in dentistry. It promotes remineralization by creating a calcium fluoride (CaF_2) layer on the enamel surface, resulting in increased enamel microhardness [44].

In the present study, while whitening mouth rinses in the Zoom group did not significantly affect surface hardness, in the Opalescence PF group, a mouth rinse containing pyrophosphate led to a statistically significant

increase in surface hardness. As a result, our fourth hypothesis was partially supported. The mouth rinse containing pyrophosphate, similar to 16% CP, also contained NaF, which may have contributed to the observed increase in surface hardness. Comparing the results of the present study with those of previous studies can be challenging due to various factors, including the origin of the enamel samples (bovine/human teeth), sample preparation protocols (abrasion of enamel surfaces or not), mouth rinse application procedures (exposure time to mouth rinse, use of artificial saliva/distilled water, application of colorants, etc.), and experimental designs, whether they are in vitro or in vivo. These variations in study designs might explain the discrepancies among results.

This in vitro study is limited because it does not account for individual physiological factors such as enamel structure and saliva content, and it does not reflect the effects of tooth brushing. Nevertheless, the results obtained within the limitations of this study will help guide dentists in selecting treatment protocols for patients seeking teeth whitening and recommending whitening mouth rinse options to maintain achieved whiteness. In conclusion, mouth rinses containing pyrophosphate can be considered an alternative to HP-containing whitening mouth rinses, as they have proven effective in maintaining whiteness and significantly increasing enamel microhardness. Clinical studies are needed to fully elucidate the whitening efficacy and impact on the enamel hardness of these products and to observe the influence of individual factors.

Conclusions

Within the limitations of the current study, the following conclusions were drawn:

1. A higher concentration of carbamide peroxide (22% CP) results in greater whitening in a shorter time compared to a lower concentration (16% CP), suggesting that more effective and faster whitening treatments can be achieved with higher concentrations in clinical settings.
2. It was found that hydrogen peroxide (HP)- and pyrophosphate-containing whitening mouthwashes show similar effectiveness in maintaining the achieved whiteness after home bleaching. Considering the disadvantages of HP, pyrophosphate-based mouthwashes could serve as an alternative agent that does not carry these drawbacks. Additionally, pyrophosphate-based mouthwashes were found to contribute to an increase in surface hardness, offering an advantage over HP-based formulations. This suggests that pyrophosphate-based products may offer a safer, more effective, and beneficial option for both maintaining whiteness and enhancing enamel hardness.
3. To fully understand the whitening efficacy of these products and their impact on enamel hardness, clinical evaluations should be conducted. This emphasizes the importance of further clinical studies to assess how these treatments perform in real-world scenarios, considering individual variations and long-term effects. Such studies would significantly contribute to improving patient care and providing evidence-based recommendations for both home-use products and in-office treatments.

Clinical significance

This study contributes valuable insights into the effectiveness of various bleaching agents and mouthwashes, providing evidence that higher concentrations of carbamide peroxide can lead to faster and more effective whitening. Additionally, the results suggest that specific mouthwashes can be useful tools for maintaining the results of home bleaching, thus improving patient satisfaction and outcomes. The study also points to the need for future clinical evaluations to better understand the long-term efficacy and safety of these products in diverse patient populations.

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Authors' contributions
All authors made substantial contributions to the conceptualization and design of the study. IG, IC, and PG contributed to the establishment of the study's materials and methods, performed the experiments, and participated in the interpretation of the obtained findings. IG also contributed to the writing of the manuscript. PG performed the statistical analysis. NC contributed to the determination of the study's materials and methods, the interpretation of the obtained findings, and the revision of the final manuscript. All authors read and approved the final version of the manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Extracted human teeth were used in this study. All participants provided informed consent. Ethics committee approval was received from the Atatürk University Faculty of Dentistry Ethics Committee (decision no. 68, dated 22.06.2022). This study was conducted in accordance with the Declaration of Helsinki and relevant institutional and national ethical guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Restorative Dentistry, Faculty of Dentistry, Atatürk University, Erzurum TR-25240, Türkiye.

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