ORIGINAL RESEARCH

Evaluation of Stain Removal Efficacy and Color Stability of Three Different Dentifrices on Artificially Stained Enamel Surface—An *In Vitro* Study

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Abstract

Aim: The aim of the present study was to assess the stain removal ability and color stability of three distinct dentifrices on artificially stained enamel surface.

Materials and methods: This study included 75 intact, healthy premolars free of dental caries that were extracted during orthodontic therapy. The samples were allowed to dry for 6 hours after being submerged in the prepared tea solution for roughly 18 hours every day. Then this procedure was repeated for seven successive days. All samples were randomly divided into three experimental groups with 25 samples in each group. Group I: control dentifrice, group II: dentifrice containing hydrogen peroxide, group III: dentifrice containing papain and bromelain. A specially designed toothbrushing simulator was used to brush every sample in the relevant group. Using a spectrophotometer and a measurement program, color measurement was evaluated after staining process after 4 weeks and 8 weeks of teeth cleaning. Using a profilometer, the surface roughness values (Ra) were assessed.

Results: After 8 weeks of brushing of stained samples, the color stability was better in dentifrice containing hydrogen peroxide (1.14 ± 0.11) followed by dentifrice containing papain and bromelain (1.22 ± 0.08) and control group (1.30 ± 0.09). And after 8 weeks of brushing of stained samples, the surface roughness was more in dentifrice containing hydrogen peroxide (0.237 ± 0.02) followed by dentifrice containing papain and bromelain (0.229 ± 0.13) and control group (0.207 ± 0.05).

Conclusion: The present study concluded that the dentifrice containing hydrogen peroxide showed a superior whitening effect on the stained enamel surface than dentifrice containing papain and bromelain and control dentifrice.

Clinical significance: The development of various dentifrice products has been greatly aided by the increased demand for an improved esthetic appearance. Teeth's natural color and any external stains that could accumulate on the tooth surface combine to determine a tooth's color. Additionally, the use of whitening dental pastes to remove external stains has grown in favor. With the development of these whitening toothpastes, dentifrices' ability to lessen or eliminate extrinsic dental stains has increased.

Keywords: Color stability, Dentifrices, Stain, Toothbrushing.

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Introduction

The concept of "extrinsic tooth discoloration" describes a stain on teeth's outer surface that comes from smoking cigarettes or consuming certain foods and drinks like wine and coffee. These factors facilitate the attachment of pigments, such as tannins and polyphenols to the surface of teeth. Saliva dissolves calcium ions from the enamel crystallites, leaving phosphate ions on the tooth's surface where they are used in the extrinsic stain adhesion mechanism.[1]

The remaining phosphate ions give the enamel's surface a negative charge. Saliva reacts negatively charged surfaces to generate the "stern layer and hydration layer," a layer formed by the positive charge of calcium counter ions. Next, on the surface of the teeth, the salivary protein grows pellicles. Negative ions from acidic protein groups, such as carboxyl, phosphate, or sulfate are consumed by positive calcium counter ions, creating an electrostatic "calcium bridge." In contrast, base proteins have positive ions that push the same calcium ions into the stern layer and directly bind with the phosphate group on the enamel surface. Following that chromogens immediately adsorb into the acquired pellicles.[2]

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Extrinsic stain removal from teeth can be accomplished using professionally administered teeth whitening treatments or at-home solutions including chewing gum, mouth rinses, bleaching gels, whitening strips, toothpastes, and brush-on agents. However, because they are inexpensive and easy to use, whitening toothpastes appear to be one of the most popular over-the-counter remedies.[3]

Whitening toothpastes are intended to be used on a daily basis to either physically or chemically clean the tooth structure by removing stains or stopping them from forming. In order to eliminate stains or stop them from building up, toothpaste contains a variety of ingredients, including as enzymes, polymers, abrasives, and surfactants. Studies conducted recently have demonstrated that the main cleaning ingredient in toothpaste formulations is abrasives.[4]

Colgate optic white, contains polyphosphates and hydrogen peroxide. As a result, it was considered to be superior in terms of stain removal.[5] Colgate regular was chosen as the control dentifrice because its relative dentine abrasive value was approximately identical to that of the test dentifrice. The Glodent dentifrice contained proteolytic enzyme extracts papain and bromelain. They disrupt and/or eliminate the protein component of the pellicle plaque layer that accumulates on the surface of teeth over time, hence eliminating stains that are attached to these proteins.[6] But Apart from the fact that color change and surface roughness are not frequently investigated in together, there is still not much solid evidence of the effectiveness of these recently developed whitening dentifrices. Hence, the present study was conducted to assess the stain removal ability and color stability of three different dentifrices on artificially stained enamel surface.

Materials and Methods

Sample Size Calculation and Preparation

The current *in vitro* study was conducted in the department of Periodontology, Kalinga Institute of Dental Sciences, Bhubaneswar, India during the year of 2023. G*Power software was used to calculate the sample size. A 95% confidence interval, 80% power, and 0.54 effect size values were used to define the parameters. A sample size of 25 samples per group was determined. This study included 75 intact, healthy premolars free of dental caries that were extracted during orthodontic therapy. Notable cracks on the tooth's surface, the existence of hypoplastic areas, white spot lesions, and dental caries were among the exclusion criteria. The teeth had a thorough ultrasonic scaling procedure, which was followed by polishing with an oral prophylactic paste and rubber cup. The sample teeth were then kept in a 1% thymol solution until they were ready for further processing.

With a low-speed cutting machine, the premolar roots were removed at the cemento-enamel junction. The buccal side of the samples was facing outward when they were inserted into a 20 × 12.5 × 9 mm brushing machine mold. Polyester resin was poured into the mold until 5 mm diameter of the buccal surface emerged from the height of the contour of the teeth. Throughout the investigation, all samples were kept in deionized water at 37°C.

Staining Procedure

Ten packets of black tea (Lipton yellow label tea bag, Unilever Plc) were submerged in 500 mL of boiling water to create the staining solution. The samples were let dry for 6 hours after being submerged in this solution for roughly 18 hours every day. After that same process was carried on 7 days in a row. Every day, a fresh staining solution was made.

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Application of Dentifrices and Stain Removal Procedure

All samples were randomly divided into three experimental groups with 25 samples in each group.

Group I: Control dentifrice (Colgate Regular, Colgate Palmolive India Ltd, Mumbai, India).

Group II: Dentifrice containing hydrogen peroxide (Colgate optic white, Colgate Palmolive Company).

Group III: Dentifrice containing papain and bromelain (Glodent, Group Pharmaceuticals Ltd, Mumbai, India).

Tooth Brushing Process

A specially designed toothbrushing simulator was used to brush every sample in the relevant group. The device is made up of a powered soft toothbrush head fixed to a stationary device that moves horizontally and applies a load of 250 gm/cm^2. Every sample underwent two rounds of brushing record. First, 4 weeks of teeth brushing; second, 8 weeks of teeth brushing. For each sample, a single new brush was utilized with a 2:1 slurry mix (pastethrin: distilled water). Following every brushing cycle, the samples were placed in artificial saliva and cleaned under running water. Subsequently, color change and surface roughness were measured for every sample three times: once after staining process, after 4 weeks and 8 weeks of teeth cleaning.

Evaluation of Color Stability

Using a spectrophotometer and a measurement program, color measurement was evaluated after staining procedure and at the conclusion of each interval. The spectrophotometer was calibrated before the measurement, and a 4 mm reflection port was used to get the data. After being taken out of the deionized water, the samples were assessed for color and blotted on paper. This approach has served as the cornerstone for the CIE Lab system.

$$\text{Color differences } (\Delta E^*) = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}.$$

The Commission Internationale de l'Eclairage L*a*b* (CIE L*a*b*) the measurement of color was done three times. Subsequently, the mean of three values were taken.

Evaluation of Surface Roughness of Different Dentifrices

The values of surface roughness (Ra) were assessed after staining process and following the two brushing cycles. A profilometer (Surftest SJ 201, Mitutoyo Co, Kawasaki, Japan) with a stylus with a 5 μm tip radius and a 90° tip angle was used to analyze the data. The stylus traversed 4.0 mm at a constant speed of 0.5 μ/s and measuring force of 4 μN. The cut-off value for surface roughness was 0.8 mm. Each sample's surface was measured three times in various directions, and the mean values were then calculated.

Statistical Analysis

Statistical analysis software (17.0 versions) was used to analyze the data. The significant differences in color stability and surface

Table 1: Assessment of color stability of different dentifrices at various duration on the stained tooth surface

	Experimental duration		
Dentifrices	*After staining process*	*After 4 weeks brushing*	*After 8 weeks brushing*
Group I: Control dentifrice	1.06 ± 0.05	1.46 ± 0.01	1.30 ± 0.09
Group II: Dentifrice containing hydrogen peroxide	1.08 ± 0.10	1.21 ± 0.17	1.14 ± 0.11
Group III: Dentifrice containing papain and bromelain	1.12 ± 0.12	1.34 ± 0.02	1.22 ± 0.08
F value	9.143	7.852	8.030
p-value	0.712	0.001	0.001

Post hoc test: At 4 weeks – $p < 0.001$ group I vs group II; At 8 weeks – $p < 0.001$ group I vs group II

Table 2: Assessment of surface roughness of different dentifrices at various duration on the stained tooth surface

	Experimental duration		
Dentifrices	*After staining process*	*4 weeks*	*8 weeks*
Group I: Control dentifrice	0.174 ± 0.02	0.201 ± 0.11	0.207 ± 0.05
Group II: Dentifrice containing hydrogen peroxide	0.179 ± 0.07	0.228 ± 0.17	0.237 ± 0.02
Group III: Dentifrice containing papain and bromelain	0.181 ± 0.10	0.212 ± 0.08	0.229 ± 0.13
F value	3.179	4.032	4.874
p-value	0.918	0.001	0.001

Post hoc test: At 4 weeks – $p < 0.001$ group I vs group II; At 8 weeks – $p < 0.001$ group I vs group II, group I vs group III

roughness (µm) were evaluated using the one-way analysis of variance (ANOVA) test, and *post hoc* comparisons were performed using the Tukey least significant difference (LSD) test. A statistically significant *p*-value is one that is less than 0.05.

Results

Table 1 presents the color stability of different dentifrices at various duration on the stained tooth surface. After staining process, the mean score of control dentifrice was 1.06 ± 0.05, dentifrice containing hydrogen peroxide was 1.08 ± 0.10 and dentifrice containing papain and bromelain was 1.12 ± 0.12. At the end of 4 weeks of brushing, control dentifrice was 1.46 ± 0.01, dentifrice containing hydrogen peroxide was 1.21 ± 0.17 and dentifrice containing papain and bromelain was 1.34 ± 0.02. At the end of 8 weeks of brushing, stained samples color stability was better in dentifrice containing hydrogen peroxide (1.14 ± 0.11) followed by dentifrice containing papain and bromelain (1.22 ± 0.08) and control group (1.30 ± 0.09). There was a significant difference found between the groups at 4th and 8th weeks after brushing. The *post hoc* test reveals the statistically significant difference found between control and dentifrice containing hydrogen peroxide group ($p < 0.001$).

Table 2 reveals the surface roughness of different dentifrices at various duration on the stained tooth surface. After staining process, the mean score of control dentifrice was 0.174 ± 0.02, dentifrice containing hydrogen peroxide was 0.179 ± 0.07 and dentifrice containing papain and bromelain was 0.181 ± 0.10. At the end of 4 weeks of brushing, control dentifrice was 0.201 ± 0.11, dentifrice containing hydrogen peroxide was 0.228 ± 0.17 and dentifrice containing papain and bromelain was 0.212 ± 0.08. At the end of 8 weeks of brushing, stained samples surface roughness was more in dentifrice containing hydrogen peroxide (0.237 ± 0.02) followed by dentifrice containing papain and bromelain (0.229 ± 0.13) and control group (0.207 ± 0.05). There was significant difference found between the groups at 4th and 8th weeks after brushing. The *post hoc* test reveals the statistically significant difference found between control vs dentifrice containing hydrogen peroxide group at 4th week and control vs dentifrice containing hydrogen peroxide group and dentifrice containing papain and bromelain at 8th week ($p < 0.001$).

Discussion

People's expectations for esthetics are rising daily in the modern world. People care more about the color of their teeth than other characteristics, despite the fact that dental esthetics is influenced by a wide range of factors. Noninvasive teeth whitening treatments have been created in response to the growing need for teeth whitening to remove discoloration. Whitening toothpastes are among the whitening treatments that patients can use on their own in addition to the whitening procedures that dentists can perform in their offices. The whitening effectiveness of toothpastes is provided by mechanical, chemical, and optical methods.[7]

A mechanical whitening effect is produced by the abrasives found in toothpastes. These abrasives include calcium carbonate, calcium pyrophosphate, sodium bicarbonate, perlite, alumina, hydrated silica, and calcium phosphate dihydrate. Only external coloring and the regions the brush comes into contact with are affected by abrasives. The structure of teeth can be eroded and abrased by abrasives. As a result, whitening toothpastes contain chemical whitening ingredients. Hydrogen peroxide, calcium peroxide, sodium citrate, sodium pyrophosphate, sodium tripolyphosphate, and sodium hexametaphosphate are examples of chemical whitening agents.[8]

Currently, there are several ways to evaluate tooth color; nevertheless, the most accurate are the objective techniques, including reflectance spectrophotometer analysis. Commonly used tools for measuring an object's color are colorimeters and spectrophotometers.[9] While colorimeters measure reflected light in only three wavelengths: red, green, and blue. Spectrophotometers assess light's reflectance throughout the visible spectrum. The spectrophotometer was used in this study to measure the samples' color changes. In order to do this, an optical fiber was used to direct light onto each sample's enamel surface. The device then

captured the whole reflected component of the light, which was quantified. As a result, the reflectance value increased and less light was absorbed as the tooth became whiter.[10]

In the current study, dentifrice containing hydrogen peroxide had superior stain removal and color stability compared with dentifrice containing papain and bromelain as well as the control group. Similarly, external stains on the enamel surface may be eliminated by abrasive agents found in whitening dentifrices, but intrinsic colorations must be eliminated by converting hydrogen peroxide or carbamide peroxide into free radicals, according to De Menezes M et al.[11] Certain teeth whitening toothpastes use different amounts of hydrogen peroxide in order to take advantage of this agent's oxidative properties. The whitening dentifrice utilized in this investigation includes hydrogen peroxide and polyphosphates. It was therefore anticipated that in terms of whitening, it would be better than the others. On the contrary, as noted by Lippert et al. and Alshara et al.,[12,13] hydrogen peroxide is unstable at low concentrations (about 1% by weight), therefore brushing reduces the action time naturally.

In comparison to the control group, dentifrice containing papain and bromelain has shown superior stain removal ability and color stability in the current investigation. Similarly, research by Lyon TC et al.[14] and Emling RC et al.[15] revealed that toothpaste containing a papain, alumina, and sodium citrate mixture significantly reduced the amount of stain. Proteolytic enzyme extracts from papain and bromelain were present in the test dentifrice used in this investigation. According to a different study by Chakravarthy PK and Acharya S,[16] they break up and/or eliminate the protein-rich pellicle plaque layer that develops on the surface of teeth over time, eliminating the stains that are attached to these proteins. Colgate regular was selected as the control dentifrice because its relative dentine abrasive value was almost identical to that of the test dentifrice. As a result, the stain removal mean values for this dentifrice group may be partially explained by these enzymes.

The present study's limitations are that surface hardness was not assessed after brushing. In subsequent research, the impact of teeth whitening toothpaste on the hardness of the enamel surface can be assessed. The samples were alone submerged in tea for staining, which is another drawback. In addition, contrary to *in vitro* studies, colorants can be diluted with saliva in the oral environment. Therefore, more *in vivo* research is required to determine how whitening dentifrices affect the surface of enamel that has been submerged in various colorants.

Conclusion

Within the limitations, it can be concluded that hydrogen peroxide-containing dentifrice had a more promising whitening effect on the stained enamel surface than control dentifrice and dentifrice with papain and bromelain. When choosing and utilizing dentifrices that contain whitening agents, patients and dental professionals can benefit from the insights gathered from the present study.

References

1. Hannig M, Joiner A. The structure, function and properties of the acquired pellicle. Monogr Oral Sci 2006;19:29–64. DOI: 10.1159/000090585.
2. Suriyasangpetch S, Sivavong P, Niyatiwatchanchai B, et al. Effect of whitening toothpaste on surface roughness and colour alteration of artificially extrinsic stained human enamel: In vitro study. Dent J (Basel) 2022;10(10):191. DOI: 10.3390/dj10100191.
3. Mehrgan S, Kermanshah H, Omrani LR, et al. Comparison the effect of charcoal-containing, hydrogen peroxide-containing, and abrasive whitening toothpastes on color stability of a resin composite; an in vitro study. BMC Oral Health 2021;21(1):594. DOI: 10.1186/s12903-021-01956-8.
4. Joiner A. Review of the extrinsic stain removal and enamel/ dentine abrasion by a calcium carbonate and perlite containing whitening toothpaste. Int Dent J 2006;56(4):175–180. DOI: 10.1111/j.1875-595x.2006.tb00091.x.
5. Hazar A, Hazar E. Effects of whitening dentifrices on the enamel color, surface roughness and morphology. Odovtos Int J Dental Sci 2023;25(2):20–29. DOI: 10.15517/ijds.2022.50981.
6. Kalyana P, Shashidhar A, Meghashyam B, et al. Stain removal efficacy of a novel dentifrice containing papain and bromelain extracts-*in vitro* study. Int J Dent Hyg 2011;9(3):229–233. DOI: 10.1111/j.1601-5037.2010.00473.x.
7. Maldupa I, Brinkmane A, Rendeniece I, et al. Evidence based toothpaste classification, according to certain characteristics of their chemical composition. Stomatologija 2012;14(1):12–22. PMID: 22617330.
8. Vaz VTP, Jubilato DP, Oliveira MRM, et al. Whitening toothpaste containing activated charcoal, blue covarine, hydrogen peroxide or microbeads: which one is the most effective? J Appl Oral Sci 2019;27:e20180051. DOI: 10.1590/1678-7757-2018-0051.
9. Horn DJ, Bulan-Brady J, Hicks L. Sphere spectrophotometer *versus* human evaluation of tooth shade. J Endod 1998;24(12):786–790. DOI: 10.1016/S0099-2399(98)80002-2.
10. Chu SJ. Use of a reflectance spectrophotometer in evaluating shade change resulting from tooth-whitening products. J Esthet Restor Dent 2003;15 Suppl 1:S42–S48. DOI: 10.1111/j.1708-8240.2003.tb00317.x.
11. De Menezes M, Turssi CP, Hara AT, et al. Abrasion of eroded root dentine brushed with different toothpastes. Clin Oral Investig 2004;8(3):151–155. DOI: 10.1007/s00784-004-0262-7.
12. Alshara S, Lippert F, Eckert GJ, et al. Effectiveness and mode of action of whitening toothpastes on enamel extrinsic stains. Clin Oral Investig 2014;18(2):563–569. DOI: 10.1007/s00784-013-0981-8.
13. Lippert F. An introduction to toothpaste-its purpose, history and ingredients. Monogr Oral Sci 2013;23:1–14. DOI: 10.1159/000350456.
14. Lyon TC, Parker WA, Barnes GP. Evaluation of effects of application of a citroxain-containing dentifrice. J Esthet Dent 1991;3(2):51–53. DOI: 10.1111/j.1708-8240.1991.tb00809.x.
15. Emling RC, Levin S, Shi X, et al. Rembrandt toothpaste stain prevention with and without the use of Peridex. J Clin Dent 1992;3:59–65. PMID: 1524688.
16. Chakravarthy PK, Acharya S. Efficacy of extrinsic stain removal by novel dentifrice containing papain and bromelain extracts. J Young Pharmacists 2012;4(4):245–249. DOI: 10.4103/0975-1483.104368.