

Physical-chemical characteristics of whitening toothpaste and evaluation of its effects on enamel roughness

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Abstract: This *in vitro* study evaluated the physical-chemical characteristics of whitening toothpastes and their effect on bovine enamel after application of a bleaching agent (16% carbamide peroxide). Physical-chemical analysis was made considering mass loss by desiccation, ash content and pH of the toothpastes. Thirty bovine dental enamel fragments were prepared for roughness measurements. The samples were subjected to bleaching treatments and simulated brushing: G1. Sorriso Dentes Brancos (Conventional toothpaste), G2. Close-UP Whitening (Whitening toothpaste), and G3. Sensodyne Branqueador (Whitening toothpaste). The average roughness (Ra) was evaluated prior to the bleaching treatment and after brushing. The results revealed differences in the physical-chemical characteristics of the toothpastes ($p < 0.0001$). The final Ra had higher values ($p < 0.05$) following the procedures. The mean of the Ra did not show significant differences, considering toothpaste groups and bleaching treatment. Interaction (toothpaste and bleaching treatment) showed significant difference ($p < 0.0001$). The whitening toothpastes showed differences in their physical-chemical properties. All toothpastes promoted changes to the enamel surface, probably by the use of a bleaching agent.

Descriptors: Dentifrices; Tooth bleaching; Toothbrushing; Dental Enamel.

Introduction

Patients increasingly seek to have an attractive smile, as it is considered to be synonymous with health.^{1,2} This growing demand for an enhanced esthetic appearance has led to great development of bleaching products.³⁻⁵ The color of teeth is influenced by a combination of their intrinsic color and the presence of any extrinsic stains that may form on the tooth surface.⁶

The active components of the whitening toothpastes include surfactants, polyphosphates and enzymes. Some of them also contain low peroxide concentrations. However, the evidence to date suggests that the primary stain removal ingredient in these toothpastes is the abrasive agents.^{4,6}

The abrasive agents are present in the toothpastes' formulation, which play an important role in cleaning teeth. However, their abrasiveness, which is influenced by particle hardness, size and shape, and also by the pH of the toothpaste, must be controlled. High amounts of abrasives in

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toothpastes may damage hard and soft tissues and dental restorations, leading to gingival recession, cervical abrasion and dentinal hypersensitivity.^{2-3, 5, 7-12} In order to ensure their effectiveness, they must have physical-chemical characteristics that allow for their therapeutic action without damaging oral tissues.¹³ Fluorine, the soluble element present in toothpastes, is able to combine with abrasive agents, rendering it insoluble and unable to exert its remineralizing property.^{1, 9, 12, 14}

Considering the whitening toothpastes, their daily use should be subject to several considerations; e.g., after the consumption of acid beverages, a high concentration of abrasives may lead to increased wear of enamel and dentine. The concomitant use of abrasive toothpastes during at-home bleaching procedures reportedly increases the roughness of dental enamel.^{11, 15-18}

The purpose of this study was to evaluate the physical-chemical characteristics of the whitening toothpastes by determining their effect on bovine enamel surfaces during brushing after treatment by a bleaching agent. The classical alternative hypothesis that was investigated was: the composition of the tested toothpastes does not interfere with enamel surface roughness after the bleaching treatment.

Methodology

Physical-chemical analysis

All the analyses were conducted in quintuplicate, and the tested toothpastes were divided into three groups:

- Group 1 (G1. Conventional toothpaste: Sorriso Dentes Brancos®),
- Group 2 (G2. Whitening toothpaste: Close-UP Whitening®), and

- Group 3 (G3. Whitening toothpaste: Sensodyne Branqueador®) (Table 1).

Desiccation loss and residue analysis

The toothpastes were weighed on a Petri dish (5.0 grams) and heated in an oven at 105 °C for 24 hours. They were then weighed again, repeating this process until the same weight was recorded in consecutive checks. Loss by desiccation was calculated from the difference between the initial and final weights.

pH analysis

The pH was measured using a digital potentiometer (DMPH-2, Digimed; São Paulo, Brazil). Measurements were performed only once for each sample at a dilution of 5.0 grams suspended in 15 mL of distilled water.

Scanning Electron Microscope (SEM)

After the toothpastes were desiccated, they were placed in porcelain crucibles and heated in a furnace at 650 °C to produce ashes, which were then analyzed under a SEM (Shimadzu SSX 550, Shimadzu do Brasil, São Paulo, Brazil), operated at 15 kV at 1000× magnification.

Evaluation of the enamel surface

Preparation of the dental fragments

The specimens were prepared from freshly extracted bovine incisors stored in artificial saliva. The vestibular portion of the dental crown was sectioned vertically and horizontally (three and four times, respectively) to obtain fragments of 4 × 4 mm. These cuts were made using a water-cooled diamond saw (ISOMET 1000- Precision Saw Buehler, Lake Bluff, USA). In preparation for cutting, the teeth were

Table 1 - Toothpastes used in the study.

Group	Toothpaste	Manufacturer	Active ingredients
G1	Sorriso Dentes Brancos (Conventional)	Colgate - Palmolive do Brasil, São Paulo, Brazil	Fluoride, calcium carbonate, sodium lauryl sulfate, tetrasodium pyrophosphate and sodium silicate
G2	Close-UP Whitening (Whitening)	Unilever do Brasil; São Paulo, Brazil	Silica, perlite, sodium lauryl sulfate, sodium fluoride and titanium dioxide
G3	Sensodyne Branqueador (Whitening)	GlaxoSmithKline do Brasil, São Paulo, Brazil	Potassium nitrate, sodium fluoride, silica, sodium bicarbonate, titanium dioxide and pyrophosphate tetrapotassium

mounted on an aluminum support using sticky wax (Figure 1A). Six enamel fragments were obtained from each bovine tooth (Figure 1B). The fragments were converted into test specimens comprising three units each. The enamel samples were embedded in acrylic autopolymerizable resin, leaving the vestibular enamel surface exposed. Thirty test specimens were prepared (n = 10, per group). The power calculation was performed using data from a previous pilot study (unpublished). If the sample size in each of the 3 groups (toothpaste) is 10 there would be 90% power for average roughness to detect a difference of 5%. The common standard deviation was 0.27 at an effect size of 0.48 and 0.05 level for a two-side test.

After the acrylic resin was embedded, the vestibular surfaces of the fragments were smoothed in a polishing machine (Aropol E; Arotec Ind. e Com. Ltda., São Paulo, Brazil). Aluminum oxide disks were used in sequential grit sizes of 400, 600, and 1,000, respectively, and then polished with a felt cloth in conjunction with abrasive pastes 6, 3, 1, and 1/2 μm along with a mineral oil coolant (Top, Gold, e Ram, Arotec Ind e Com Ltda., São Paulo, Brazil). These procedures were conducted in order to obtain homogeneous surfaces for treatment (Figure 1C).

Roughness measurements prior to and after treatment

After the samples were polished, initial enamel

surface roughness parameters (initial and final) were obtained by a digital profilometer (Surftest 301, Mitutoyo Sul Americana, Suzano, Brazil). The profilometer was set for a sampling surface of 1.25 mm, with a cutoff value of 0.25 mm. Two points in each fragment of enamel were measured to obtain the initial roughness values for the enamel surface. This measurement was carried out transversely to the direction in which the fragment would be brushed. Thus, the average roughness (Ra) was obtained. After treatment of the test specimens following the same procedure, the roughness was evaluated again (final roughness).

Bleaching treatment and brushing with toothpastes

The first enamel fragment was treated with 16% carbamide peroxide bleaching agent (Whiteness Perfect, FGM, Joinvile, Brazil) for a period of 10 hours. The second fragment was treated with the same bleaching agent for the same period of time, and it also received a 1-min topical application of 2% neutral fluoride gel (Flugel, DFL, Jacarepaguá, Brazil). The third enamel fragment was treated with artificial saliva. After treatment, the enamel samples were brushed using the tested toothpastes.

The simulated brushing using the toothpastes and application of bleaching agent and fluoride were carried out 4 times. Each simulation comprised

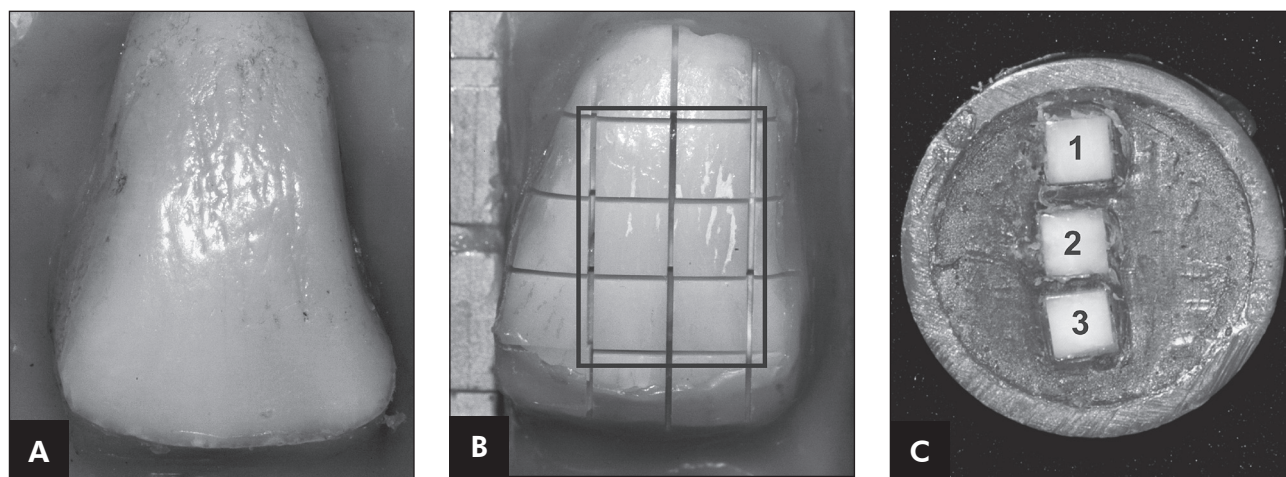


Figure 1 - (A) Bovine tooth inserted into an aluminum base with sticky wax. **(B)** Six enamel fragments (4 x 4 mm) were obtained from each bovine tooth. **(C)** Fragments of enamel embedded in acrylic resin were smoothed in a polishing machine: 1 - Bleaching; 2 - Bleaching + Fluoride; 3 - Saliva.

400 cycles (back and forth movements). Simulated brushing was carried out under a load of 200 g in a universal brushing machine (MSEt - 1500W, Marcelo Nucci ME; São Carlos, Brazil), using a toothbrush (32 mm × 11 mm head; 3 × 9 rows of tufts; soft artificial nylon bristles with rounded tips).

The toothpastes were diluted in distilled water at a ratio of 1:2 by weight, taken up in syringes and taken to the brushing machine. The machine was set to inject 2 ml of the diluted toothpaste at 10-second intervals, at a speed of 4.5 cycles per second. Each simulated brushing was carried out during the daytime, after which the samples were again immersed in artificial saliva at 37 (± 1) °C.

All laboratory procedures were conducted by two researchers.

Statistical analysis

Physical-chemical characteristics of the toothpastes (desiccation loss, ash content and pH tests) were evaluated by one-way ANOVA with Bonferroni's post-hoc test. Comparisons between the

toothpastes and bleaching treatment for the final roughness were tested by two-way ANOVA with Bonferroni's post-hoc test. The initial and final roughness values found for each group were compared by paired Student's *t*-test. An alpha value of ≤ 0.05 was used to indicate statistically significant differences among the groups. All analyses were performed using a software program (GraphPad Prism 5.00, GraphPad Software, San Diego, USA).

Results

Physical-chemical characteristics of the toothpastes

The results for percentage of desiccation loss, ash content and pH values revealed statistically significant differences ($p < 0.0001$) among the groups (Table 2).

In the SEM evaluation, G2 and G3 displayed agglomerates of irregular particles that were sharp-tipped and of undefined shape, while G1 showed rounded and more clearly defined particles (Figure 2).

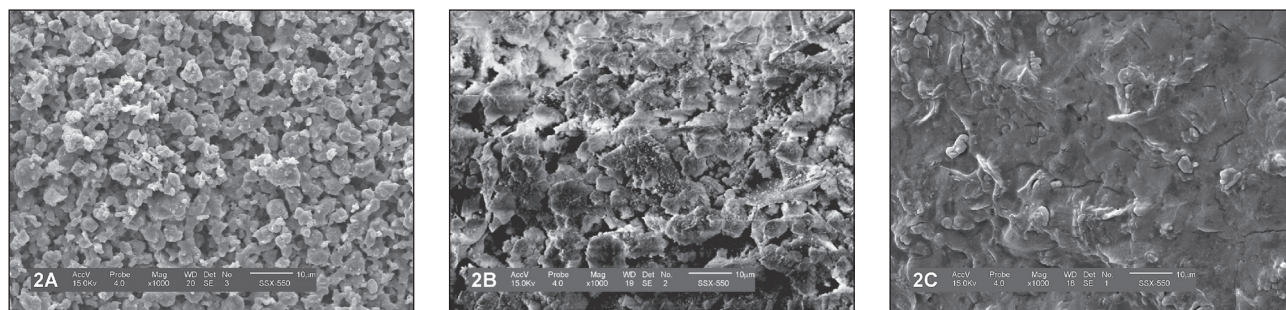


Figure 2 - SEM photomicrographs showing the toothpaste ashes. **(A)** Sorriso Dentes Brancos (G1. Conventional toothpaste), showing agglomeration of inorganic substances, round or oval-shaped and uniform. **(B)** Close-Up Whitening (G2. Whitening toothpaste). Presence of agglomeration of inorganic substances, sharp-tipped and amorphous. **(C)** Sensodyne Branqueador (G3. Whitening toothpaste); although the inorganic particles melted during calcination, irregular and amorphous particles are still visible. (bar = 10 μm).

Table 2 - Physical-chemical characteristics of the toothpastes (mean and standard deviation).

Toothpaste	Loss by desiccation (%)	Ash content (%)	pH values
G1 - Conventional toothpaste (Sorriso Dentes Brancos)	31.91 ± 1.12 ^a	36.67 ± 1.97 ^a	10.09 ± 0.14 ^a
G2 - Whitening toothpaste (Close-UP Whitening)	44.73 ± 1.12 ^b	17.64 ± 1.23 ^b	7.87 ± 0.08 ^b
G3 - Whitening toothpaste (Sensodyne Branqueador)	65.83 ± 2.21 ^c	16.00 ± 3.09 ^b	9.12 ± 0.16 ^c

Vertically, different letters indicate statistically significant differences among bleaching treatments ($p < 0.001$). One-way ANOVA with Bonferroni's post-hoc test.

Analysis of enamel roughness

The final average roughness (Ra) had higher values following the bleaching treatment and brushing with toothpastes for G1 and G2. G3 showed statistical difference only following saliva treatment (Figure 3).

The mean of the Ra did not show statistically significant differences, considering toothpaste groups and bleaching treatment. Interaction (toothpaste and bleaching treatment) showed statistically significant difference (Table 3).

Discussion

The toothpastes that were studied showed different physical-chemical characteristics and changes on the enamel surface after treatments. Thus, the classical alternative hypothesis was rejected.

Several studies have evaluated the relation between the abrasive potential of toothpastes and alterations on enamel and restorative materials.^{2-3, 8,14}

However, the application of bleaching agents followed by brushing with abrasive toothpastes has not been extensively evaluated.^{3, 7, 17-18}

The desiccation loss test showed different values among the groups, indicating toothpastes with different compositions. G1 (Sorriso Dentes Brancos®) presented the lowest loss by desiccation and the highest values of ash content (solid residues). This toothpaste, applied on the enamel surface increased the average roughness (Ra) parameter. The ash content and solid residues of toothpaste may determine its potential to alter the surface enamel, since higher values were a sign of alterations in roughness.¹³

Considering pH, there were differences among the groups, but the pH in all of the three toothpastes was higher than 7 (neutral). An alkaline pH has a tendency to cause less change to the dental surface, while toothpastes with lower pH have showed greater alteration. On the other hand, in this study, G1 had the highest pH and promoted extensive

Figure 3 - Mean (standard error) of initial and final roughness parameter (Ra). Results of the paired Student's *t*-test for the three toothpastes after bleaching treatment (B. Bleaching; B/F. Bleaching + Fluoride; S. Saliva). *Significant difference ($p < 0.05$).

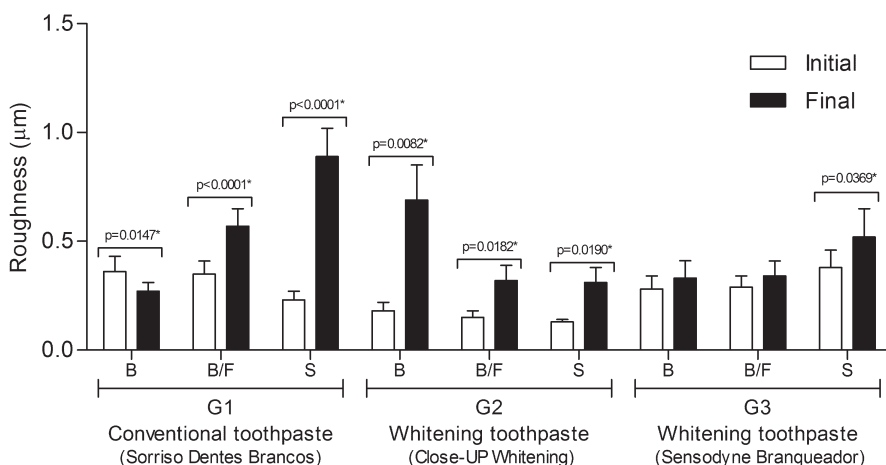


Table 3 - Mean and standard deviation of roughness parameter (Ra) for bovine enamel following brushing with different toothpastes and bleaching treatments.

Toothpaste	Bleaching Treatment		
	Bleaching	Bleaching/Fluoride	Saliva
G1 - Conventional toothpaste (Sorriso Dentes Brancos)	0.27 ± 0.14 ^{Aa}	0.57 ± 0.24 ^{Aa}	0.89 ± 0.40 ^{Ba}
G2 - Whitening toothpaste (Close-UP Whitening)	0.69 ± 0.51 ^{Ab}	0.32 ± 0.22 ^{B^{ba}}	0.31 ± 0.22 ^{B^b}
G3 - Whitening toothpaste (Sensodyne Branqueador)	0.33 ± 0.26 ^{Aa}	0.34 ± 0.22 ^{Aa}	0.52 ± 0.41 ^{Ab}

Horizontally, different capital letters indicate statistically significant differences among bleaching treatments ($p < 0.05$). Vertically, different small letters indicate statistically significant differences among toothpastes ($p < 0.05$). Two-way ANOVA with Bonferroni's post-hoc test.

change of the enamel surface. This finding suggests that the pH may be an important factor when considering the impact of the toothpaste on the enamel surface.^{13, 15} G2 (Close-UP Whitening®) presented a pH closest to 7 and altered the enamel surface when brushing was preceded by application of the bleaching agent.

The ash SEM analysis for G2 and G3 (Sensodyne Branqueador®) toothpastes revealed agglomerations of particles with irregular contours that were sharp tipped and often of irregular shape. G1 presented spherical agglomerated particles. These findings indicate that particle size also influences the effect of toothpaste on the dental surface.^{5, 8, 13}

In this study, G1 was the only toothpaste whose composition contained calcium carbonate. Toothpastes with this composition are less abrasive.¹³ However, in the present research, G1 did not prove to be less abrasive. G2 (abrasive: silica) had resulted in an increase in roughness. Previous findings have shown that silica exhibits strong abrasive properties.⁸ Only in G3 (abrasive: sodium bicarbonate) did bleaching treatment prior to brushing not result in significant surface enamel alterations.

The application of a bleaching agent on the enamel structure is, *per se*, a factor that alters the surface characteristics.¹⁷ Different bleaching agents containing the same concentration of carbamide peroxide had different effects on dental enamel.¹⁸ Enamel chemical changes could be observed after 10%, 20% and 30% hydrogen peroxide treatment.¹⁶ In another study, enamel surface showed no mechanical, morphologic, or chemical changes following bleaching *in situ* with three different carbamide peroxide agents.¹⁹

Therefore, considering that the appropriate concentration of bleaching gel is still under discussion, in the present study only one concentration of carbamide peroxide (16%) was used throughout the study for the sake of consistency.

Considering the initial and final roughness, the combination of whitening toothpaste and bleaching agent produced more changes to the enamel surface in G1 and G2.

Comparison of the final roughness between teeth brushed with the tested toothpastes and bleaching treatments did not show significant differences. Therefore, when the final roughness parameter data were grouped for statistical analysis (toothpastes and treatments), it was not possible to identify statistical differences, but the interaction (toothpaste and bleaching treatment) had shown statistically significant difference. Thus, the interaction factors were analyzed individually, showing significant difference between toothpastes after bleaching treatment. Toothpaste compositions could be responsible for different changes to the enamel surface.^{3-5, 10-12}

Fluoride's ability to form a protective film on the enamel surface has been established.^{1, 7, 9, 14} However, the presence of fluoride in the bleaching gels did not increase mineral recovery.²⁰ Similarly, in the present study, the application of fluoride gel for one minute after the bleaching agent did not add a protective effect. On the other hand, more studies are necessary about this subject.

Conclusion

Regarding physical-chemical characteristics, the whitening toothpastes showed higher loss by desiccation and lower ash content and pH values than the control group.

All toothpastes (whitening and control) were able to promote alterations to the enamel surface. The use of a bleaching agent before brushing with toothpaste could be a determining factor in the modifications revealed by the roughness parameter.

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