

Brushing Effect with Whitening Dentifrices on Dental Enamel: Whitening and Microhardness Analysis

Efeito da Escovação com Dentifrícios Clareadores no Esmalte Dentário: Análise de Clareamento e Microdureza

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Abstract

There are whitening dentifrices that claim to reduce or eliminate extrinsic stains by incorporating abrasive systems. This study aimed to assess the whitening potential and surface microhardness on enamel after different whitening dentifrices use. 120 bovine enamel blocks were used for 6 groups: G1- distilled water; G2- Colgate Total 12 Whitening (Colgate®); G3- Rembrandt Deeply White (Johnson&Johnson®); G4- Rembrandt Intense Stain (Johnson&Johnson®); G5-PeroxiCare (Arm&Hammer®); G6- CompleteCare (Arm&Hammer®). The evaluations of color and microhardness were realized before and after the staining accomplishment and after 5,000, 10,000, and 15,000 brushing cycles. The data were submitted to a one-factor analysis of variance (ANOVA) and the differences were analyzed by the Tukey test with a significance level of 5%. Regarding color, groups G3, G4, G5, and G6 showed the highest whitening potential, whereas groups G1 and G2 showed the lowest whitening potential. Additionally, group G3 displayed the highest superficial hardness, whereas group G6 had the lowest superficial hardness. At the end of 15.000 brushing cycles, dentifrices G3, G4, G5 and G6 showed similar whitening potential. Only G3 and G6 have peroxides in their composition, which achieved greater whitening action after 15,000 cycles. Dentifrices composed only of abrasive particles provided a rapid pseudo-whitening effect followed by color stabilization. Only G3 showed no suggestive mineral loss.

Keywords: Toothbrushing. Dentifrices. Dental Enamel. Tooth Bleaching.

Resumo

Existem dentifrícios clareadores que afirmam reduzir ou eliminar manchas extrínsecas por meio da incorporação de sistemas abrasivos. O objetivo deste estudo foi avaliar o potencial clareador e a microdureza da superfície de do esmalte após o uso de diferentes dentifrícios clareadores. Foram usados 120 blocos de esmalte bovino em 6 grupos: G1 – água destilada; G2 – Colgate Total 12 Whitening (Colgate®); G3 – Rembrandt Deeply White (Johnson&Johnson®); G4 – Rembrandt Intense Stain (Johnson&Johnson®); G5 – PeroxiCare (Arm&Hammer®); G6 – CompleteCare (Arm&Hammer®). As avaliações de cor e microdureza foram realizadas antes e depois da realização do manchamento e após 5.000, 10.000, e 15.000 ciclos de escovação. Os dados foram submetidos a uma análise de variância de um fator (ANOVA) e as diferenças foram analisadas pelo teste de Turkey com um nível de significância de 5%. Com relação à cor, os grupos G3, G4, G5, e G6 apresentaram o maior potencial de clareamento, enquanto os grupos G1 e G2 apresentaram o menor potencial de clareamento. Além disso, o grupo G3 apresentou a maior dureza superficial, enquanto o grupo G6 apresentou a menor dureza superficial. Ao final de 15.000 ciclos de escovação, os dentifrícios G3, G4, G5, e G6 apresentaram potencial clareador semelhante. Apenas G3 e G6 possuem peróxidos em sua composição, o que atingiu maior ação clareadora após 15.000 ciclos. Os dentifrícios compostos apenas por partículas abrasivas propiciaram rápido efeito pseudoclareador e estabilização da cor. Apenas G3 não apresentou perda mineral sugestiva.

Palavras-chave: Escovação dentária. Dentifrícios. Esmalte Dentário. Clareamento Dental.

1 Introduction

Dental bleaching is a popular procedure that aims to achieve brighter smiles and the industry offers dentifrices, gels, strips, and in-office as treatments that use concentrated whitening agents under professional supervision¹. The teeth's color is determined by intrinsic pigmentation that occur before and after the teeth's eruption², and the presence of extrinsic stains on the surface³ caused by poor brushing technique, smoking, pigmented foods, aging, and the use of certain cationic agents like chlorhexidine or metal salts⁴⁻⁶.

There are several whitening dentifrices products available in the market that claim to reduce or eliminate extrinsic stains by incorporating abrasive systems that remove the acquired

film and the extrinsic stain itself⁶. The active components of whitening dentifrices include surfactants, polyphosphates, enzymes, and often low concentrations of peroxide that acts releasing oxygen-free radicals that are responsible for an oxidation reaction, which is the principle of the bleaching technique^{7,8}. However, some evidences indicate that abrasive agents are the main ingredients for removing stains in these dentifrices^{9,10}.

Brushing teeth with dentifrices is effective in reducing the build-up of plaque and in removing stains. However, it is important to note that several products contain ingredients that can cause abrasion and roughness on the surfaces of restorations and hard tissues^{6,10,11}.

It is essential to evaluate the impact of using whitening dentifrices with different compositions on dental enamel due to the limited research available regarding their actual effects on teeth and the increasing availability of dentifrices products marketed as whiteners. The aim of this in vitro study was to evaluate the effect of dentifrices with different compositions on tooth enamel by microhardness assess and its tooth bleaching effect.

2 Material and Methods

This research was conducted on bovine teeth and, therefore, subjected to approval by the Animal Research Ethics Committee of the Federal University of Alagoas (UFAL) under Project N°. 020/2013.

2.1 Experimental design

For this study, 120 bovine teeth (Figure 1a) were collected, the debris were manually removed, and the root portions were extracted using double-sided diamond discs (KG Sorensen, Ind. Com. Ltda, Barueri, SP, Brazil) (Figure 1B and 1C), and the crowns were stored in distilled water. The 120 enamel blocks (5.0 x 5.0 mm) were extracted from the flattest area of the teeth's vestibular surface. The coronal portion was cut using high-concentration diamond discs (Extec 4" x 0.012" x 1/2, Extec, USA) attached to a metallographic cutter (Labcut 1010, Extec, USA) (Figure 1d and 1d). The size of each dental block was confirmed using a micrometer (Figure 1f). The blocks were attached to an acrylic disc with the enamel surface facing downwards (Figure 1g) and 400-grit sandpaper (APL4 metallographic sandpaper; Arotec S.A, Brazil) was used in a polishing machine (APL4 Metallographic Polisher; Arotec S.A, Brazil) for the enamel blocks planning (Figure 1h).

Figure 1 - Methodological Sequence for Enamel Blocks Manufacture

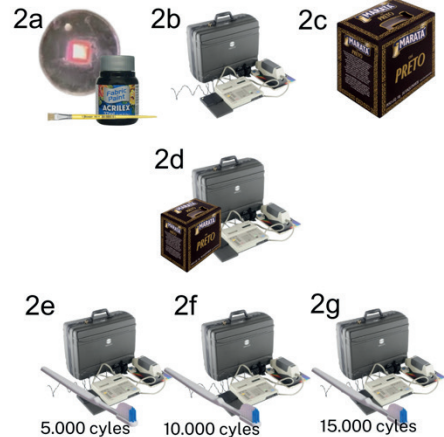


Source: research data.

The dental blocks were equally divided into a group for color assessment (Figure 2) and another for microhardness evaluation (Figure 3). For the blocks designated for color evaluation, the acrylic resin was painted with matte black paint (Figure 2a), and only the enamel surface was exposed to prevent external

light from interfering with the color measurement process. For microhardness evaluation, the enamel surface of the blocks was planned and polished using 400, 600, and 1200-grit sandpapers sequentially (APL4 metallographic sandpapers; Arotec S.A, Brazil) and final polish using a self-adhesive metallographic polishing cloth soaked in alumina solution (Figure 3a and 3b).

Figure 2 - Methodological Sequence for Color Analysis



Source: research data.

Figure 3 - Methodological Sequence for Microhardness Analysis



Source: research data

2.2 Color analysis

A spectrophotometer (MINOLTA CR-321, Japan) was used to obtain baseline color data before the staining and brushing stages (Figure 2b) in digital CIELAB values (L^* , a^* , b^*). According to the Commission Internationale de l'Eclairage (CIE) L^* represents brightness, a^* the variation from green to red and b^* the variation from blue to yellow. Positive a^* values indicate shift towards red and negatives shift towards green. Positive b^* values indicate shift towards yellow and the negatives shift towards blue^{12,13}.

Five color evaluations were conducted: before staining (baseline), after staining, after 5,000, 10,000 and 15,000 brushing cycles (Figure 2b, 2d-2g). Before the baseline reading, the test specimens for color evaluation were randomly divided into 6 groups according to the dentifrice used (Table 1).

Table 1 - Technical description of the dentifrices by the manufacturer

Groups	Dentifrice	Compounds	Manufacturer
G1	-	The negative control: ideionized distilled water	-
G2	Colgate Total 12 Whitening	Sodium Fluoride (1450 ppm Fluoride), Triclosan 0.3%. Sodium Lauryl Sulphate, Sorbitol; Hydrated Silica, Gantrez, Saccharin Sodium, Aromatic Composition, Dyes, Water, Triclosan, Fluoride, Carrageenan, Sodium Hydroxide, Dyes CI 77891, CI 77019 and CI 42090.	Colgate®
G3	Rembrandt Deeply White	Sodium monofluorophosphate 0.884%, glycerin, hydrated silica, urea peroxide, aluminum hydroxide, sodium citrate, propylene glycol, cocoamidopropyl betaine, papain, sodium lauryl sulfate, carbomer, sodium saccharin, calcium disodium EDTA.	Johnson & Johnson®
G4	Rembrandt Intense Stain	Sodium fluoride 0.243%, water, glycerin, hydrated silica, sorbitol, tetrapotassium pyrophosphate, titanium dioxide, sodium lauroyl sarcosinate, cellulose gum, lauryl glucoside, PVP, cocamidopropyl betaine, sodium saccharin, sucralose.	Johnson & Johnson®
G5	PeroxiCare	Sodium fluoride 0.243%, sodium bicarbonate, PEG-8, PEG/PPG-116/66 copolymer, sodium carbonate peroxide, tetrasodium pyrophosphate, silica, sodium saccharin, flavor, sodium lauryl sulfate, lauroyl sarcosinate.	Arm & Hammer®
G6	CompleteCare	Sodium fluoride 0.24%, sodium bicarbonate, PEG-8, tetrasodium pyrophosphate, PEG / PGG 116/66 copolymer, sodium carbonate peroxide, silica, sodium saccharin, flavor, water, sodium lauroyl sarcosinate, sodium lauryl sulfate.	Arm & Hammer®

Source: research data.

The test specimens were stained using black tea prepared at a ratio of 10 grams of tea per 1 liter of distilled water and boiled for two minutes. The experiment lasted for five consecutive days with daily tea changes at 9:00 AM and 3:00 PM. At 9:00 AM, the test specimens were individually placed in 20 mL of black tea and remained until 3:00 PM. They were then removed from the tea, washed with distilled water, and immersed in artificial saliva for one hour. After that, the test specimens were placed back in 20 mL of freshly prepared black tea solution, where they remained until 9:00 AM the following day. This entire exchange process was repeated for five consecutive days^{12,14}.

For the simulated brushing test, each product was diluted in a ratio of 3:1 (300 ml of distilled water to 100 g of dentifrice)^{13,15,16}. The dentifrice was weighed using a precision electronic scale (Bel Mark 330), and distilled water was measured using a volumetric pipette. The dilution process occurred on a magnetic stirrer.

During the brushing test, the MSEt brushing machine (1500w) was used, which has a metal bar for securing the test specimens (enamel blocks) and dentifrices. Soft-bristle brushes (Tek®) were cut and positioned on the machine so that the bristles were perpendicular to the specimen. The machine was adjusted to operate at a temperature of 37 °C during the experimental stage, with a pressure of 200g, performing back-and-forth movements at a speed of 60 cycles per minute.

The test specimens were attached to the metal bar of the brushing machine in such a way that approximately 1 mm of the enamel surface was in contact with the bristles of the brushes used. They were then subjected to brushing cycles totaling 15,000 cycles, which corresponds to approximately 15 months of brushing by a healthy individual¹⁷. After every 5,000 brushing cycles, the test specimens were re-analyzed for color changes.

During the brushing stage, the dentifrice solution was injected into the test specimens every 20 seconds using

syringes to ensure consistent exposure throughout the brushing cycles.

During the intervals between the brushing stages, the test specimens were kept in artificial saliva. The saliva was constantly agitated using an orbital shaker (Tecnal TE 420) at a temperature of 37 °C and a speed of 100 rpm. Following this, the specimens underwent brushing cycles, and color readings were taken.

2.3 Microhardness analysis

The Microdurometer HMV-2000 (Shimadzu, Japan) equipped with a Knoop indenter was used. Three indentations were made by applying a force of 50 grams for 10 seconds. The microhardness of the surface of each block was determined by calculating the average of these indentations. The microhardness was measured at four different times: before brushing, and after 5,000, 10,000, and 15,000 brushing cycles¹⁸.

Before conducting microhardness testing, the test specimens were randomly divided into 6 groups based on the dentifrice used (Table 1). After randomizing the test specimens, the initial microhardness evaluation was conducted. Subsequently, the brushing procedure was performed as described for color analysis. After every 5,000 brushing cycles, the test specimens were re-evaluated for microhardness. The data obtained were subjected to a one-way analysis of variance test (ANOVA), and any significant differences were analyzed using the Tukey test with a significance level of 5%.

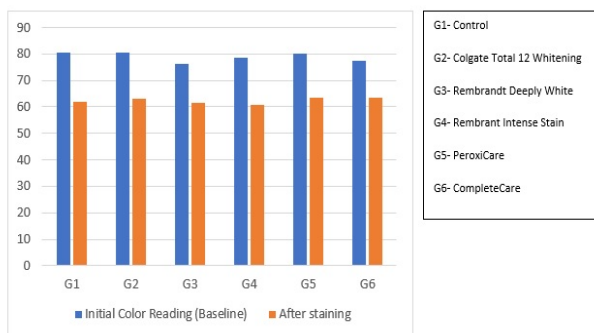
3 Results and Discussion

Studies analyzing the effects of teeth-whitening products became common in the scientific community^{7,19} and some of these products can have adverse effects on enamel surfaces⁸. Based on recent studies^{20,21}, abrasive is the primary component in whitening dentifrices for stain removal, and hydrated silica

and baking soda are effective abrasives for this purpose and some dentifrice formulations may contain peroxides as well¹. However, the misinformation about the potential harm of these products is increasing in society due to careless usage. In this study, the specimens were stained for five consecutive days using a black tea solution because it is one of the most effective substances for teeth discoloration²⁰.

The average “L” value during the initial readings (80.40, 80.64, 76.32, 78.47, 80.01 and 77.42, for the six respective dentifrices) are represented in Figure 4. The luminosities of the enamel blocks before the experiment were statistically similar ($p=0.1155$), indicating homogeneity. This outcome is critical to the study because it enables the comparison of the average “L” values among the groups during the following brushing stages.

Figure 4 - Initial Luminosity Read (Baseline) and After the Staining Step



Source: research data.

It can be observed that there was no significant difference between the average L^* values among the dentifrices after the staining stage (62.11, 62.98, 61.39, 60.88, 63.6, 63.39, for the six respective dentifrices). This indicates that all test specimens underwent the staining process similarly and homogeneously ($p>0.05$). In the post-staining stage compared with the first 5,000 brushing cycles ($\pm 2,5$ months) it was noted that dentifrices G1, G4, G5 and G6 had lesser color variation compared to G2 and G3. The G5 and G6 had greater color variation and were also similar to each other (Figure 5).

Figure 5 - Total Color Variation (ΔE) after Staining and with 5,000 Brushing Cycles (ANOVA, $p > 0.05$)

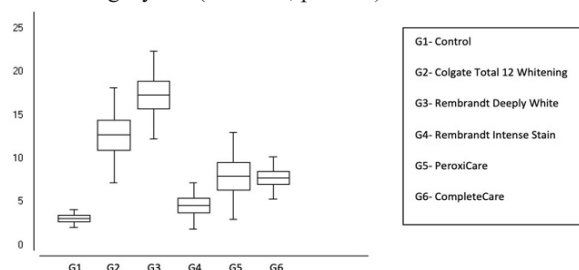


Figure 5: Total Color Variation (ΔE) after Staining and with 5,000 Brushing Cycles (ANOVA, $p > 0.05$).

Source: research data.

When comparing 10,000 cycles to the post-staining stage, G3 had significantly higher total color variation than the

other dentifrices, as shown in Figure 6. After analyzing the gradual changes in tooth color between brushing cycles, G2 showed the highest total color variation between 5,000 and 10,000 cycles and it suggests that the dentifrice has greater whitening potential, probably due to the abrasive component in its composition, which had more significant initial impact. Over time, the whitening effect of G3 and G6 became more pronounced, and this can be attributed to the chemical reaction of the peroxide contained in the composition of these dentifrices, which are responsible for the gradual whitening action.

Figure 6 - Total Color Variation (ΔE) after Staining and with 10,000 Brushing Cycles (ANOVA, $p > 0.05$)

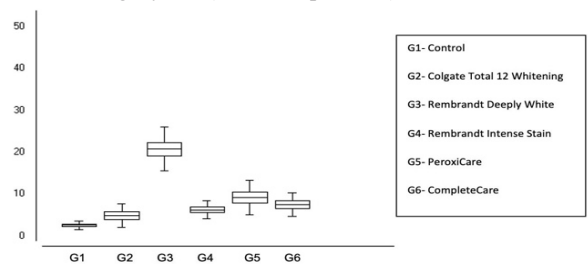


Figure 6: Total Color Variation (ΔE) after Staining and with 10,000 Brushing Cycles (ANOVA, $p > 0.05$).

Source: research data.

According to Figure 7, it is noticeable that dentifrices G3, G4, G5 and G6 showed higher color variation, and they were statistically similar to one another. On the other hand, G1 and G2 had lower color variations and were similar. As mentioned, the concentration of whitening agents in dentifrices products is quite low and as a result, it may take a longer period to observe any noticeable color changes. The dentifrice containing only abrasives (G2) may have caused the initial whitening effect and then stabilized, while the dentifrices containing peroxides continued to whiten teeth, resulting in a greater whitening effect after 15,000 brushing cycles.

Figure 7 - Total Color Variation (ΔE) after Staining and with 15,000 Brushing Cycles (ANOVA, $p > 0.05$)

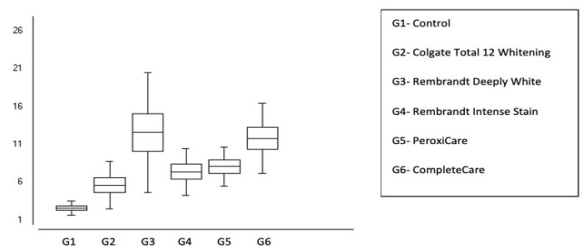


Figure 7: Total Color Variation (ΔE) after Staining and with 15,000 Brushing Cycles (ANOVA, $p > 0.05$).

Source: research data.

Many articles suggest that dentifrice marketed as ‘whitening’ doesn’t whiten teeth, but only removes surface stains through abrasion²³. This contradicts the results of a particular study^{24,25}, which found that the dentifrice they used did change the color of enamel. The change probably occurred due to the removal of surface stains, which causes a ‘pseudo-whitening’ effect by increasing the amount of light reflected on

the enamel surface. However, this effect isn't a true whitening, as studies show that abrasives cannot remove the chromatic molecules located in the deeper regions of dental tissues.

A gradual evaluation between brushing cycles, rather than between cycles and the post-staining stage, it can be observed that dentifrice G1 exhibited lower color variation, not differing statistically from G3, G4, G5 and G6. G2 showed the highest total color variation. About the color variation between 10,000 and 15,000 brushing cycles, it can be observed that G1, G2, G4 and G5 had lower color variation and were similar to each other. Meanwhile, G3 and G6 showed higher total color variation and were statistically similar.

Dentifrices abrasiveness is influenced by several factors such as the hardness of the abrasive agent, particle size and shape, and pH²⁶. The mean microhardness values (-11.23, -47.10, 43.70, -155.46, -55.13 and -360.33 for the six respective dentifrices) are represented in Figure 8, ANOVA ($p < 0.0001$). It has been observed that dentifrices containing whitening agents increased the enamel surface roughness²⁵, this agrees with G6 values as a peroxide-abrasive-based dentifrice showed a decrease in superficial microhardness, suggesting significant mineral loss and lower surface microhardness. It is worth noting that G3 had higher hardness, but there was no statistical difference from the other groups.

Figure 8 - Microhardness Means after 5,000 Brushing Cycles (ANOVA, $p = 0.09$)

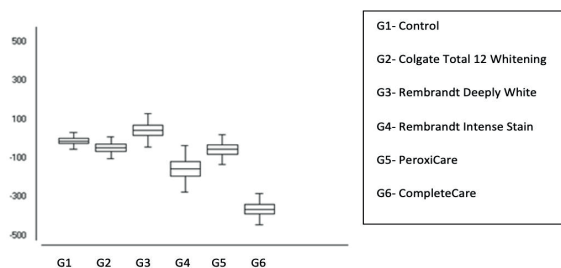
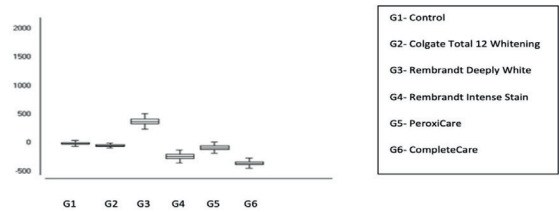


Figure 8: Microhardness Means after 5,000 Brushing Cycles (ANOVA, $p = 0.09$).

Source: research data.

Some authors argue that the fluoride present in dentifrice can interact with abrasive agents and make it insoluble and ineffective in the remineralization process^{27,28}. This could be one of the reasons why we did not observe any mineral repair caused by the peroxides and abrasives in the dentifrice used in the experiment. After 5,000 additional cycles to explore if there were any changes in hardness with an increased number of cycles, the mean microhardness values (-10.56, 46.00, 376.53, -236.06, -84.40 and -355.30 for the six dentifrices) were found, ANOVA ($p < 0.0001$).

Figure 9 - Microhardness Means after 10,000 Brushing Cycles



Source: research data.

After more 5,000 brushing cycles, totaling 15,000 cycles, the mean microhardness values (22.93, -31.30, 344.96, -227.13, -125.66, -354.86, for the six respective dentifrices) were found as represented in Figure 10, ANOVA ($p < 0.0001$). G6 exhibited lower surface microhardness, which suggests greater mineral loss. However, the difference between G6 and G4 was not significant and both dentifrices were significantly different from the rest. On the other hand, G3 showed higher surface microhardness, indicating a possible gain in minerals. However, this conclusion cannot be definitively drawn as that type of assessment was not conducted.

Figure 10 - Microhardness Means after 15,000 Brushing Cycles

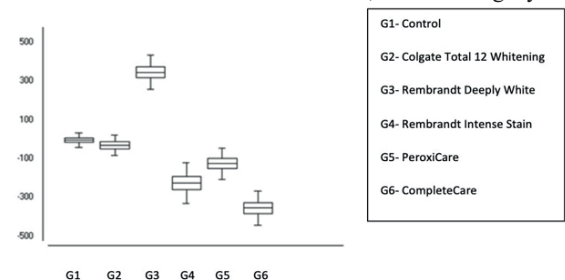


Figure 10: Microhardness Means after 15,000 Brushing Cycles.

Source: research data.

All dentifrices except G3 indicate possible mineral loss. However, several factors must be considered while interpreting the results. These factors include the type of abrasive substance present in each dentifrice, the molecular complexity of dentifrice, and their propensity for fluoride aggregation. Additionally, the chemical reaction mechanism that occurs during brushing with various types of dentifrices and the various conditions that lead to fluoride undergoing chemical processes must be considered and is recommended to conduct specific studies to analyze these phenomena.

After 5,000 cycles, group G6 experienced a significant reduction of the surface hardness, indicating a noticeable mineral loss as compared to the other groups. This suggests a lower superficial microhardness, which remained consistent throughout the analysis after 15,000 brushing cycles. Furthermore, group G3 showed higher surface hardness initially, but it was not statistically different from the other groups. However, after 10,000 cycles, there was an increase in microhardness that continued for 15,000 cycles, and it was

statistically different from the other groups. The reason for this increase in surface hardness is unknown and must be the subject of future research.

The available literature on the impact of peroxides in whitening dentifrice on dental enamel remains limited. Due to the lack of extensive research in this area, it is difficult to have a detailed discussion on the effectiveness of whitening dentifrice containing peroxides in its formulation.

4 Conclusion

Based on the results obtained, we can conclude that in this in vitro study, Rembrandt Deeply White, Rembrandt Intense Stain, PeroxiCare, and CompleteCare dentifrices showed greater whitening potential. Brushing with Rembrandt Deeply White dentifrice resulted in higher superficial microhardness on bovine dental enamel, while CompleteCare dentifrice resulted in lower superficial microhardness.

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