


## EVALUATION OF SURFACE ROUGHNESS IN BOVINE TEETH AFTER THE APPLICATION OF TWO IN-OFFICE BLEACHING PROTOCOLS

Avaliação da rugosidade superficial de dentes bovinos após a aplicação de dois protocolos de clareamento de consultório

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## RESUMO

O clareamento dental é uma prática comum para a remoção de manchas dentárias, mas seus efeitos na rugosidade superficial do esmalte ainda são pouco estudados. Este estudo in vitro teve como objetivo avaliar as alterações na rugosidade do esmalte bovino após diferentes protocolos de clareamento

dental. Trinta dentes incisivos bovinos foram divididos em cinco grupos experimentais, recebendo diferentes combinações de clareamento e tratamentos dessensibilizantes. A rugosidade superficial foi medida antes e após o tratamento utilizando o índice Ra. Os resultados indicaram uma leve redução na mediana da rugosidade após o tratamento ( $P = 0,447$ ), sem diferença estatisticamente significativa. O poder do teste foi de 0,050, sugerindo que a amostra foi insuficiente para detectar diferenças pequenas. A literatura sugere que alterações sutis na rugosidade podem ocorrer, mas muitas vezes sem relevância clínica.

**Palavras-chave:** Clareamento dental; rugosidade de superfície; peróxido de hidrogênio.

## ABSTRACT

Dental bleaching is a common practice for removing dental stains; however, its effects on enamel surface roughness remain underexplored. This in vitro study aimed to evaluate changes in the surface roughness of bovine enamel following different dental bleaching protocols. Thirty bovine incisor teeth were divided into five experimental groups, receiving various combinations of bleaching and desensitizing treatments. Surface roughness was measured before and after treatment using the Ra index. Results showed a slight reduction in median roughness after treatment ( $P = 0.447$ ), with no statistically significant difference. The test power was 0.050, indicating that the sample size was insufficient to detect small differences. Literature suggests that subtle changes in roughness may occur but are often clinically irrelevant.

**Keywords:** Dental bleaching; surface roughness; hydrogen peroxide.

## INTRODUCTION

Tooth whitening has established itself as one of the most sought-after aesthetic interventions in dental practice due to its effectiveness in removing intrinsic and extrinsic stains associated with factors such as diets, poor oral hygiene habits, aging, and the use of substances such as tobacco (CAREY, 2014; ITTATIRUT *et al.*, 2014; LI *et al.*, 2024; MAJEED *et al.*, 2015). This procedure is widely adopted not only for its ability to enhance dental aesthetics but also for its minimally invasive nature, providing satisfactory results with a low risk of complications (RODRÍGUEZ-MARTÍNEZ *et al.*, 2019; WIEGAND *et al.*, 2005).

However, understanding the mechanism of action of whitening agents continues to generate significant scientific interest, as it is essential to optimize outcomes and minimize potential adverse effects. The underlying mechanism of tooth whitening relies on the ability of oxidizing agents, such as hydrogen peroxide and carbamide peroxide, to generate free radicals (BABOT-MARQUILLAS *et al.*, 2022; KWON & WERTZ, 2015). These highly reactive radicals penetrate the layers of the dental enamel, breaking the chemical bonds of chromophore pigments present in its structure (HEGEDÜS *et al.*, 1999; KWON *et al.*, 2012). The depth of penetration of whitening agents and the stability of the effect depend on several factors, including agent concentration, application time, and the condition of the dental enamel, which must be strictly controlled to ensure effective and safe results (ZHAO *et al.*, 2023).

Although the aesthetic effects of tooth whitening are widely recognized, the physical and structural modifications in dental enamel, particularly surface roughness, have been less explored (FERNANDES *et al.*, 2020). Studies indicate that whitening can alter enamel microstructure, resulting in increased roughness, which may compromise both dental aesthetics and functionality (PIMENTA-DUTRA *et al.*, 2016; TRENTINO *et al.*, 2015). Increased roughness may facilitate plaque accumulation, potentially compromising oral health and reducing the durability of aesthetic results, as rougher surfaces promote additional wear and increase susceptibility to new stains (HOSOYA *et al.*, 2003; HOSSAM *et al.*, 2013).

The analysis of surface roughness emerges as a crucial aspect for understanding the long-term effects of tooth whitening, as well as for contributing to the development of therapeutic protocols that promote sustainable aesthetic results while preserving the structural integrity of the enamel. This study aimed to investigate changes in enamel surface roughness resulting from whitening, using the Ra standard as a quantitative analysis method.

## MATERIALS AND METHODS

### *Study Characterization*

This in vitro study was conducted to evaluate changes in the surface roughness of bovine enamel following the application of different dental bleaching protocols. Thirty permanent central bovine incisor teeth were used, obtained from authorized slaughterhouses and handled in accordance with current ethical guidelines.

### ***Sample Preparation***

Initially, the teeth were subjected to the mechanical removal of periodontal tissue remnants and other residues using periodontal curettes. After manual cleaning, the teeth were immersed in a 0.5% sodium hypochlorite solution for 10 minutes, followed by thorough rinsing with distilled water to remove organic residues completely.

Standardized fragments measuring 7 × 7 mm were then obtained from the middle third of the labial surface of each tooth. Sectioning was performed using a high-speed rotary motor (Kavo, Curitiba, PR, Brazil) equipped with double-sided diamond-coated flexible discs (KG Sorensen, Barueri, SP, Brazil) under continuous irrigation with distilled water to prevent overheating and enamel damage. The dimensions of the fragments were verified using a digital caliper (Mitutoyo, Suzano, SP, Brazil) to ensure sample uniformity. After preparation, the fragments were stored in isotonic saline solution (0.9% NaCl) at 4°C for 15 days for initial stabilization.

### ***Storage Medium***

Following stabilization, the specimens were transferred to an artificial saliva storage medium containing potassium chloride (1.2 g/L), sodium chloride (0.7 g/L), magnesium chloride hexahydrate (0.2 g/L), calcium chloride dihydrate (0.3 g/L), monobasic potassium phosphate (0.2 g/L), and sodium fluoride (0.02 g/L). The pH was adjusted to 6.8 using hydrochloric acid and sodium hydroxide, simulating oral conditions. The samples were maintained in an incubator at 37°C, with constant agitation at 50 rpm, and the solution was refreshed every 48 hours to prevent ionic saturation and biofilm formation.

### ***Experimental Group Distribution***

The samples were randomly divided into five experimental groups (n = 6) according to the bleaching and desensitizing protocols described in Table 1.

Group	Desensitization Protocol	Bleaching Agent	Number of Samples
G1	Not applied	Not applied	6
G2	Yes (potassium nitrate and fluoride)	Potenza Bianco 38%	6
G3	Not applied	Potenza Bianco 38%	6
G4	Yes (potassium nitrate and fluoride)	Whiteness HP Maxx 35%	6
G5	Not applied	Whiteness HP Maxx 35%	6

### ***Desensitization Protocol***

For the groups subjected to the desensitization protocol (G2 and G4), Desensibilize KF 2% gel (FGM Produtos Odontológicos, Joinville, SC, Brazil), containing potassium nitrate and sodium fluoride, was used. The product was applied to the sample surfaces and allowed to act for 10 minutes, following the manufacturer's instructions. Afterward, the gel was removed with gauze moistened in distilled water.

### ***Bleaching Protocol***

**Figure 1:** Bovine tooth specimens after sectioning and preparation, embedded in utility wax for stabilization and proper positioning during experimental procedures. The wax was used to ensure uniform fixation of the samples, facilitating handling and the application of treatments in the bleaching and desensitization protocols. The samples were subsequently assigned to experimental groups according to the treatments described:



### **Control Group (G1):**

- **Procedure:** No bleaching or desensitization protocol was performed.
- **Application:** Only prophylaxis with pumice and water was performed to remove potential surface impurities.
- **Objective:** This group served as a control, allowing comparisons with the results of other groups treated with bleaching agents.

**Group G2 (Potenza Bianco 38% with desensitization protocol):**

- **Procedure:** Initial prophylaxis with pumice and water.
- **Bleaching treatment:** Following prophylaxis, a uniform layer of Potenza Bianco 38% bleaching gel was applied to the buccal and interproximal surfaces of the teeth. The layer thickness was approximately 1 mm.
- **Application time:** The gel remained on the samples for 25 minutes, with constant monitoring to ensure the removal of any oxygen bubbles.
- **Objective:** To evaluate the effectiveness of Potenza Bianco 38%, including the effect of the desensitization protocol applied before the bleaching gel.

**Group G3 (Potenza Bianco 38% without desensitization protocol):**

- **Procedure:** Prophylaxis with pumice and water, without desensitization.
- **Bleaching treatment:** Application of Potenza Bianco 38% bleaching gel, identical to G2, with a uniform 1-mm-thick layer.
- **Application time:** The gel was maintained on the tooth surface for 25 minutes, monitored for bubble removal.
- **Objective:** To evaluate the effect of Potenza Bianco 38% without desensitization, in comparison with G2.

**Group G4 (Whiteness HP Maxx 35% with desensitization protocol):**

- **Procedure:** Initial prophylaxis with pumice and water.
- **Desensitization treatment:** Desensibilize KF 2% gel was applied for 10 minutes, following the manufacturer's guidelines.
- **Bleaching treatment:** Following desensitization, Whiteness HP Maxx 35% bleaching gel was applied in three consecutive 15-minute cycles, with a layer thickness of 0.5 to 1 mm. The gel was agitated on the tooth surface using a microbrush to release oxygen bubbles and improve enamel contact.

- **Objective:** To evaluate the bleaching effect of Whiteness HP Maxx 35%, including the impact of the desensitization protocol.

#### **Group G5 (Whiteness HP Maxx 35% without desensitization protocol):**

- **Procedure:** Prophylaxis with pumice and water, without desensitization.
- **Bleaching treatment:** Application of Whiteness HP Maxx 35% bleaching gel according to the manufacturer's protocol, in three consecutive 15-minute cycles, totaling 45 minutes.
- **Objective:** To evaluate the effect of Whiteness HP Maxx 35% without desensitization, in comparison with G4.

#### **Group G6 (Additional control without bleaching):**

- **Procedure:** Prophylaxis with pumice and water, without bleaching or desensitization.
- **Objective:** To assess the effect of isolated prophylaxis on enamel roughness without the interference of bleaching or desensitizing agents.

#### ***Surface Roughness***

Surface roughness was measured using a profilometer from Time Group Inc. (model TA 630) at the LabMat - IFPI. Topographical assessment was performed at seven points, with a scanning range of 2.5 mm.

#### ***Quality Control and Ethics***

All procedures adhered to the ethical guidelines outlined in Resolution No. 11.794/2008. The study was submitted to and approved by the Ethics Committee on the Use of Experimental Animals at Centro Universitário Santo Agostinho (CEUA/UNIFSA), under protocol number [0725/22].

## RESULTS

### *Data Distribution*

The normality of the initial and final surface roughness data was assessed using the Shapiro-Wilk test. Results indicated that the data did not follow a normal distribution ( $P < 0.050$ ), justifying the use of non-parametric statistical methods for subsequent analyses. Statistical analyses were performed using SigmaPlot 12 software, which enabled the application of appropriate tests for non-normal data, ensuring the robustness and reliability of the findings.

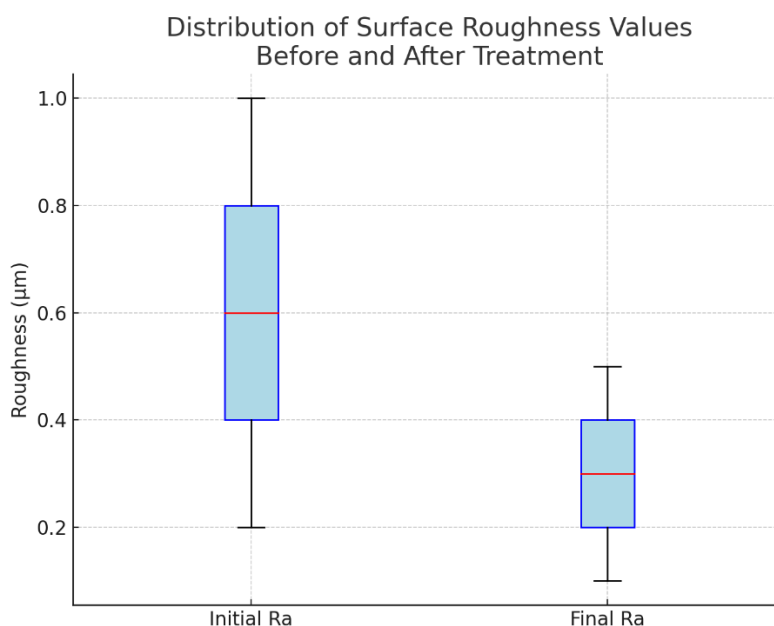
### *Surface Roughness Before and After Treatment*

To evaluate differences in surface roughness (Ra) between the initial and final time points, the Wilcoxon Signed Rank test was applied. Initial roughness values exhibited a median of 0.294  $\mu\text{m}$  (interquartile range: 0.173–0.978  $\mu\text{m}$ ), while final roughness values showed a median of 0.272  $\mu\text{m}$  (interquartile range: 0.148–0.643  $\mu\text{m}$ ). Despite a slight reduction in median roughness values after treatment, statistical analysis revealed that this difference was not significant ( $P = 0.447$ ).

Metric	N (Total)	Missing	Median	25th Percentile	75th Percentile
Initial Ra	30	0	0.294	0.173	0.978
Final Ra	30	0	0.272	0.148	0.643

The boxplot (Figure 2) illustrates the dispersion and overlap of data at both analyzed time points. Initial roughness values exhibited greater variability, with a broader interquartile range, compared to final values. However, this reduction in dispersion was not sufficient to indicate a significant change in roughness after treatment.





**The statistical power of the test was calculated as 0.050, indicating that the sample size was insufficient to detect small differences, if they exist. The ideal power of 0.800 suggests that future studies should consider larger sample sizes to ensure greater statistical robustness.**

## DISCUSSION

The analysis of surface roughness before and after dental treatment, while showing a slight reduction in median roughness, did not reveal a statistically significant difference. This finding aligns with the scientific literature, which reports varying results regarding the impact of dental bleaching on enamel surface roughness. While some studies indicate an increase in roughness after bleaching (HAUSS MONTEIRO *et al.*, 2019; WONGPRAPARATANA *et al.*, 2018; YUSOF *et al.*, 2020), others have found no significant changes to the surface (DA ROSA *et al.*, 2016; KOLSUZ OZCETIN & SURMELIOGLU, 2020; MOUROUZIS *et al.*, 2021).

Furthermore, the study by Borges *et al.* (2021) also found no significant differences in bleaching efficacy when comparing different in-office protocols, reinforcing that, under the conditions evaluated, the tested protocols demonstrated equivalent performance in both whitening efficacy and enamel preservation. This variability in findings can be attributed to multiple factors, such as the composition of bleaching agents, application technique, treatment duration, and the initial condition of the dental surface.

Another important consideration is that enamel surface roughness is influenced by several variables, including enamel quality (e.g., whether it is more or less mineralized), the presence of pre-existing surface defects, and even the patient's overall oral health. In teeth that already exhibit some degree of wear or irregularity, treatment may have a more pronounced impact, promoting a reduction in surface roughness. However, in teeth with better structural integrity, changes in surface texture may be less perceptible.

It is noteworthy that, as indicated by the power analysis, which yielded a value of 0.050, the sample size in this study was insufficient to detect small differences, even if they exist. A power of 0.050 reflects a relatively high probability of committing a Type II error, meaning the potential failure to detect a real effect. To ensure greater statistical robustness and the ability to identify small variations in roughness, future studies should consider using larger sample sizes, which would enable the detection of significant differences with greater precision.

Although the results of this study suggest a slight trend toward reduced surface roughness after treatment, the lack of statistical significance and the low-test power highlight the need for more robust studies with larger sample sizes to validate or refute these findings. Only through more comprehensive analyses will it be possible to draw more consistent and clinically applicable conclusions.

Additionally, the findings of this study are consistent with the existing literature and underscore the importance of considering methodological variables, such as bleaching agent composition and experimental conditions, when interpreting the effects of dental bleaching on enamel. Future investigations should address these issues more thoroughly, contributing to a deeper understanding of clinical impacts and aiding in the development of safer and more effective protocols.

## CONCLUSION

In conclusion, the results of this study indicate a slight trend toward reduced surface roughness after treatment, without, however, achieving statistical significance. Similarly, the comparison between groups with and without desensitization revealed no significant differences. These findings suggest that while treatment may have a potential effect in smoothing the dental surface, the observed changes are likely small and of limited clinical relevance.



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