

# Surface analysis of bovine teeth submitted to dental bleaching: in vitro study

Joyce Caroline da Silva, Celena de Sousa Dias, Luanne Mara Rodrigues de Matos, Thais Oliveira Cordeiro

Department of Odontology, Santo Agostinho University Center, Teresina-PI/Brazil.

Received: 11 Sep 2025,

Received in revised form: 09 Oct 2025,

Accepted: 13 Oct 2025,

Available online: 17 Oct 2025

©2025 The Author(s). Published by AI  
Publication. This is an open-access article  
under the CC BY license

**Keywords—** Tooth whitening, Bleaching  
agents, Hydrogen peroxide, pH analysis,  
Bovine teeth

**Abstract**—Tooth whitening is a procedure that lightens the color of a tooth by applying a chemical agent to oxidize the organic pigmentation of the tooth. The agents hydrogen peroxide and carbamide peroxide are oxidizing agents, which, after diffusion, dissociate to produce free radicals that affect pigmented organic molecules. Objective: To evaluate the action of bleaching agents on the dental surface of bovine teeth. Through an in vitro laboratory experiment, the action of bleaching agents (Whiteness HP 35% - FGM and Potenza Bianco Pro 35% - PHS) on the tooth surface was evaluated, from which 30 bovine teeth were collected, sanitized with distilled water, stored in saliva for seven days, cut by separating the root from the crown with a standardized 10x10 mm cut in the straight piece with a diamond disc, then immersed in saliva for another seven days, where they were divided into three groups (samples), defined in control group (GC), test groups I and II (GT I and TG II). Prophylaxis was performed on all teeth in the groups (samples) with prophylactic paste and a straight Robinson brush. Subsequently, the teeth in the control group (CG) were immersed again in artificial saliva for another seven days. After seven days, the pH of the two bleaching agents was descriptively analyzed, and the analysis of free energy/wetting of the groups (samples) was also carried out. The pH of Potenza Bianco Pro 35% bleach was 6.0 and its wettability was 119.85. The pH of Whiteness HP 35% bleach was 8.5 and its wettability value was 119.66. The bleaching agent Potenza Bianco Pro – PHS is more acidic and the Whiteness HP – FGM is alkaline. The wettability of the whitening agent Potenza Bianco Pro – PHS is similar to Whiteness HP – FGM > 90°.

## I. INTRODUCTION

Teeth are an integral part of facial aesthetics and are involved in complex social, cultural, and psychological interactions. It is commonly perceived that whiter teeth enhance the beauty of a person's smile. As a result, the demand for whiter and brighter teeth is increasing among today's patients. Teeth whitening is one of the fastest-growing areas in aesthetic and restorative dentistry. In light of this, there is a need to delve deeper into understanding

how the dental surface behaves during the action of whitening agents[1].

Tooth aesthetics and color are important topics for society. This includes both dental professionals who aim to select the correct tooth shade and restorative material, and patients who wish to improve the appearance of their smile. Tooth color is influenced by a combination of its intrinsic shade and the presence of any extrinsic stains that may form on the tooth surface[2].

Whitening is a procedure that involves lightening the color of a tooth through the application of a chemical agent that oxidizes organic pigmentation in the tooth. Teeth are whitened by materials such as hydrogen peroxide (HP) and carbamide peroxide (CP), which diffuse initially through the enamel and dentin. These oxidizing agents, after diffusing, dissociate to produce free radicals that target pigmented organic molecules, reducing light absorption and creating a "whitening effect"[1].

It is extremely important to evaluate the action of whitening agents on the dental surface through an in vitro study. The scope of this study was to evaluate the effects of whitening agents on the dental surface of bovine teeth.

## II. METHODS

Thirty bovine teeth (n=30) were collected as a convenience sample from a slaughterhouse in the city of Teresina, PI, with authorization from the Animal Use Ethics Committee of Centro Universitário Santo Agostinho (CEUA/UNIFSA), under protocol no. 6064/22. Since biological material was involved, the study was conducted in accordance with Resolution No. 003/2015 and the appointment ordinance No. 007/2015, which aim to enforce the provisions of Law No. 11.794 of October 8, 2008, and other applicable regulations regarding the use of animals in teaching and research, especially the resolutions of the National Council for the Control of Animal Experimentation (CONCEA).

After extraction, the teeth were thoroughly washed with distilled water and immersed in 500 mL of artificial saliva (1% CMC; 0.12% potassium chloride; 0.0052% magnesium chloride; 0.0084% sodium chloride; 3% sorbitol; 3% monobasic potassium phosphate; 0.0146% calcium chloride; q.s.p. distilled water to 1000 mL) for seven days. Next, the crowns were separated from the roots using diamond discs (American Burrs) attached to a straight handpiece with a micromotor (KaVo), making standardized cuts of 10x10 mm, measured with a millimeter ruler for identification. The samples were cleaned and stored in containers submerged in artificial saliva for another seven days, after which they were divided into three groups (samples), with n=10 in each group: control group (CG), and test groups I and II (TG I and TG II).

Prophylaxis was performed on all sample teeth using Shine prophylactic paste (tutti-frutti flavor) – Maquira and a straight Robinson brush – Preven, attached to a low-speed contra-angle handpiece with a KaVo micromotor. The control group (CG) teeth were then re-immersed in artificial saliva for an additional seven days. The hydrogen peroxide and thickening agents from both whitening products (Whiteness HP 35% – FGM and Potenza Bianco Pro 35% –

PHS) were mixed using the mixing tray and spatula included in the kit, in a 3:1 drop ratio, according to each manufacturer's instructions.

The pH of the two whitening agents was analyzed descriptively using universal pH strips – KASVI. Following this, the physicochemical properties of the enamel surface were characterized by contact angle measurements using the sessile drop method to determine surface free energy. A fixed-volume automatic pipette – Kacil was used to dispense 0.5 µL of each liquid onto the enamel surface of each bovine tooth fragment using a 0.5 mm gauge needle.

Images were captured using a Canon Rebel T5i camera with a 100 mm macro lens. After seven days, additional images of the control group (CG) were taken with distilled water applied to the dental surface to analyze surface free energy/wettability. Once the laboratory phase was completed, the images were processed using SurfTens 4.7 Automatic software (OEG GmbH, Frankfurt, Oder, Germany), where settings were configured for "distilled water" and "4-point analysis." In the software, the image was opened, zoomed in, and centered. Then, four (4) points were marked: the first exactly at the intersection between the specimen surface and the drop; the second and third at two random points on the drop's surface; and finally, the fourth at the opposite point of intersection between the drop and the specimen surface[3]. After marking the last point, the software automatically calculated the contact angle and displayed the result.

### Statistical

Each image was analyzed in triplicate by two researchers. Finally, the collected data were presented as means ± standard error of the mean and subjected to statistical testing using GraphPad Prism software.

## III. RESULTS

The Potenza Bianco Pro 35% whitening agent exhibited an acidic pH, whereas the Whiteness HP 35% whitening gel showed an alkaline pH (Table 1).

Table 1. Descriptive data of the pH of the tested substances.

	Distilled water	Potenza bianco Pro35%	Whiteness hp 35%
pH	7,0	6,0	8,5

Source: Authors (2025).

There was no statistically significant difference ( $p > 0.05$ ) between the control group and the tested whitening

agents in terms of contact angle/wettability. The comparisons between the groups are listed in Table 2.

Table 2. Means (standard deviations) of the wettability of the whitening agents.

	Distilled Water (GC)	Potenza Bianco Pro35%(GTI)	Whiteness HP 35% (GT II)
CONTACT ANGLE	92,47± (0,66) A	119,85 ± (2,17) A	119,66± (0,29) A

Means followed by the same uppercase letters indicate no statistically significant difference in the rows ( $p > 0.05$ ). Source: Authors (2025).

This study evaluated the effects of whitening agents on the surface of bovine teeth. The pH of the Potenza Bianco Pro 35% whitening agent was acidic, whereas the pH of the Whiteness HP 35% whitening agent was alkaline. Furthermore, the mean wettability values showed no statistically significant difference between the two test groups. Thus, the null hypothesis that whitening agents do not act equally on the dental surface is confirmed by the pH test. However, both whitening agents studied exhibited similar wettability values on the dental surface.

Dentists use high-concentration whitening gels due to their intense color change in the initial applications. Because the free radicals generated are not only selective to chromophores, but also interact with dental structures during diffusion, they can cause tooth sensitivity both during and after the procedure.

The application of whitening gels with acidic pH causes tooth sensitivity, which leads to the recommendation of whitening agents with neutral or alkaline pH. Therefore, the whitening agent in test group I (Whiteness HP 35% – FGM) is the most suitable for whitening treatments, as its alkaline pH reduces adverse effects related to sensitivity caused by other agents available on the market. As more products are introduced, the focus has been primarily on the overall impact of whitening agents on enamel surface morphology and mechanical action, rather than the contribution of peroxide pH to initiating a destructive chain reaction during the whitening process [4].

According to laboratory studies, enamel exposed to whitening products with different pH levels shows an increased risk of demineralization and root resorption after prolonged exposure to highly acidic products with pH values below 5.2 or highly basic products [5].

Thus, when evaluating the agents used in this study through the pH test, it was found that the GT I agent had a pH close to 5.2, corroborating the occurrence of adverse effects such as sensitivity and demineralization of the tooth surface. The most common adverse effect of most peroxide-based whitening procedures is tooth sensitivity, and efforts have been made to overcome the sensitivity caused by whitening treatments. As a result, fluoride, potassium nitrate, amorphous calcium phosphate (ACP), or hydroxyapatite nanoparticles (n-HAP) have been introduced into whitening agents to reduce the occurrence of hypersensitivity or demineralization [6].

However, even when using a desensitizing agent, it is still necessary to have pH-neutral or alkaline products available on the market in order to minimize the side effects of whitening treatment, such as post-whitening sensitivity, as presented by Whiteness HP 35%. Wettability was defined as the ability of a liquid to wet a solid. This can be exemplified as a drop of liquid resting on a solid surface, on which the liquid may or may not spread. Water, being a polar liquid, tends to spread over a surface with high surface energy and lead to the formation of a drop in areas with low energy. The analysis of the contact angle of a sessile drop, demonstrated by the angle of the section of the drop of a liquid material on a surface in equilibrium, is a means of quantifying this behavior [7].

The degree of spreading in which the fluid droplet flows over the solid surface is called wetting. The formation between the droplet-sphere and the substrate plane is the contact angle, which is consequently the measure of wetting. If the angle is small, wetting is considered good; when the angle is greater than 90 degrees, wetting is considered poor. When there is strong attraction between the molecules of the substance and the solid, this means good wetting, and surface energy also influences this attraction [8].

Given this, the variable investigated was similar for the two whitening agents under study and, in addition, showed poor results, since they presented an angle  $> 90^\circ$ . Although the samples were prepared rigorously and following the entire pre-established laboratory sequence, the experimental results obtained in this *in vitro* study still need to be confirmed in future clinical investigations on the surface of human teeth, since the present study evaluated the action of whitening agents on the surface of bovine teeth.

#### IV. CONCLUSION

- The Potenza Bianco Pro -PHS whitening agent is acidic, while Whiteness HP -FGM is alkaline;

- The wettability of the Potenza Bianco Pro - PHS whitening agent is similar to that of Whiteness HP - FGM > 90°.

#### DATA AVAILABILITY STATEMENT

The authors confirm that all data generated or analyzed during this review are included in this article.

#### CONFLICT OF INTEREST STATEMENT

The authors declare that they have no financial conflicts.

#### ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the Santo Agostinho University Center for the institutional support provided throughout the completion of this work. The infrastructure offered, access to academic resources, and the environment conducive to research were fundamental to the development of this article.

#### REFERENCES

- [1] Mounika A, Mandava J, Roopesh B, Karri G.(2017) Clinical evaluation of color change and tooth sensitivity with in-office and at-home whitening treatments. *Indian J Dent Res*;29:423-7.
- [2] Joiner A, Luo W.(2017) Tooth color and whiteness: A review. *J Dent. Dec*; 67S: S3-S10. doi: 10.1016/j.jdent.2017.09.006.
- [3] Neves JG, Danelon M, Pessan JP, Figueiredo LR, Camargo ER, Delbem ACB.(2018) Surface free energy of enamel treated with sodium hexametaphosphate, calcium, and phosphate. *Arch Oral Biol. Jun*; 90: 108-112. DOI:10.1016/j.archoralbio.2018.03.008.
- [4] Costa JL, Nogueira BR, de Oliveira Junior OB, Pretel H.(2021) Association of microabrasion and tooth whitening with LED/laser system in the treatment of incisor hypomineralization: 3-year followup. *Photodiagnosis Photodyn Ther. Mar*;33:102197. doi: 10.1016/j.pdpdt.2021.102197.
- [5] Alkahtani R, Stone S, German M, Water House P. A (2020) Review on dental whitening. *JDent. Sep*;100:103423. doi: 10.1016/j.jdent.2020.103423.
- [6] Kutuk ZB, ErginE, CakirFY, GurganS (2018). Effects of in-office bleaching agent combined with different desensitizing agents on enamel. *J Appl Oral Sci. Nov 8*;27:e20180233. doi: 10.1590/1678-7757-2018-0233. PMID: 30427477; PMCID: PMC6223786.
- [7] Dantas, Marta Cléa Costa et al.(2015). Effect of argon plasma therapy on teeth exposed to sodium hypochlorite: analysis of hydrophilicity. *RFO UPPF [online]*. vol. 20, no. 1, pp. 34-38. ISSN 1413-4012.
- [8] Fejerskov O; Kidd E (2011) Dental caries: the disease and its clinical treatment. *Ed. Santos*.p.216.