Beyond The White Smile: In Vitro Insights into Carbamide Peroxide Bleaching by Confocal Microscopy

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Abstract

1.Background/Objectives: Tooth bleaching with carbamide peroxide (CP) is one of the most common aesthetic procedures in modern dentistry, yet its effects on enamel ultrastructure remain a matter of concern. This in vitro study aimed to qualitatively assess microstructural changes in human enamel following in-office bleaching with two professional CP formulations—Opalescence Quick 45% and Pola Office+ 37.5%—in accordance with ISO 28399:2021 guidelines. *2.Methods*: Forty extracted human molars and premolars were sectioned to obtain paired specimens, with one half treated and the other serving as control. Bleaching protocols were performed under standardized conditions, and samples were analyzed using laser scanning confocal microscopy (CLSM) with three-dimensional reconstruction to evaluate enamel prism organization, porosity, and dentinal tubule exposure. *3.Results*: Both agents induced morphological alterations, including partial prism disorganization, increased porosity, and widening of interprismatic spaces. Opalescence Quick, with its higher CP concentration, produced more pronounced structural effects compared with Pola Office+, which exhibited a more moderate impact. Despite these changes, enamel integrity was not severely compromised. *4.Conclusion:* These findings suggest that carbamide peroxide bleaching can be considered safe when performed under professional supervision, particularly when complemented with remineralizing strategies. Future studies should investigate long-term effects and interactions with restorative materials under clinical conditions.

Keywords: tooth bleaching; carbamide peroxide; enamel microstructure; confocal laser scanning microscopy; in-office bleaching; enamel porosity; dental aesthetics

INTRODUCTION

Human social interactions have traditionally emphasized the importance of physical appearance, with the face being the most visible and expressive part. Among facial features, the eyes and mouth are most strongly linked to perceived attractiveness, with smiling serving as a central element of nonverbal communication. The absence of an aesthetically pleasing smile can negatively affect self-esteem, and tooth color has been consistently identified as a critical determinant of smile attractiveness. Dental aesthetics therefore often prioritize both tooth color and shape as key parameters in evaluating smile appeal. Individuals who express satisfaction with the appearance of their teeth, including their color and form, are more likely to project confidence and extroversion, whereas discolored, missing, or damaged teeth may contribute to social discomfort, reduced self-confidence, and negative psychosocial outcomes [1].

In contemporary dentistry, patients increasingly seek not only oral health but also the aesthetic benefits of a flawless smile. Dental appearance plays a crucial role in self-perception and social interactions, with tooth whiteness in particular being strongly associated with improved quality of life before and after treatment [2]. Tooth whitening has become one of the most sought-after aesthetic procedures in modern dentistry, reflecting the growing demand for minimally invasive treatments that enhance the appearance of the smile. Tooth discoloration may result from intrinsic factors linked to pathological processes, but it is more commonly associated with extrinsic factors such as the consumption of coffee, tea, wine, and other pigmented foods, smoking, or the misuse of antibiotics. Substances including tannins, furfurals, carotenoids, artificial dyes, and tetracyclines can alter enamel and dentin structures, producing visible stains [2]. While bleaching procedures using hydrogen peroxide or carbamide peroxide are widely employed to counteract these effects, they are not without drawbacks: tooth sensitivity, gingival irritation, and even reversible pulpitis have been reported, largely due to microstructural alterations such as surface porosity, prism disorganization, or enamel defects. Furthermore, aggressive whitening protocols may disrupt the organic–inorganic balance of the tooth matrix and potentially influence the oral microbiome. Thus, while tooth whitening offers clear aesthetic and psychosocial benefits, it also raises concerns regarding enamel integrity and long-term oral health, emphasizing the need for careful evaluation of its safety and efficacy.

The increasing popularity of tooth whitening has driven the development of diverse treatment modalities, ranging from professionally applied in-office whitening (OW) and dentist-supervised at-home whitening (HW) to over-the-counter (OTC) and do-it-yourself (DIY) products used without clinical oversight [3]. Among these approaches, peroxide- and carbamide-based bleaching agents remain the most widely employed. Nevertheless, their effects on dental ultrastructure are not yet fully elucidated, highlighting the need for further investigation into their impact on enamel integrity and long-term safety [4].

Among the various methods available, bleaching agents based on carbamide peroxide remain widely used due to their efficacy and relative ease of application in clinical settings. Carbamide peroxide is widely employed in various concentrations for both professional in-office whitening procedures and dentist-supervised at-home bleaching. Compared to hydrogen peroxide (H_2O_2), it demonstrates a slower degradation rate, allowing prolonged contact with the tooth surface when administered through customized dental trays. Upon decomposition, peroxides release highly reactive free radicals that oxidize organic chromophores—molecules responsible for extrinsic tooth discoloration caused by substances such as coffee, red wine, and tea. This oxidation process breaks down larger pigmented

compounds into smaller molecules that absorb fewer wavelengths of visible light, thereby producing a perceptibly lighter shade of the teeth [5].

With the growing demand for cosmetic dentistry, it has become increasingly important to evaluate the structural impact of carbamide peroxide-based bleaching agents in order to optimize both treatment efficacy and patient safety. Although these agents are widely used, their potential to alter enamel microstructure necessitates thorough investigation. Laser scanning confocal microscopy (CLSM), combined with three-dimensional reconstruction, provides a powerful tool for assessing enamel surface topography, prism organization, and defect depth under controlled conditions. In this context, the effects of different in-office bleaching protocols on enamel surface characteristics are assessed in accordance with ISO 28399:2021 guidelines [6], which establish safety and performance standards for external tooth whitening products.

Aim and objectives

This study compares the effects of two professional carbamide peroxide formulations—Opalescence Quick 45% and Pola Office+ 37.5%—on dental enamel integrity. The analysis focuses on identifying microstructural changes such as increased porosity, prism disorganization, microcrack formation, and dentinal tubule exposure. By evaluating both treated samples and matched controls originating from the same tooth, the design minimizes biological variability and ensures a reliable comparison of whitening-induced alterations. In this way, the investigation provides a systematic assessment of how product composition and oxidizing agent concentration may influence enamel morphology. The overarching aim of this in vitro study is to determine the structural impact of carbamide peroxide-based whitening agents on enamel through the use of laser scanning confocal microscopy (LSCM). This advanced imaging modality allows for detailed visualization of tissue architecture and the detection of subtle morphological modifications resulting from bleaching treatments. Beyond characterizing the extent of these changes, the study seeks to generate qualitative insights into the safety profile of high-concentration carbamide peroxide gels when applied under conditions simulating clinical dental practice, ultimately informing evidence-based recommendations for their responsible use.

MATERIAL AND METHODS

Ethical Approval and Specimen Collection

Human molars and premolars extracted for clinical reasons were used in this study, in full compliance with established ethical protocols and with the approval of the Ethics Committee of the "Victor Babeş" University of Medicine and Pharmacy, Timisoara (Approval No. 09/11.03.2024). All procedures were conducted in accordance with the principles of the Declaration of Helsinki and the standards of Good Practice in Biomedical Research, ensuring rigorous and ethically sound selection of dental specimens.

Sample Size Calculation

The required sample size was determined using G*Power 3.1 software, with parameters set at a significance level (α) of 0.05, a statistical power of 0.80, and a medium effect size (d = 0.5), consistent with values reported in the literature concerning structural alterations of enamel after bleaching. Based on these calculations, a minimum of 40 specimens was established, evenly distributed between two experimental groups (n = 20 treated with Opalescence Quick 45% and n = 20 treated with Pola Office+ 37.5%). To compensate for potential specimen loss or individual variability, the initial number of samples was increased by approximately 20%.

Sample Preparation

All teeth were obtained from an anonymized tissue bank in accordance with current ethical regulations. Following extraction, soft tissues were carefully removed by periodontal curettage, and specimens were stored in distilled water under controlled temperature for no longer than 30 days to preserve hydration and structural integrity. This storage method was intended to maintain the physicochemical properties of enamel as close as possible to those found in a natural biological environment, avoiding dehydration artifacts or mineral alterations. For additional preservation, the samples were immersed in a 0.1% thymol solution for five days, a commonly used approach to prevent microbial growth while maintaining tissue integrity. Representative specimens used in the study are shown in Figure 1.

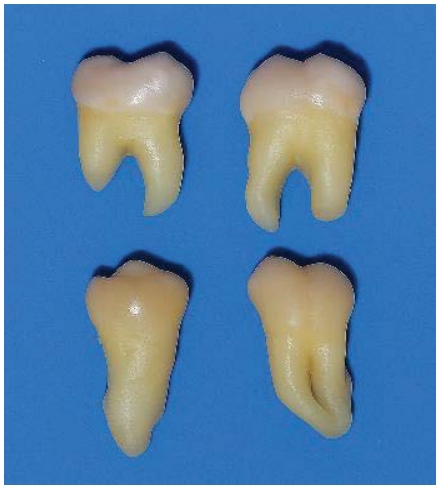

Figure 1. Representative human molars and premolars collected from the tissue bank for experimental use

Each extracted tooth was initially examined under ×10 magnification (OMS2356, Zumax Medical Co., Ltd., Suzhou, Jiangsu, China), and specimens presenting carious lesions, cracks, restorations, or enamel defects were excluded. To obtain standardized samples, the teeth were sectioned horizontally 2 mm coronally to the cementoenamel junction using a diamond disc with continuous water irrigation to prevent overheating and structural alterations. The crowns were subsequently divided longitudinally along the cervico-occlusal axis, producing two symmetrical halves. A unique identification code was assigned to each pair, with one half designated for bleaching treatment and the other retained as an untreated control. This allocation resulted in two experimental groups, each exposed to a different carbamide peroxide-based bleaching agent, and allowed for direct intra-tooth comparison of enamel morphological changes according to the composition and concentration of the whitening products. All fragments were embedded in autopolymerizing acrylic resin (UNIFAST Trad, GC America), and the exposed enamel surfaces were sequentially polished for two minutes per step with Soft-lex abrasive discs (3M ESPE, USA) of decreasing grit sizes: 42 μm, 30 μm, and 15 μm. After each polishing stage, specimens were rinsed thoroughly with distilled water to remove residual abrasives.

To simulate intraoral conditions and maintain enamel hydration throughout the entire experimental process, all samples were stored in artificial saliva at 37 °C in a biological incubator. This protocol was initiated immediately after extraction and maintained continuously during specimen preparation, bleaching treatment, and all subsequent analyses, including spectrophotometric evaluation and laser scanning confocal microscopy (LSCM). To ensure chemical stability and prevent microbial contamination, the artificial saliva solution was renewed every 48 hours. The composition of the solution included sodium bicarbonate

(2190 mg), potassium phosphate (1270 mg), magnesium chloride (125 mg), calcium chloride (441 mg), potassium chloride (820 mg), sodium fluoride (4.5 mg), nipasol (100 mg), nipagin (10 mg), sorbitol (24 mg), carboxymethylcellulose (8 mg), and distilled water (1000 mL), with the pH adjusted to a physiological value of 7.0. The solution was prepared in a closed-circuit pharmacy following the standardized protocol described by Vilhena et al. [7].

Bleaching Procedure

The experimental group included specimens treated with two professional carbamide peroxide-based whitening gels commonly used in dental practice:

- Opalescence Quick (Ultradent Products Inc., USA) – 45% Carbamide Peroxide

Opalescence Quick (Figure 2) is a chemically activated professional whitening gel containing 45% carbamide peroxide, with a neutral to slightly alkaline pH (5.6–7.2). Unlike light-activated systems, this formulation does not require external illumination. Carbamide peroxide gradually decomposes to release hydrogen peroxide, enabling a controlled oxidative reaction on the enamel structure. For each specimen, the gel was applied in a uniform layer approximately 1 mm thick over the exposed enamel surface. The treatment protocol consisted of three consecutive applications, each lasting 15 minutes, resulting in a total exposure time of 45 minutes per sample. After every application, the gel was completely removed, and the enamel surface was thoroughly rinsed with distilled water to eliminate any residual reactive components.

Figure 2. Presentation of the Opalescence Quick 45% bleaching gel – carbamide peroxide–based, chemically activated, light-free system.

- Pola Office+ (SDI, Australia) – 37.5% Carbamide Peroxide

Pola Office+ (Figure 3) is another in-office bleaching agent containing 37.5% carbamide peroxide, formulated with a neutral pH and desensitizing components such as fluoride and potassium nitrate to minimize post-treatment sensitivity. Similar to Opalescence Quick, it is chemically activated and does not require photoactivation. The gel was applied in a thin layer over the enamel surface, with the treatment protocol consisting of two applications of 20 minutes each. Between applications, the enamel was thoroughly rinsed with distilled water to remove any residual material. Pola Office+ is characterized by its slow release of hydrogen peroxide, providing a gradual and controlled oxidative effect on intrinsic pigments.

Figure 3. Presentation of the Pola Office+ 37.5% bleaching gel – carbamide peroxide–based, chemically activated, light-free system

All treated specimens were stored in saline solution throughout the experiment in order to prevent dehydration and structural alterations that may occur in a dry environment. Prior to the application of bleaching gels, dental surfaces were mechanically cleaned to remove organic residues and microbial biofilm, followed by thorough rinsing with distilled water. After treatment, representative enamel fragments were sectioned and mounted on glass slides for detailed microscopic evaluation using laser scanning confocal microscopy (LSCM). The exact composition of the two bleaching products employed in this study is presented in the table below (Table 1).

Table 1. Composition of the bleaching agents used in the study

Product name	Composition	Manufacturer	Lot number
Opalescence Quick	45%carbamide peroxid, pH = 5.6–7.2	Ultradent Products Inc., South Jordan, SUA	BWNV9
Pola Office+	37.5% carbamide peroxide, pH = 6.5–7	SDI Limited, Victoria, Australia	PO+2375-01

Confocal Microscopy Evaluation

Specimen analysis was performed using a laser scanning confocal microscope Olympus Fluoview FV1000 (Figure 4), equipped with UPLSAPO objectives of 10× (NA 0.40) and 20× (NA 0.75), allowing for detailed assessment of morphological changes induced by bleaching treatments. Excitation was carried out at wavelengths of 405 nm and 635 nm, depending on the objective used, with corresponding emission detection at 461 nm and 647 nm. With the 10× objective, images were acquired as high-resolution Z-stacks (1024 × 1024 pixels) at an optical sectioning step size of 1.5 µm, enabling three-dimensional reconstruction of the enamel surface using Bitplane Imaris v7.4 software. For the 20× objective, analysis was conducted using the same excitation (635 nm) and emission (647 nm) parameters, but at a higher resolution (1600 × 1600 pixels), with an optical sectioning interval of 1.2 µm. The scanning speed was set at 2 µs/pixel, laser transmissivity at 65%, and the photomultiplier tube (PMT) voltage adjusted to 629 V to optimize image contrast and clarity. This configuration provided high-precision three-dimensional reconstructions, highlighting structural alterations in enamel. To quantify these morphological variations, fluorescence intensity was measured in four distinct regions of each specimen—mesial, distal, buccal, and lingual/palatal—and the mean value was used for comparison between treated and control groups. Additional examinations at higher magnification (×40) were performed in reflection mode, using immersion oil and a He-Ne laser with a wavelength of 633 nm. Initially, low-magnification images were acquired to provide general orientation of the specimen, followed by a 3× digital zoom to allow detailed observation of prismatic microstructure. CLSM images revealed distinct variations in enamel surface morphology depending on the bleaching protocol applied. Areas with well-organized prisms appeared more translucent under laser illumination, reflecting uniform mineralization, whereas poorly organized inter-prismatic regions produced characteristic reflective patterns, resulting in a honeycomb-like appearance.

Figure 4. Laser scanning confocal microscope Olympus Fluoview FV1000 used for morphological evaluation of enamel specimens

Data Processing

As the methodology applied in this study was designed exclusively for the qualitative assessment of structural alterations in dental enamel using laser scanning confocal microscopy (LSCM), no conventional quantitative statistical analysis was performed. The interpretation of results was based on direct visual comparisons between bleaching-treated specimens and their corresponding control halves from the same tooth. Through this comparative approach, significant morphological changes were identified, including prism disorganization within the enamel structure, increased porosity, and widening of dentinal tubules. This non-invasive and descriptive evaluation method enabled a detailed observation of the structural effects induced by bleaching treatments, without the need for numerical measurements or statistical inference tools.

RESULTS

In Figure 5, the images on the left represent control specimens, showing a well-organized enamel prism structure and a uniform reflection of laser light. In contrast, the images on the right correspond to treated fragments, where evident morphological changes can be observed, including increased porosity, disorganization of the prismatic architecture, and areas of variable fluorescence intensity. These alterations qualitatively illustrate the oxidative impact of carbamide peroxide on the dental surface, as revealed by confocal microscopy.

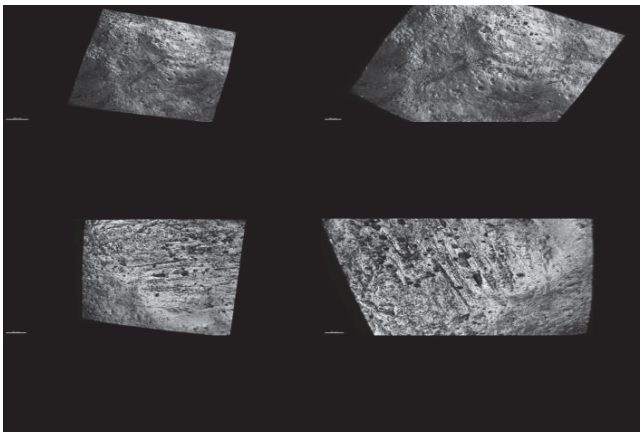

Figure 5. CLSM images illustrating enamel structure before and after bleaching treatment with carbamide peroxide (Opalescence Quick 45%)

Figure 6 presents four micrographs obtained through laser scanning confocal microscopy (CLSM), illustrating the morphological changes in dental enamel before and after treatment with the Pola Office+ 37.5% carbamide peroxide bleaching gel. The first two images (left) correspond to control specimens, which display a regular prismatic architecture and uniform reflectivity. In contrast, the treated samples (right) reveal partial prism disorganization, uneven fluorescence patterns, and a tendency toward widening of interprismatic spaces—features suggestive of early oxidative alterations induced by the bleaching agent. These observations support the moderate structural impact of Pola Office+, confirming a controlled interaction with enamel under simulated clinical application conditions.

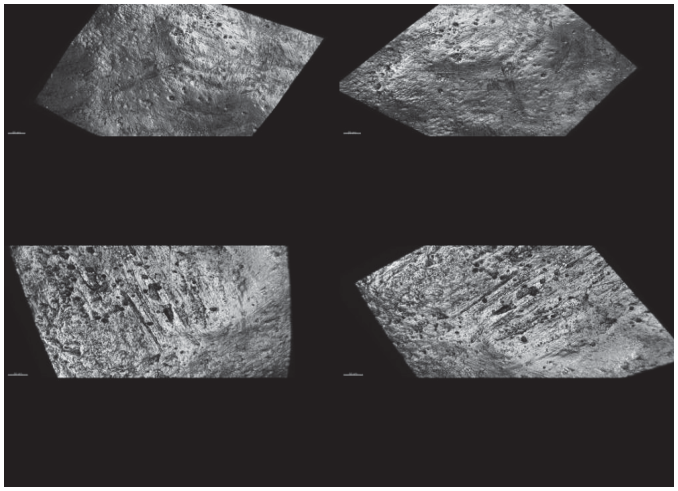

Figure 6. Structural appearance of dental enamel treated with Pola Office+ 37.5% – comparative CLSM images (control vs. treated specimen)

DISCUSSIONS

In today's culture, strongly influenced by social media, the appearance of a bright white smile is increasingly valued and often associated with health, self-confidence, and attractiveness. Dental aesthetics, closely linked to perceptions of beauty and overall well-being, has become an essential component of modern restorative practice [8]. A well-maintained, luminous smile contributes to positive social impressions, psychological well-being, and satisfaction in personal interactions [9]. Tooth whitening is a widely used clinical procedure aimed at improving tooth color and, consequently, the aesthetic outcome and patient satisfaction [10]. The effectiveness of bleaching treatments is influenced by several factors, including the type of whitening agent used, the pH of the oral environment, the application protocol, and the nature of the dental discolorations.

In this study, laser scanning confocal microscopy (CLSM) was employed to evaluate structural changes of enamel surfaces following exposure to peroxide-based bleaching agents. CLSM combines optical imaging with computational processing to generate high-resolution, real-time three-dimensional reconstructions of enamel surfaces. By using a laser beam to excite fluorophores, this technique enables visualization of peroxide diffusion through enamel, dentin, and biofilms, providing detailed two-dimensional images that highlight structural alterations. Compared with scanning electron microscopy (SEM) and other histological methods, CLSM offers significant advantages, particularly in assessing penetration depth and the distribution of bleaching agents within dental tissues. Moreover, CLSM is widely used in microbiological research for quantifying bacterial presence within

dentinal tubules, further demonstrating its versatility as a non-destructive and highly precise analytical method [11].

An important finding of this study was the difference in enamel response depending on the bleaching product used. Opalescence Quick 45%, due to its higher carbamide peroxide concentration, produced more pronounced alterations in enamel prism structure compared with Pola Office+ 37.5%. This observation is consistent with reports in the literature, which indicate that the efficacy of bleaching agents is directly proportional to the concentration of the active substance; however, higher concentrations are also associated with an increased risk of adverse effects, such as heightened sensitivity, morphological changes, and mineral loss [3,4].

With regard to patient perception and expectations of tooth whitening, studies show that there is increasing social pressure for an aesthetic smile, which often leads to frequent and sometimes excessive use of bleaching products. Therefore, understanding the long-term effects on enamel is essential. In a systematic review conducted by Rodríguez-Martínez et al. (2019), the need for balance between rapid aesthetic results and the preservation of oral health was emphasized [12]. Recent literature also highlights the importance of post-treatment remineralization strategies. Studies employing fluoride toothpastes or calcium-phosphate solutions after bleaching have reported favorable effects on restoring enamel hardness and reducing porosity induced by whitening procedures [13,14]. Consequently, the inclusion of remineralizing products in post-treatment protocols is considered a justified measure and is recommended by specialists in modern dental aesthetics [15]. Although the in vitro design of the present study did not allow for a direct evaluation of dentin hypersensitivity, it is important to note that high-concentration hydrogen peroxide-based agents are frequently associated with transient sensitivity in clinical practice. To mitigate this adverse effect, dentists commonly adopt preventive strategies such as limiting exposure time, using neutral pH formulations, spacing treatment sessions, and applying desensitizing agents before or after bleaching procedures.

Among the most effective desensitizing substances are 5% potassium nitrate, sodium fluoride varnishes, and casein phosphopeptide–amorphous calcium phosphate complexes (CPP-ACP). A recent meta-analysis confirmed that both potassium nitrate and CPP-ACP significantly reduce post-bleaching sensitivity compared with untreated control groups [16]. Other clinical studies have demonstrated that topical application of CPP-ACP (e.g., MI Paste®) or fluoride reduces thermal and tactile sensitivity following professional bleaching, without compromising treatment efficacy [16,17]. These findings underline the importance of incorporating desensitizing protocols as a standard practice in bleaching treatments, particularly when high-concentration agents are used.

With regard to the durability of whitening outcomes, numerous studies confirm that color stability is strongly influenced by diet, oral hygiene, and patient habits. Highly pigmented foods and beverages (such as coffee, red wine, and tea) may accelerate re-staining, particularly during the first two weeks post-treatment—a period in which adherence to the so-called "white diet" is recommended [14]. In addition, periodic use of maintenance products containing mild bleaching agents may help to prolong the initial whitening effect. The present study aligns with the conclusions of a recent meta-analysis evaluating the impact of different bleaching modalities on enamel microstructure, which reported that in-office treatments with professional products are generally safer than those carried out without clinical supervision [18]. Thus, in-office bleaching with carbamide peroxide, when performed according to recommended protocols, provides an effective balance between efficacy and preservation of dental structure.

An essential aspect to be further emphasized concerns the technology used to evaluate enamel structural changes—laser scanning confocal microscopy (LSCM). This non-invasive

method offers excellent resolution for the visualization of dental microstructures and enables three-dimensional observation of alterations produced by bleaching treatments. Compared with scanning electron microscopy (SEM), LSCM provides the advantage of preserving specimen integrity while highlighting subtle variations in reflectivity and fluorescence, which are indicative of prismatic microstructure disorganization [19,20]. In our study, qualitative analysis using LSCM revealed that, regardless of the bleaching agent applied, an increased exposure of dentinal tubules was observed following treatment compared with baseline conditions. These microstructural changes were consistently identified across all treatment groups, reflecting accentuated surface irregularities and visible exposure of dentinal tubules after bleaching. While this investigation focused on surface morphology and qualitative enamel changes visualized with LSCM, we recognize the importance of assessing peroxide penetration depth and associated demineralization. Naim et al. [21] conducted an in vitro quantitative evaluation of enamel demineralization depth after bleaching, using fluorescence to measure lesion penetration. Their results demonstrated that structural alterations may extend to subsurface enamel layers and vary according to peroxide concentration and application time. Although our methodology did not include depth measurements, future research should integrate such analyses to better characterize enamel responses to bleaching agents and to more accurately assess long-term risks to structural integrity [21].

From a clinical perspective, the microstructural changes observed in enamel may have significant implications for patient care. Increased surface roughness and porosity favor the retention of acids and dental biofilm, thereby enhancing the risk of erosion and caries, especially in acidic oral environments [21–23]. These structural alterations may also facilitate fluid migration through enamel, intensifying post-bleaching sensitivity [24]. Moreover, compromised enamel surfaces can negatively affect the adhesion of restorative materials: several studies have shown a significant reduction in bond strength—up to 60%—when restorations are placed immediately after bleaching, with substantial improvement only after a waiting period of 7–21 days [21,24]. These findings highlight the need for increased clinical caution: dentists should delay bonding procedures, recommend careful post-whitening care, and, where appropriate, apply remineralizing agents to restore enamel integrity and reduce sensitivity.

Differences between in-office and at-home whitening procedures represent another relevant topic of discussion. High-concentration carbamide peroxide (CP) gels (above 30%), such as those used in this study, offer the advantage of rapid and controlled action but carry a higher risk of structural alterations if not properly administered. By contrast, at-home whitening with gels containing 10–16% CP requires longer treatment times but is generally associated with milder effects on enamel [10]. Thus, the choice of whitening method should take into account both the patient's aesthetic expectations and the baseline condition of the dental structures.

Another key element is the pH of bleaching products. Studies have shown that neutral or slightly alkaline formulations are associated with a significantly lower risk of demineralization and better preservation of enamel hardness [23,25]. The products analyzed in the present study comply with these characteristics, supporting the notion that adverse effects can be minimized through the use of chemically optimized formulations. It is also important to note that pH influences the release kinetics of active oxidizing agents from CP, which directly affects both the efficacy and safety of the whitening procedure. An emerging factor in assessing the safety of bleaching treatments is their interaction with pre-existing restorative materials in the oral cavity. Carbamide peroxide has been shown to compromise the surface integrity of composite resins—particularly microhybrid and nanocomposites—by increasing surface roughness and reducing gloss [26]. Therefore, prior to bleaching, it is essential to identify and protect existing restorations, especially in the anterior region.

Furthermore, for patients with extensive restorations, replacement may be required post-treatment to achieve a uniform color match between natural teeth and restorative materials.

Recent literature has also introduced the concept of "biomimetic whitening," referring to formulations enriched with remineralizing agents such as nanostructured hydroxyapatite or active fluorides. These additives are designed to compensate for oxidative effects and to support enamel repair in parallel with stain removal [27]. This research direction is promising, as it may significantly reduce adverse effects associated with conventional bleaching and pave the way for gentler and more personalized protocols. Finally, individual biological variability of enamel must be considered. Differences in thickness, degree of mineralization, porosity, and wear influence how each tooth responds to whitening. The present study attempted to control this variability by using paired samples (control and treated) from the same tooth; however, in clinical practice, individual responses may vary significantly. This reality underscores the importance of personalized evaluation and careful clinical monitoring during and after whitening procedures [28,29].

This study presents several limitations that should be considered when interpreting the results. The in vitro design restricts direct clinical extrapolation, as artificial saliva cannot fully replicate the complex enzymatic, immunological, and buffering properties of natural saliva, nor the dynamic oral environment influenced by salivary flow, temperature changes, mastication, and pH fluctuations. The analysis was limited to a small number of commercial bleaching products, reducing the generalizability of the findings across all formulations. Biological variability among extracted teeth, despite careful selection, also represents a potential confounding factor. Furthermore, assessments were performed immediately after bleaching, without long-term follow-up to evaluate potential remineralization or recovery processes. CLSM provided high-resolution qualitative insights but lacks quantitative surface roughness parameters; integrating profilometry, SEM, Raman spectroscopy, or micro-CT could strengthen future analyses. Finally, complementary mechanical tests such as microhardness or bond strength evaluations would provide clinically relevant data. Despite these limitations, the study contributes valuable information on the structural effects of carbamide peroxide bleaching and emphasizes the need for safe and evidence-based clinical protocols.

CONCLUSIONS

Carbamide peroxide-based tooth whitening remains an effective and widely accessible aesthetic procedure, with a measurable impact on patients' quality of life. Using laser scanning confocal microscopy, this study qualitatively demonstrated moderate morphological changes in enamel, such as partial prism disorganization and increased porosity, without severe compromise of tissue integrity. Differences between Opalescence Quick 45% and Pola Office+ 37.5% highlighted the role of concentration and exposure time in shaping structural outcomes, underscoring the need for careful product selection and adherence to clinical protocols. Overall, carbamide peroxide whitening can be considered safe when performed under professional supervision, particularly when supported by remineralizing strategies and post-treatment care. Future research should focus on long-term in vivo effects, interactions with restorative materials, and the development of biomimetic formulations capable of balancing aesthetic efficacy with enamel preservation.

Conflicts of Interest

The authors declare no conflict of interest.

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