In Vitro Color Stability of Bleached Teeth Using Peroxide-Free Bleaching Gel, Carbamide Peroxide and Hydrogen Peroxide

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Abstract

This in vitro study investigates the effects of various bleaching agents on the color stability of extracted human teeth in two different staining media, Pepsi and saffron. Twenty human incisors were randomly allocated into four groups: a control group that was not treated with bleach, two groups treated with different concentrations of carbamide peroxide (22% and 16%), and a group treated with a peroxide-free bleaching agent containing phenolmidoperoxycaproic acid (PAP). Color measurements were taken at baseline, after bleaching, and following staining with either Pepsi or saffron. Statistical analysis revealed the bleaching treatments significantly affected the color, with the PAP-based bleaching agent demonstrating the lowest level of color change. Post hoc analysis confirmed significant differences between all pairs of groups, with Group 4 (PAP-based) showing the lowest mean color change. The study highlights the efficacy of peroxide-free bleaching agents and underscores its potential as a safe and effective alternative to traditional peroxide-based bleaching agents. Limitations include the in vitro nature of the study and the relatively small sample size. Future research should explore the long-term effects of different bleaching agents and staining conditions on safety and tooth color stability.

INTRODUCTION

Dental discoloration has been attributed to various factors, such as extrinsic stains from food, beverages, and tobacco, as well as intrinsic discoloration due to aging, trauma, or medication. Bleaching agents are commonly used for dental aesthetics, offering a non-invasive approach to addressing tooth discoloration. However, the impact of bleaching agents on tooth color stability under different conditions remains a topic of interest.

Tooth bleaching is a significant aspect of cosmetic dentistry, used to enhance the color of teeth and remove stains. This process commonly involves using peroxide-based bleaching agents, such as hydrogen peroxide and carbamide peroxide [1]. These agents function by breaking down the chromogens responsible for tooth stains, resulting in a visibly whiter appearance [2]. The use of peroxide-based bleaching agents is well-documented and is deemed safe when administered by dentists.

Numerous studies have investigated the effectiveness of whitening products containing peroxide-based agents; the outcomes varied based on the concentrations utilized. A 2016 study assessed the efficacy, color stability, and tooth sensitivity of combined bleaching techniques using 20% or 35% hydrogen peroxide for in-office protocols. The findings indicated that both concentrations provided effective and stable whitening over 12 months, with the 20% hydrogen peroxide protocol having a lower risk and intensity of tooth sensitivity [3].

A systematic review has also addressed whether at-home bleaching with more concentrated carbamide peroxide gels is as effective and safe as using a 10% carbamide peroxide gel. After analyzing 13 studies, the review concluded that at-home bleaching with 10% carbamide peroxide demonstrated similar

bleaching efficacy with lower risk and intensity of tooth sensitivity compared to more concentrated carbamide peroxide gels. The evidence supporting these outcomes was considered moderate quality [4].

Although peroxide-based bleaching agents are generally considered safe and effective when used correctly, they can have some adverse effects. The most frequently reported side effects include gingival irritation and mild-to-moderate temporary tooth sensitivity [5–8]. Among individuals undergoing external dental whitening, 15–78% may experience tooth discomfort, and gingival irritation is a common side effect [5]. Excessive or improper use of these products, particularly those with a low pH, can have erosive effects on the tooth surface [9]. Misuse, improper application, or incorrect whitening products could also have unfavorable effects [10].

In contrast, compounds with oxygen other than hydrogen peroxide (HP) react differently with pyridinium chlorochromate (PCA), especially in epoxidation. PCA is adept at targeting double bonds, particularly in high electron-density molecules, making it effective against tooth discoloration-causing compounds [11]. Its ability to induce epoxidation, especially in aromatic rings, makes PCA valuable in industrial bleaching, including in HiSmile products. Moreover, phenolmidoperoxycaproic acid (PAP), used as a tooth-whitening agent, offers various advantages as a PCA [12]. Unlike oxygen species (ROS), its oxidant chemistry follows the epoxidation pathway. This property eliminates irritation to the soft tissues of the mouth, eliminating the necessity for a gingival barrier to protect them. This mechanistic difference highlights the potential of PAP to achieve tooth whitening while reducing the adverse effects commonly associated with traditional peroxide-based bleaching agents, making it a promising alternative in cosmetic dentistry.

However, despite the distinct mechanistic advantages of PAP, its efficacy as a tooth-whitening agent remains uncertain. Limited research and clinical studies have explored the effectiveness of PAP compared to well-established hydrogen peroxide-based bleaching agents. Further investigations are required to evaluate its whitening efficacy, color stability over time, and potential side effects. While its unique mechanism of action is promising, comprehensive studies are needed to establish the use of PAP in tooth-whitening protocols and determine its overall efficacy and safety in cosmetic dentistry applications.

PURPOSE OF THE STUDY

This study aims to comprehensively investigate the effects of various bleaching agents on the color stability of extracted human teeth in two different staining media, Pepsi and saffron.

METHODS AND MATERIALS

Study design.

This in vitro study was approved by the Ethics Committee of the King Saud University, Riyadh, Saudi Arabia (Approval No. E-24-8615). The informed consent was obtained from all participants who volunteered to provide the extracted human teeth used in the study for experimental purposes. Moreover,

this study considered the modified CONSORT Checklist for reporting in vitro studies of dental materials [13].

Sample preparation.

Twenty human incisor teeth, devoid of enamel defects, fillings, or cracks, were utilized for this research. The crown was separated from the root using a water-cooled low-speed diamond bur. Following this, tooth pulp extraction was performed with a spoon excavator. Each specimen was then fixed within a ring using auto-polymerizing acrylic resin. To standardize the enamel surface and establish parallel planar surfaces, the samples underwent polishing with water-cooled low-speed Sof-Lex polishing discs (3M ESPE) [14].

Intervention protocol

The intervention protocol randomly allocated the samples into four groups (n = 8), each subjected to specific bleaching treatments. No bleaching occurred in Group 1 (Control), so it was the baseline reference. Group 2 underwent home bleaching using a 22% carbamide peroxide gel (Whiteness Perfect, FGM, Santa Catarina, Brazil), while Group 3 received a similar home bleaching treatment employing a gel containing 16% carbamide peroxide (FLASH, WHITEsmile, Birkenau/Germany). In Group 4, a novel approach was employed, using a peroxide-free bleaching agent containing PAP (HiSmile, Queensland, Australia). Each bleaching procedure was performed following the manufacturer's instructions. All applications were conducted by the same operator, using a micro-brush to cover all labial surfaces of each sample with the bleaching gel. The treatment regimen involved two-hour daily applications for ten days. After each treatment session, all specimens were thoroughly washed under running water and subsequently stored individually in distilled water at 37°C.

For the control group, specimens were immersed in distilled water at 37°C for two weeks; the water was changed daily. This standardized intervention protocol ensures consistency across groups, allowing for a comprehensive evaluation of the bleaching effects on the dental specimens.

Staining protocol

Each group was randomly divided into two equal subgroups, each consisting of a sample size of 10 (n = 16), and a staining solution, Pepsi or saffron, was applied. Post-bleaching, the specimens were immersed in a vial filled with the appropriate solution and placed in an incubator at 37°C for 24 hours, simulating the effects of one month of consumption of saffron and Pepsi. In the case of the Saffron group, 0.2 g of saffron leaves was dissolved in 500 mL of boiled distilled water, while the Pepsi group used the beverage straight from the bottle. Following the incubation period, the specimens were thoroughly rinsed and dried.

Color measurement

Color measurements were conducted using the Vita Easyshade V spectrophotometer (Vita Zahnfabrik, Bad Sackingen, Germany), adhering to the CIELAB system (Fig. 1). Readings were taken at the baseline

(T0), after the bleaching session (T1), and following the staining protocol (T2). The baseline refers to the time before the application of any bleaching protocols. The color differences between T1 and T0 and T2 and T0 were represented as ΔE1* and ΔE2*, respectively, and were computed using the formula: $\Delta E^* = [(\Delta L1^*)^2 + (\Delta a1^*)^2 + (\Delta b1^*)^2]^{1/2}$. This formula considers the differences in lightness (ΔL1*), green-to-red axis (Δa1*), and blue-to-yellow axis (Δb1*) between the specified times.

STATISTICAL ANALYSIS

The collected data were inputted into a Microsoft Excel spreadsheet, and subsequently, the data was exported to SPSS 22 for complete analysis. The mean change in lightness before and after treatment was calculated to assess the extent of bleaching exhibited by the materials after the experiment. The tested products underwent comparison using analysis of variance (ANOVA) within their respective groups and post hoc analysis. The predetermined significance level for this analysis was set at $p \leq 0.05$.

RESULTS

Table 1
Descriptive statistics of study groups

Groups	N	ΔE1(Mean, SD)	ΔE2(Mean, SD)
Group 1- Control	8	6.26 ± 0.20	7.40 ± 0.32
Group 2 - FMG	8	5.34 ± 0.24	5.48 ± 0.25
Group 3 - FLASH	8	5.35 ± 0.23	5.40 ± 0.24
Group 4 - HiSmile	8	4.29± 0.16	4.47 ± 0.23

Table 1 summarizes the key measures of color perception changes (ΔE1 and ΔE2) in the four study groups (Control, FMG, FLASH, HiSmile). Group 1 exhibited the highest color change, while Group 4 showed the lowest.

Table 2
The mean value in color change (ΔE) observed in the examined specimens after their submersion in diverse solutions.

Groups	Staining	ΔE1(Mean, SD)	ΔE2(Mean, SD)
Group 1- Control	Saffron	6.11 ± 0.07	7.14 ± 0.34
	Pepsi	6.42 ± 0.16	7.66 ± 0.26
Group 2 - FMG	Saffron	5.17 ± 0.11	5.33 ± 016
	Pepsi	5.52 ± 0.21	5.64 ± 0.23
Group 3- FLASH	Saffron	5.32 ± 0.23	5.37 ± 027
	Pepsi	5.39 ± 0.29	5.44 ± 0.22
Group 4 - HiSmile	Saffron	4.22 ± 0.19	4.50 ± 0.45
	Pepsi	4.36 ± 0.11	4.44 ± 0.12

In the current investigation, baseline readings were recorded before applying the tooth-whitening protocols, followed by assessments after the tooth-whitening procedure and subsequent processing of the samples in various solutions. The color change was measured using a spectrophotometer (Table 2).

Table 3
Post Hoc analysis One-way ANOVA test between the groups for ΔE1

(I) group	(J) group	Mean Difference (I-J)	SE	*p*-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Group1	Group 2	0.92000*	0.10711	< 0.001	0.6276	1.2124
	Group 3	0.90875*	0.10711	< 0.001	0.6163	1.2012
	Group 4	1.97250*	0.10711	< 0.001	1.6801	2.2649

Our one-way ANOVA results indicated significant differences in the color change (ΔE1) of specimens across all groups after bleaching compared to the baseline. Group 4 demonstrated a notably higher mean color change than Groups 1, 2, and 3, showing a substantial difference of 1.97 ($p < 0.005$) compared to Group 1. Additionally, significant differences were observed among the groups in terms of color changes following the staining protocol (ΔE2). Group 4 displayed particularly high differences: 2.92 ($p < 0.005$) compared to Group 1, 1.01 compared to Group 2 ($p < 0.005$), and 0.93 compared to Group 3 ($p < 0.005$) (Table 3, 4).

DISCUSSION

This study aimed to comprehensively investigate the effects of various bleaching agents on the color stability of extracted human teeth in two different staining media, Pepsi and saffron. The study used an in vitro study design, randomly allocating samples into distinct groups, intervention protocols following the manufacturer's instructions, and color measurements using a spectrophotometer. The primary goal of the study was to assess the efficacy and safety of different bleaching agents, particularly focusing on a peroxide-free option containing PAP, represented by the HiSmile product.

The study revealed significant color changes (ΔE1) among the experimental groups after the bleaching procedures. As anticipated, the control group, containing no bleaching agents, exhibited the highest color change, confirming the natural discoloration process. However, the peroxide-based bleaching agents, 22% carbamide peroxide (FMG) and 16% carbamide peroxide (FLASH), demonstrated comparable efficacy in tooth whitening. The absence of a significant difference between the two concentrations suggests that 22% and 16% carbamide peroxide are equally effective in addressing tooth discoloration.

The similar levels of tooth discoloration observed from 22% and 16% carbamide peroxide treatments can be attributed to various factors supported by existing literature. A study by Knežević et al. (2022) has highlighted the efficiency of carbamide peroxide in improving permeability and penetrating deeper into tissues, facilitating the reversal of chromatic discoloration through oxidation reactions [15]. Another study has emphasized the effectiveness of carbamide peroxide in home bleaching, indicating its potential for discoloration based on a literature review [16]. Additionally, Durán et al. (2018) have found that concentrations of 10% and 16% carbamide peroxide, when combined with potassium oxalate and fluoride, were not associated with tooth sensitivity, suggesting that the 16% concentration might already be optimal without added benefits [17].

Moreover, Alrashoud et al. (2022) have reported that different concentrations of carbamide peroxide can lead to different levels of peroxide leakage, suggesting that saturation effects may occur at higher concentrations [18]. Santana et al. (2021) have noted that enamel opacity increase due to HP action is a significant factor in the bleaching effect, which could explain why increasing concentrations beyond 16% may not enhance efficacy proportionally [19]. Furthermore, the study by Dewiyani et al. (2023) has demonstrated that in-office bleaching with higher concentrations of HP led to a higher prevalence of tooth sensitivity compared to at-home bleaching with 15% carbamide peroxide, indicating a balance between concentration and adverse effects [20].

These studies collectively suggest that the lack of a significant difference in tooth discoloration between 22% and 16% carbamide peroxide could be influenced by factors such as penetration efficiency, enamel permeability, saturation effects, and the balance between efficacy and adverse effects associated with higher concentration.

The standout result emerged from the HiSmile group, which used a peroxide-free bleaching agent containing PAP. This group exhibited the lowest color change (ΔE1), suggesting a potential alternative in cosmetic dentistry. This result is attributed to the unique properties of PAP, including its distinct mechanism of action, reduced soft tissue irritation, elimination of ROS, potential for enhanced color

stability, and the overall consumer-friendly experience. Collectively, these factors make HiSmile a promising alternative in cosmetic dentistry. The unique mechanistic advantages of PAP, particularly its epoxidation pathway, seem to manifest as superior color stability [21]. Thus, HiSmile is a promising alternative to traditional peroxide-based agents and has potential as a novel bleaching agent.

The staining protocol introduced Pepsi and saffron as extrinsic stain agents, causing a significant color change (ΔE2) in all groups. The inevitability of color change after teeth whitening, whether conducted in a dental office or at home, is well-documented. Ugurlu et al. have reported that after two years of tooth whitening, tooth color tends to revert to its original state. A color rebound may be observed following bleaching treatment, irrespective of the HP concentration used. The findings of this study agree with previous research in the field [22]. This highlights the susceptibility of bleached teeth to external staining agents, necessitating ongoing oral care practices post-bleaching. However, even after staining, the HiSmile group exhibited the lowest color change, indicating a potential for enhanced color stability over time. This outcome supports the proposed benefits of the mechanism of action of PAP, highlighting its resistance to external staining factors.

The findings of this study have practical implications for cosmetic dentistry. While peroxide-based bleaching agents remain effective and widely accepted, the emergence of PAP-containing products such as HiSmile introduces a potential paradigm shift. The reduced color change observed in the HiSmile group implies enhanced safety and efficacy, addressing concerns related to the tooth sensitivity and soft tissue irritation associated with traditional bleaching agents.

The design of this study, incorporating real-world staining media such as Pepsi and saffron, adds value. In contrast to many previous studies that used common staining agents such as coffee, tea, and red wine[23], our research explored the impact of saffron on tooth discoloration. This unique choice was motivated by the prevalence of saffron in Saudi coffee and desserts and the awareness that global populations might not be fully cognizant of its potent discoloration effects on teeth.

Saffron, a spice known for its vivid color and distinct flavor, holds cultural significance in Saudi Arabia, particularly in preparing traditional coffee and desserts. By incorporating saffron into our study, we aimed to focus on a lesser-explored facet of tooth discoloration, acknowledging the diverse dietary and cultural practices that influence oral health. This approach broadens the scope of understanding regarding the potential sources of tooth stains, especially in regions where saffron plays a significant role in culinary traditions.

However, the study has several limitations to be considered. Firstly, the in vitro nature of the study may not fully replicate the complex oral environment, including factors such as salivary flow, oral pH, and bacterial interactions, which could influence the bleaching outcomes differently in vivo [18, 19]. Additionally, the study focused on a specific set of bleaching agents and staining media, and these results may not be generalizable to all commercially available products and diverse staining conditions [20, 21]. Furthermore, the sample size was relatively small, and the findings may benefit from validation in larger sample populations to enhance the robustness of the conclusions [20, 22]. Future research should

address these limitations by conducting in vivo studies with larger sample sizes, longer follow-up periods, and a broader range of bleaching agents and staining conditions to provide a more comprehensive understanding of the effects of bleaching agents on tooth color stability and safety.

Future studies could also explore the influence of different application techniques, concentrations, and exposure times on tooth color stability and potential adverse effects. Moreover, developing novel bleaching agents with improved color stability, reduced tooth sensitivity, and minimal adverse effects should be a focus of future research to enhance the options available for dental professionals and patients.

This study contributes valuable insights into the evolving landscape of tooth-whitening protocols. The results suggest that PAP, represented by HiSmile, has promise as a safe and effective alternative to traditional peroxide-based bleaching agents. The observed color stability and resistance to staining hint at the potential of PAP to redefine cosmetic dentistry practices. However, further rigorous research, including long-term clinical studies, is imperative to solidify the position of PAP in tooth-whitening protocols and ensure its efficacy and safety in diverse clinical scenarios.

CONCLUSION

In summary, this study provides crucial insights into tooth-whitening protocols, specifically focusing on evaluating the efficacy and safety of PAP as a peroxide-free alternative, represented by HiSmile. The lowest color change was observed in the HiSmile group, suggesting the potential of PAP in cosmetic dentistry. This study uniquely included real-world staining agents, Pepsi and saffron, enriching our understanding of tooth discoloration under diverse dietary and cultural influences. Despite the acknowledged limitations, the findings encourage a paradigm shift by positioning PAP-containing products as a safer and more effective alternative to traditional peroxide-based agents. This study motivates future research and innovation in cosmetic dentistry, offering a new approach that considers real-life staining conditions and provides a valuable perspective for clinicians and patients seeking optimal tooth whitening with minimized adverse effects.

Declarations

The study approved by the Ethics Committee of the King Saud University, Riyadh, Saudi Arabia (Approval No. E-24-8615). The informed consent was obtained from all participants who volunteered to provide the extracted human teeth used in the study for experimental purposes.

Consent for publication:

Not applicable

Availability of data and materials:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests:

the authors declare that they have no competing interests.

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Authors' contributions:

Abdul Aziz Al Kheraif contributed to the conception and design of the study, drafting the manuscript and supervision. Tasneem R. Adam and Aisha Wasi contributed to sample preparation, carried out the experiments, performed statistical analysis and interpretation of the data. All the authors were involved in critically revising and approving the final version submitted for publication.

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References

1. Fiorillo L, et al. Dental whitening gels: strengths and weaknesses of an increasingly used method. Gels. 2019;5(3):35.
2. Abouassi T, Wolkewitz M, Hahn P. Effect of carbamide peroxide and hydrogen peroxide on enamel surface: an in vitro study. Clin Oral Invest. 2011;15:673–80.
3. Rezende M, et al. Combined bleaching technique using low and high hydrogen peroxide in-office bleaching gel. Oper Dent. 2016;41(4):388–96.
4. De Geus JL, et al. At-home bleaching with 10% vs more concentrated carbamide peroxide gels: a systematic review and meta-analysis. Oper Dent. 2018;43(4):E210–22.
5. Tredwin CJ, et al. Hydrogen peroxide tooth-whitening (bleaching) products: review of adverse effects and safety issues. Br Dent J. 2006;200(7):371–6.
6. Li Y, Greenwall L. Safety issues of tooth whitening using peroxide-based materials. Br Dent J. 2013;215(1):29–34.
7. Maran BM, et al. In-office bleaching with low/medium vs. high concentrate hydrogen peroxide: A systematic review and meta-analysis. J Dent. 2020;103:103499.
8. Carneiro TdS, et al. In-office dental bleaching in adolescents using 6% hydrogen peroxide with different application tips: randomized clinical trial. J Appl Oral Sci. 2023;31:e20230216.

9. Rodríguez-Martínez J, Valiente M, Sánchez-Martín MJ. Tooth whitening: From the established treatments to novel approaches to prevent side effects. J Esthetic Restor Dentistry. 2019;31(5):431–40.
10. Gofur NRP et al. *Impact in Oral Cavity due to the Use of Hydrogen Peroxyde in Dental Treatment.*
11. Pascolutti M, de Oliveira D. A Radical-Free Approach to Teeth Whitening. Dentistry J. 2021;9(12):148.
12. Bizhang M, et al. Effectiveness of a new non-hydrogen peroxide bleaching agent after single use-a double-blind placebo-controlled short-term study. J Appl Oral Sci. 2017;25:575–84.
13. Faggion CM Jr. Guidelines for reporting pre-clinical in vitro studies on dental materials. J Evid Based Dent Pract. 2012;12(4):182–9.
14. Altınışık H, et al. The Effect of In-Office Bleaching with Different Concentrations of Hydrogen Peroxide on Enamel Color, Roughness, and Color Stability. Materials. 2023;16(4):1389.
15. Knezevic N, et al. Clinical Testing of Walking Bleach, In-Office, and Combined Bleaching of Endodontically Treated Teeth. Medicina. 2022;59(1):18.
16. Mahardiansyah R, Harniati ED, Sari IA. Effectiveness of Adding Home Bleaching Time With 10% Carbamide Peroxide: Literature Review. J Res Social Sci Econ Manage. 2022;1(11):1890–8.
17. Durán G et al. *Evaluation of color change using CIE LAB coordinates on tooth structure subjected to different concentrations of peroxide carbamide.* Revista clínica de periodoncia, implantología y rehabilitación oral, 2018. 11(3): p. 143–6.
18. AlRashoud A, et al. Effectiveness and Adverse Effects of Dental Bleaching with 10–16% vs. 30–37% Carbamide Peroxide: A Systematic Review. Archives Pharm Pract. 2022;13(2–2022):104–9.
19. Santana TR et al. Role of enamel and dentin on color changes after internal bleaching associated or not with external bleaching. J Appl Oral Sci, 2020. 29.
20. Dewiyani S, Binarti I, Ergitamanda K. Potential Outcome, Enamel Surface Roughness and Tooth Sensitivity of in Office Bleaching and at Home Bleaching. Int J Pharm Bio Med Sci. 2023;3(1):29–37.
21. Grillon M, et al. Vitro Evaluation of Tooth-Whitening Potential of Peroxide-Free OTC Dental Bleaching Agents. Dentistry J. 2023;11(4):89.
22. Ugurlu M, Al-Haj N, Husain, Özcan M. Color Change after 25% Hydrogen Peroxide Bleaching with Photoactivation: A Methodological Assessment Using Spectrophotometer versus Digital Photographs. Materials. 2022;15(14):5045.
23. Villalta P, et al. Effects of staining and bleaching on color change of dental composite resins. J Prosthet Dent. 2006;95(2):137–42.

Figures

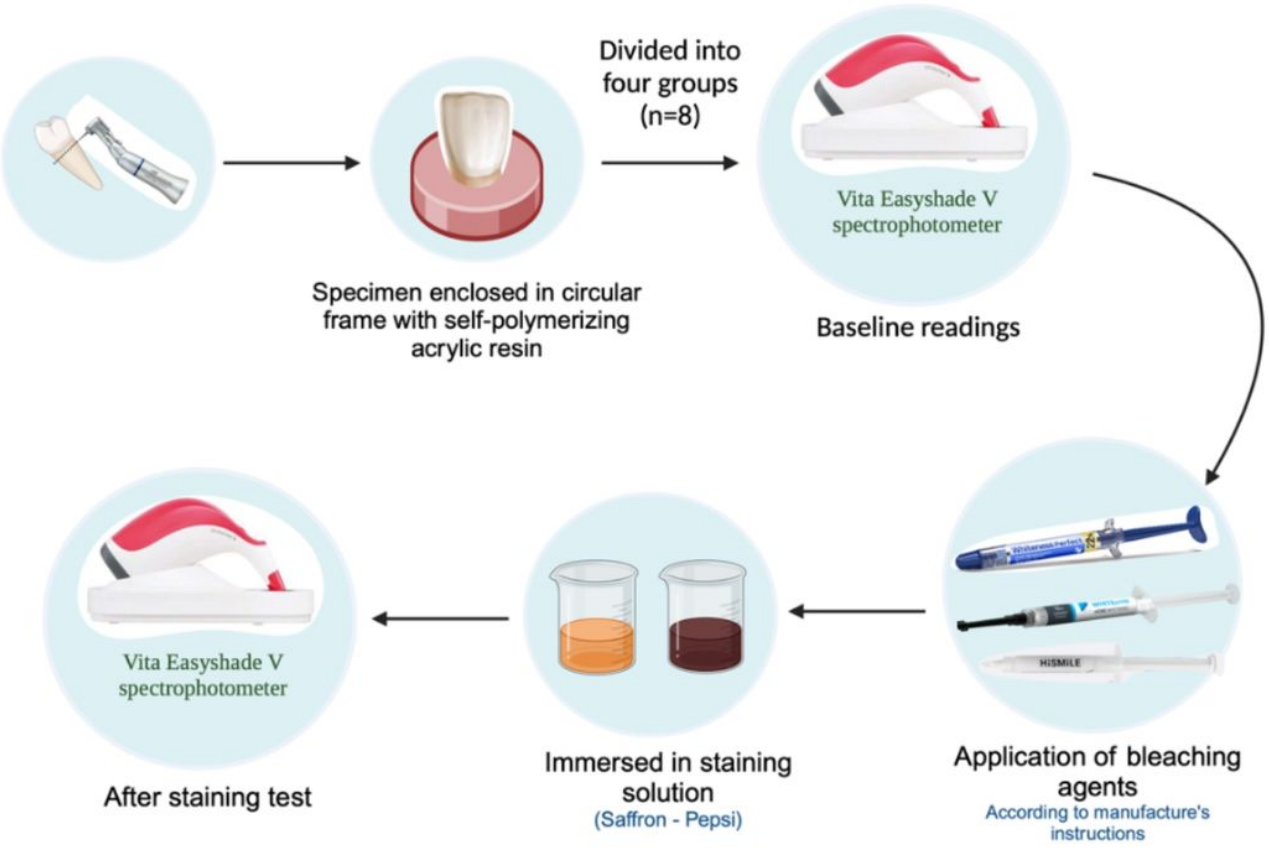

Figure 1

Flowchart depicting the methods and materials involved in the study