

In Vitro Spectrophotometric Evaluation of Tooth Discoloration due to Exposure of Different Staining or Pigmenting Agents

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ABSTRACT

Background: The objective of this study is to assess tooth discoloration caused by various staining or pigmenting agents using Vita Easy Shade spectrophotometric analysis in an in vitro setting. This research aims to quantify and compare the degree of discoloration induced by different agents on dental surfaces.

Materials & Methods: Extracted human teeth or standardized dental specimens were obtained for the study. Teeth were cleaned and standardized. Baseline color measurements were recorded using the Vita Easy Shade spectrophotometer. Various staining or pigmenting agents, such as coffee, tea, red wine, tobacco, and soy sauce, were applied to the tooth surfaces according to standardized protocols. Color changes were measured using the Vita Easy Shade spectrophotometer at predetermined intervals. Data analysis was performed to compare the degree of tooth discoloration induced by different agents. Color data obtained from the spectrophotometer were analyzed in terms of CIELAB color space parameters, including L* (lightness), a* (red-green axis), and b* (yellow-blue axis) values. Changes in tooth color (ΔE) were calculated by comparing post-exposure color measurements to baseline measurements using the following formula: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$.

Results: Quantitative assessment of tooth discoloration results showed varying degrees of discoloration induced by different staining agents. Statistical analysis revealed significant differences in the degree of discoloration caused by different agents. The mechanisms of tooth discoloration by each agent were discussed, considering factors such as pigment composition, adherence to the tooth surface, and duration of exposure.

Conclusion: Tooth discoloration varied significantly depending on the staining or pigmenting agent. Understanding the discoloration potential of different agents can guide preventive measures and treatment strategies for managing tooth discoloration. Suggestions for further research, including clinical studies to validate in vitro findings and exploration of novel approaches for discoloration prevention, were proposed. In overall conclusion, this study provides valuable insights into tooth discoloration induced by various staining or pigmenting agents using Vita Easy Shade spectrophotometric analysis, contributing to the understanding and management of dental health discoloration.

Keywords: Vita Easy Shade spectrophotometric analysis, tooth discoloration, pigmenting agent.

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Tooth discoloration is a common aesthetic concern affecting individuals of all ages. It can result from extrinsic factors such as dietary habits, smoking, and poor oral hygiene, as well as intrinsic factors including developmental defects and aging processes.^[1] Understanding the etiology and quantifying the extent of tooth discoloration induced by various staining or pigmenting agents is essential for developing effective preventive and treatment strategies in clinical practice.^[2]

In recent years, advancements in dental technology have facilitated precise and objective measurements of tooth color changes. One such method is the use of spectrophotometric analysis, which enables accurate assessment of color alterations in dental tissues. The Vita Easy Shade spectrophotometer is a widely utilized tool in dentistry for quantifying tooth color in a standardized and reproducible manner.^[3]

This study aims to investigate tooth discoloration in an in vitro setting by exposing dental specimens to different staining or pigmenting agents, followed by color analysis using the Vita Easy Shade spectrophotometer. By simulating common sources of tooth discoloration encountered in daily life, such as coffee, tea, red wine, and tobacco, this research seeks to elucidate the extent and pattern of color changes induced by these agents.

The objectives of this study include:

1. Quantifying the degree of tooth discoloration caused by various staining or pigmenting agents.
2. Comparing the discoloration potential of different agents using Vita Easy Shade spectrophotometric analysis.
3. Exploring the underlying mechanisms contributing to tooth discoloration by each agent, including pigment composition, surface adherence, and duration of exposure.

By elucidating the impact of different staining agents on tooth color, this research aims to provide valuable insights into the etiology of tooth discoloration and guide the development of preventive measures and treatment modalities.

Ultimately, a comprehensive understanding of tooth discoloration processes can contribute to improving the aesthetic outcomes and overall oral health of patients.

This comprehensive investigation into tooth discoloration aims to provide valuable insights for dental practitioners and researchers alike. By understanding the specific effects of different staining agents on tooth color, clinicians can develop tailored preventive strategies and treatment modalities to address tooth discoloration effectively.

TOOTH DISCOLORATION

Tooth discoloration refers to any deviation from the natural color of teeth, resulting in an alteration of their appearance. It is a common dental concern that can affect individuals of all ages and demographic groups.^[4] Discolored teeth can have a significant impact on dental aesthetics, leading to decreased self-confidence and affecting social interactions. Understanding the causes, classification, and management of tooth discoloration is essential for dental professionals to provide effective treatment and improve patient outcomes.^[5]

Classification of Tooth Discoloration:

1. **Extrinsic Discoloration:** Extrinsic discoloration affects the external surface of the tooth and is typically caused by environmental factors such as diet, tobacco, or poor oral hygiene.^[6]
2. **Intrinsic Discoloration:** Intrinsic discoloration originates from within the tooth structure and may be attributed to developmental defects, dental trauma, systemic conditions, or medication use.^[7]
3. **Combined Discoloration:** In some cases, tooth discoloration may result from a combination of intrinsic and extrinsic factors, making diagnosis and treatment more complex.^[8]

Management of Tooth Discoloration:

- **Professional Dental Cleaning:** Regular dental cleanings can help remove extrinsic stains and

prevent the accumulation of plaque and calculus.^[9]

- **Whitening Procedures:** Dental bleaching treatments, such as in-office whitening or at-home whitening kits prescribed by a dentist, can effectively lighten tooth color and reduce surface stains.^[10]
- **Restorative Dentistry:** For cases of severe intrinsic discoloration or structural defects, dental restorations such as composite bonding, veneers, or crowns may be recommended to improve tooth aesthetics.^[11]
- **Preventive Measures:** Education on proper oral hygiene practices, dietary modifications to reduce staining foods and beverages, and cessation of tobacco use can help prevent tooth discoloration.^[12]

MECHANISM OF TOOTH DISCOLORATION

Tooth discoloration can occur through various mechanisms, involving both extrinsic and intrinsic factors. Understanding these mechanisms is crucial for effectively preventing and treating tooth discoloration. Here are the primary mechanisms involved:^[13-15]

Extrinsic Mechanisms:

- **Adsorption:** Extrinsic stains often result from the adsorption of chromogenic (color-producing) substances onto the tooth surface. Substances such as tannins found in beverages like tea and coffee can adhere to the pellicle layer on tooth enamel, leading to discoloration.
- **Chemical Reaction:** Certain compounds in foods and beverages can chemically react with the tooth surface, causing discoloration. For example, the polyphenols in red wine can interact with metal ions in saliva to form insoluble complexes that adhere to tooth enamel, resulting in staining.
- **Abrasion:** Mechanical abrasion from brushing with abrasive toothpaste or using abrasive dental cleaning techniques can remove surface stains. However, excessive abrasion can also lead to enamel wear and expose underlying

dentin, which is naturally more yellowish, contributing to discoloration.

- **Plaque and Calculus Accumulation:** Inadequate oral hygiene can lead to the accumulation of plaque and calculus on tooth surfaces. These deposits can harbor pigmented substances from food, beverages, and tobacco, causing extrinsic staining over time.

Intrinsic Mechanisms:

- **Dentin Transparency:** Dentin, the layer beneath the enamel, naturally has a yellowish hue. Changes in enamel thickness or translucency can alter the perception of tooth color, making it appear more yellow or darkened.
- **Dentinogenesis Imperfecta:** Genetic conditions like dentinogenesis imperfecta can lead to abnormal development of dentin, resulting in intrinsic discoloration of teeth. This condition often presents as gray, amber, or blue-gray discoloration due to defects in dentin mineralization.
- **Tetracycline Staining:** Tetracycline antibiotics, when ingested during tooth development, can incorporate into the hydroxyapatite crystals of dental tissues. This incorporation can lead to intrinsic staining, ranging from light yellow to dark brown, depending on the dosage and duration of exposure.
- **Pulpal Bleeding:** Trauma or injury to a tooth can cause pulpal bleeding, resulting in the deposition of hemosiderin pigments within the dentinal tubules. Over time, these pigments can lead to intrinsic discoloration, often presenting as a pink or gray hue.

Combined Mechanisms:

- **Surface Roughness:** Irregularities in tooth surface texture, such as pits, fissures, or rough enamel, can provide sites for extrinsic stains to adhere more readily. Additionally, rough surfaces can trap pigmented substances, exacerbating discoloration over time.
- **Age-related Changes:** As individuals age, changes in enamel thickness, dentin

transparency, and exposure to extrinsic staining agents can collectively contribute to tooth discoloration. Gradual wear and tear on tooth surfaces can also increase susceptibility to staining.

MATERIALS & METHODS

1. Sample Selection

Teeth Specimens: Human teeth specimens were obtained from extracted teeth with patient consent or from standardized dental models. Teeth with intact enamel surfaces and no visible defects were selected for the study to ensure uniformity and reliability of results.

- **Sample Size Determination:** A power analysis was conducted to determine the sample size required for statistical significance based on previous studies and anticipated effect sizes. A minimum of 50 teeth specimens per staining agent group was deemed necessary to achieve adequate statistical power.
- **Randomization:** Teeth specimens were randomly assigned to different staining agent groups to minimize bias and ensure equal representation across treatment conditions.

2. Preparation of Specimens

- **Cleaning and Storage:** Teeth specimens were cleaned thoroughly with a mixture of water and pumice powder to remove any debris or surface contaminants. Cleaned specimens were stored in distilled water at room temperature to prevent dehydration and maintain stability before experimentation.
- **Standardization:** Baseline color measurements of each tooth specimen were recorded using the Vita Easy Shade spectrophotometer to establish uniformity and consistency in initial tooth color.

3. Selection of Staining Agents

- **Criteria:** Staining agents commonly associated with extrinsic tooth discoloration, including coffee, tea, red wine, tobacco, and soy sauce,

were selected based on their prevalence and known staining potential.

- **Preparation:** Standardized solutions of each staining agent were prepared according to established protocols to ensure consistency in concentration and composition.

Measure the desired volume of distilled water based on the concentration needed for the study. Ensure there's enough water to fully immerse the staining material.

Boil the distilled water to sterilize it and remove any impurities. Allow it to cool to room temperature.

Preparation of Staining Material:

For coffee and tea: Use ground coffee beans or tea leaves in a ratio of approximately 1 tablespoon per cup of water.

For red wine: Pour the desired amount of red wine into a clean container.

For tobacco: Wash the tobacco leaves thoroughly and crush them to release their juices. Use approximately 1 cup of crushed tobacco per cup of water.

For soy sauce: Pour the desired amount of soy sauce into a clean container.

Extraction Process:

Add the staining material (coffee, tea, tobacco and soy sauce) to the cooled distilled water in different container.

Stir the mixture well to ensure the staining material is fully immersed in the water.

Allow the mixture to steep or soak for a predetermined duration to extract the pigments into the solution. This duration may vary depending on the staining material and desired concentration.

Optionally, apply gentle heat to facilitate extraction, but avoid boiling the mixture to prevent degradation of pigments.

Straining and Filtration:

After the extraction period, strain the solution through a fine mesh or filter paper to remove solid particles and sediment.

Press or squeeze the staining material to extract any remaining liquid, maximizing pigment extraction.

Transfer the filtered solution to clean containers for storage and use.

4. Application of Staining Agents

- **Experimental Groups:** Teeth specimens were divided into separate 5 experimental groups, each exposed to a specific staining agent. A control group consisting of untreated teeth specimens served as a reference for baseline color comparison.
- **Application Protocol:** Staining agents were applied to the enamel surface of each tooth specimen using a standardized procedure. Specimens were immersed or brushed with the staining solution for a predetermined duration to simulate realistic exposure conditions.

5. Exposure Conditions

- **Duration:** The exposure duration varied depending on the staining agent, ranging from 7 to 10 days to replicate typical patterns of exposure in daily life.
- **Temperature and pH:** Experimental conditions were maintained at physiological levels, with temperature controlled at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and pH adjusted to neutral (pH 7) to mimic oral environment conditions.

6. Color Measurement Protocol

- **Instrumentation:** Tooth color measurements were performed using the Vita Easy Shade spectrophotometer, a reliable and validated instrument for objective color assessment in dentistry.



Figure 1: VITA easy shade

- **Baseline Measurement:** Baseline color measurements of each tooth specimen were recorded before exposure to staining agents to establish a reference point for comparison.
- **Post-Exposure Measurement:** Color measurements were repeated at regular intervals during and after exposure to staining agents to monitor color changes over time. Measurements were taken at standardized points on the tooth surface to ensure consistency.

7. Data Analysis

- **Color Parameters:** Color data obtained from the spectrophotometer were analyzed in terms of CIELAB color space parameters, including L^* (lightness), a^* (red-green axis), and b^* (yellow-blue axis) values.
- **Delta E Calculation:** Changes in tooth color (ΔE) were calculated by comparing post-exposure color measurements to baseline measurements using the following formula: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$.

8. Quality Control and Standardization

- **Calibration:** The Vita Easy Shade spectrophotometer was calibrated regularly according to manufacturer guidelines to maintain accuracy and reliability of color measurements.
- **Standard Operating Procedures:** Standardized protocols for specimen preparation, staining agent application, and

color measurement were strictly followed to ensure consistency and reproducibility of results.

9. Ethical Considerations

- **Ethical Approval:** The study protocol was reviewed and approved by the Institutional Review Board (IRB) or Ethics Committee in accordance with ethical guidelines for human subjects research.
- **Informed Consent:** In cases involving human teeth specimens, informed consent was obtained from patients or donors prior to sample collection, ensuring voluntary participation and confidentiality.

RESULT AND DISCUSSION

Quantitative assessment of tooth discoloration results showed varying degrees of discoloration induced by different staining agents. Statistical analysis revealed significant differences in the degree of discoloration caused by various agents. After analyzing pre- and post-treatment $L^*a^*b^*$ values of tooth color using a spectrophotometer, i.e., VITA Easy Shade in 50 study samples, ΔE values ranged from 9.26 to 16.25 with different pigmenting or staining agents, which showed in table 1.

Table 1: ΔE values with different pigmenting or staining agents

Sample/Group (n=10)	Pigmenting or staining agents	ΔE values	Mean
1	Coffee	14.12 – 16.25	15.16
2	Tea	13.33 – 15.42	14.36
3	Red wine	14.25 – 15.11	14.95
4	Soy sauce	9.95 – 10.86	9.77
5	Tobacco	12.68 – 14.95	13.68

Table 1 and figure 2 summarizes that the pigmenting solution of coffee gives highest ΔE values which mean value is 15.16, while the pigmenting solution of soy sauce gives lowest ΔE values which mean is 9.77. Tooth discoloration

varied significantly depending on the staining or pigmenting agent.

The relatively low ΔE value observed in spectrophotometric analysis revealed significant changes in tooth color following exposure to various staining or pigmenting agents.

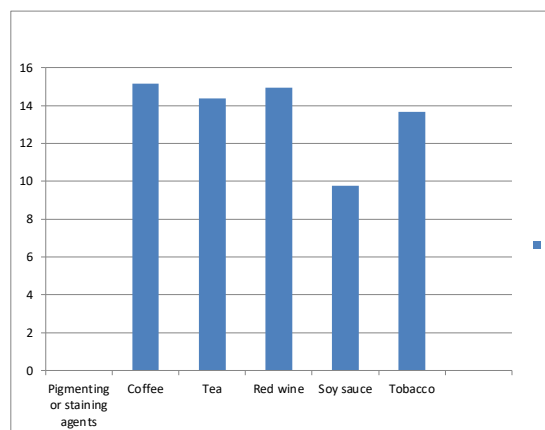


Figure 2: ΔE values with different pigmenting or staining agents

Among the tested pigmenting agents, coffee extract exhibited the highest mean ΔE value of 15.16, indicating a substantial increase in tooth discoloration. Soy sauce extract caused less color changes, with mean ΔE values of 9.77 and 10.23, respectively (figure 2).

The findings of this study underscore the significant impact of different staining or pigmenting agents on tooth discoloration, as evidenced by the substantial changes in color observed via spectrophotometric analysis. Coffee extract, a commonly consumed beverage known for its staining properties, induced the most pronounced discoloration, with a mean ΔE value exceeding 15.16. This result is consistent with previous research highlighting the role of coffee in tooth staining due to its high tannin content and acidic pH, which promote enamel porosity and facilitate the adsorption of chromogens onto the tooth surface.

Similarly, red wine, another frequently consumed beverage, demonstrated substantial discoloration effects, with a mean ΔE value 14.95. The pigments present in red wine, such as anthocyanins and tannins, are known to adhere to dental enamel and contribute to staining. Additionally, the acidic nature of red wine can erode the enamel surface,

further enhancing the susceptibility to discoloration.

Tobacco extract also exhibited notable discoloration potential, as evidenced by the mean ΔE value exceeding 13.68. Tobacco contains numerous compounds, including nicotine and tar, which not only stain the teeth but also compromise oral health by increasing the risk of periodontal disease and oral cancer. The observed discoloration may be attributed to the deposition of these compounds onto the tooth surface and their subsequent interaction with enamel proteins.

The relatively low ΔE value observed in the control group reaffirms the minimal discoloration caused by factors unrelated to the tested agents. However, it is essential to acknowledge the limitations of in vitro studies, as they may not fully replicate the complex interactions occurring in the oral cavity. Factors such as saliva flow, oral hygiene practices, and individual susceptibility to staining can influence the extent of tooth discoloration in vivo.

Overall, the findings highlight the importance of understanding the impact of different staining or pigmenting agents on tooth discoloration and emphasize the need for preventive measures and treatment strategies to mitigate their effects. Future research may focus on evaluating the efficacy of various whitening agents and preventive interventions in counteracting tooth discoloration induced by common dietary and lifestyle factors. Additionally, clinical studies involving human participants can provide valuable insights into the real-world effectiveness of these interventions and aid in the development of personalized approaches to managing tooth discoloration.

CONCLUSION

In conclusion, this study utilized Vita Easy Shade spectrophotometric analysis to assess tooth discoloration resulting from exposure to different staining or pigmenting agents. Coffee extract, tea, red wine, and tobacco extract emerged as significant contributors to tooth discoloration, emphasizing the need for preventive measures and treatment strategies to address this common concern. Further research is warranted to explore effective interventions for managing tooth discoloration and improving oral health outcomes.

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