

Degree of pigmentation of dental teeth when immersed in different beverages: Coffee, Dark Soft Drink, and red wine, after bleaching with 16% Carbamide Peroxide: An *In Vitro* study

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Abstract

Dental bleaching with 16% carbamide peroxide improves dental color aesthetics, although it may temporarily increase enamel susceptibility to pigmentation. Beverages such as coffee, red wine, and dark soft drinks contain compounds that promote staining. This study evaluated their effect on dental color stability after bleaching.

This was an *in vitro* experimental study that assessed the degree of pigmentation in 60 extracted human teeth subjected to dental bleaching with 16% carbamide peroxide. The samples were divided into a control group and three experimental groups exposed to coffee, dark soft drink, and red wine. Color measurements were taken before bleaching, after bleaching, and after immersion in the beverages using digital spectrophotometry (VITA Easyshade® V). Color change was analyzed using the ΔE_{BLEACH} and ΔE_{STAIN} parameters. Data were tested for normality and compared using ANOVA or non-parametric tests. This design allowed evaluation of post-bleaching susceptibility to pigmentation under controlled conditions.

The statistical analysis showed that all four groups began with similar chromatic conditions, which allowed subsequent changes to be attributed to bleaching and beverage staining. The bleaching protocol was effective in all samples, with ΔE values close to 12 and no statistically significant differences among groups. Subsequently, exposure to chromogenic beverages produced clear changes in the L, a, and b coordinates, confirmed by descriptive analyses, plots, and ANOVA. These variations demonstrated that each beverage affects enamel brightness and hue differently.

When directly comparing pigmentation between Measurement 2 and Measurement 3, Coca-Cola showed the highest ΔE_{STAIN} , followed by red wine and coffee, while the control group remained stable. Results indicated that Coca-Cola produces stronger darkening and yellowing, red wine increases the red component, and coffee produces moderate changes. ANOVA confirmed highly significant differences among beverages. Overall, the data established a clear chromatic trajectory and concluded that Coca-Cola has the greatest post-bleaching staining potential.

The analysis confirmed that all groups started with similar dental colors, ensuring comparability. After bleaching, the chromatic change was uniform and showed no significant differences among groups. The staining stage did show marked differences, with Coca-Cola producing the greatest pigmentation, followed by red wine and coffee. The L*, a*, and b* parameters showed distinct behaviors depending on the beverage, with more abrupt changes in the experimental groups. Overall, time and chromogenic beverages were determining factors in the final variability of dental color.

Keywords: Dental bleaching, carbamide peroxide, dental pigmentation, staining beverages, tooth whitening, color stability

Introduction

Dental bleaching is a widely used treatment for patients who present intrinsic and/or extrinsic discolorations, or who simply wish to improve their current shade. Changes in tooth shade occur due to the accumulation of organic molecules formed by conjugated chains with single and double bonds. Bleaching agents act by breaking these chains through oxidation reactions, which causes cleavage of the double bonds in the conjugated structure (Farawati *et al.*, 2020).

Tooth color is a critical property in smile aesthetics; however, substances such as coffee, dark soft drinks, and red wine can darken enamel due to chromogenic pigments and chromatic components that adhere to the tooth surface (Carey, 2019; Joiner, 2019) [7]. In particular, red wine and coffee contain highly pigmented tannins that compromise dental translucency (Kwon *et al.*, 2021) [18]. In addition, low-pH beverages such as cola can slightly erode the enamel surface, favoring greater pigment absorption (Li *et al.*, 2019) [19]. Understanding these mechanisms is essential to evaluate how they influence the aesthetic effectiveness of dental treatments.

Dental bleaching with carbamide peroxide at concentrations such as 16% is one of the most commonly used methods in

esthetic dentistry because it releases hydrogen peroxide that oxidizes chromatic compounds, lightening the tooth (Ferraz *et al.*, 2020) [10]. Nevertheless, after this treatment, enamel may be more vulnerable to pigment re-absorption due to a slightly porous or altered surface (Attia *et al.*, 2020) [4]. Several studies have shown that after bleaching with carbamide peroxide or hydrogen peroxide, enamel undergoes temporary surface alterations that make it more susceptible to extrinsic staining. Melo *et al.* (2022) [20] reported that high-concentration bleaching agents reduce enamel microhardness and create a more porous and irregular surface due to partial demineralization. This surface change facilitates adherence of chromogens found in staining beverages such as coffee, red wine, or dark soft drinks. However, the authors observed that enamel structure tends to recover gradually between 7 and 14 days after treatment, especially when hydration is maintained and natural remineralization is promoted by saliva or by remineralizing agents with calcium and fluoride. Similar findings have been described by Attia *et al.* (2020) [4], who likewise reported that bleaching-induced roughness and microhardness loss are reversible over time; however, during the early post-treatment period enamel remains more vulnerable to extrinsic pigmentation. This highlights the

need to evaluate how chromogenic beverages affect color stability after the bleaching procedure is completed.

A recent *in vitro* study by Sarembe *et al.* (2022) [21] investigated how chlorhexidine (CHX) mouthrinse potentiates staining by chromogenic beverages. It has been proposed that dietary chromogens precipitate on adsorbed cations, such as those from chlorhexidine, forming a pigmented layer on enamel (Sarembe *et al.*, 2022) [21]. Their results show that beverages such as red wine, coffee, and also dark soft drinks due to their low pH promote significant staining, even after simulated brushing (Sarembe *et al.*, 2022) [21].

In light of the above, this study aimed to evaluate the degree of pigmentation in extracted teeth bleached with 16% carbamide peroxide after exposure to different beverages (coffee, dark soft drink, and red wine) in order to determine the impact of these beverages on post-bleaching dental color stability.

Methodology

This study used a comparative cross-sectional *in vitro* experimental design. The objective was to evaluate the degree of pigmentation in extracted human teeth that were bleached with 16% carbamide peroxide and subsequently exposed to different staining beverages (coffee, dark soft drink, and red wine). The procedure was carried out in five phases: baseline color measurement, application of the bleaching agent, post-bleaching color measurement, immersion in staining solutions, and final color assessment.

The sample consisted of 60 extracted human teeth divided into two groups: 15 teeth in the control group and 45 teeth in the experimental group. Teeth without caries, restorations, fractures, cervical lesions, or endodontic treatment were included. Samples were obtained from young patients aged 15 to 30 years, and no more than six months had elapsed since extraction for orthodontic or periodontal reasons. Teeth with pulp exposure, dyschromias, erosion, abrasion, abfraction, structural enamel/dentin alterations, or prior damage were excluded.

Teeth were obtained from dental clinics in the city. Collection included only teeth extracted for orthodontic or periodontal indications, with no relationship between extraction and the research. After extraction, the teeth were stored in sterile containers with physiologic saline solution and transported to the Universidad Hemisferios research laboratory, following the institution's biosafety standards.

To ensure reliability and validity of the measurements, the digital spectrophotometer was calibrated according to the manufacturer's instructions, and data were recorded as coordinate values. The 60 teeth were dried with absorbent paper and then embedded in acrylic as follows: a plastic tube mold (20 mm wide × 14 mm high) was cut, filled with transparent acrylic, and the teeth were positioned leaving the crown exposed; the acrylic base was then polished. Samples were numbered and classified according to study group.

First, all specimens underwent cleaning of the vestibular surface using a prophylaxis brush and a pumice-and-water mixture.

Baseline color was then determined using a spectrophotometer (VITA Easyshade® V), obtaining L*, a*, and b* components representing lightness, red-green component, and yellow-blue component, respectively. To improve precision, each measurement was taken three times

per tooth and the values were averaged. The device was calibrated with a standard white card before each recording. From these measurements, the primary variable—color change (ΔE)—was calculated. Ideally, the ΔE_{00} formula was used because it is the most clinically accurate; if not available, the classic ΔE^*_{ab} was applied. Two change indicators were generated: ΔE_{BLEACH} (difference between baseline and post-bleaching) to evaluate initial treatment efficacy, and ΔE_{STAIN} (difference between post-bleaching and the value after immersion in beverages) to analyze susceptibility to pigmentation. These variables were continuous quantitative measures. In addition, the percentage of samples exceeding clinical perceptibility/acceptability thresholds was calculated, generating a categorical variable indicating the presence or absence of clinically relevant change.

For dental bleaching, Whiteness Perfect (16% carbamide peroxide) was applied to the vestibular surface for 6 hours per day, followed by rinsing with abundant water and storage in distilled water for 24 hours. This procedure was repeated for 10 consecutive days, after which a new color measurement was taken at 24 hours.

The specimens were distributed into two groups: Group 1 (control, n=15), immersed only in distilled water, and Group 2 (experimental, n=45), divided into three subgroups of 15 teeth corresponding to the chromogenic solutions. In the experimental group, samples were immersed daily for 2 hours in dark soft drink (Coca-Cola), red wine (Clos Merlot), and coffee (Minerva), and then kept in distilled water at room temperature. This process continued for 4 days, with daily renewal of the solutions.

At the end, dental color was measured again at the middle third of the vestibular surface of each specimen, recording the average of three readings per tooth.

Thus, the study comparatively evaluated dental pigmentation at three time points: before bleaching (baseline), after treatment with 16% carbamide peroxide, and after 4 days of exposure to one of four solutions (distilled water, coffee, dark soft drink, and red wine).

After the study, teeth were placed in hermetically sealed containers with 5% sodium hypochlorite for 24 hours for disinfection. They were then disposed of as biological waste according to the institutional hazardous waste management protocol through an authorized company for collection and final disposal.

Statistical analysis began with a detailed description of the data (mean, standard deviation, median, interquartile range, minimum, and maximum) and visualization using boxplots, violin plots, and time-trajectory plots. Normality (Shapiro–Wilk) and homogeneity of variances (Brown–Forsythe) were assessed.

The main comparison focused on ΔE_{STAIN} among beverages, as this variable indicates how much teeth stained after bleaching. If parametric assumptions were met, one-way ANOVA was applied; if heteroscedasticity was present, Welch's ANOVA was used; and if the distribution was not normal, Kruskal–Wallis or permutation ANOVA was used. Post-hoc comparisons among beverages were performed with multiplicity corrections (Tukey, Games–Howell, or Dunn–Holm).

Additionally, a mixed repeated-measures model (time as within-subject factor and beverage as between-subject factor) was used to simultaneously evaluate the effect of bleaching and beverage exposure, as well as their

interaction. Each tooth had a random intercept, a flexible covariance structure was used, and standard errors were estimated robustly. When needed, transformations or non-parametric aligned-rank models were applied.

Finally, sensitivity analyses were performed by excluding outliers and evaluating results also with ΔE^*ab when necessary. Comparative tables and plots were reported, including assumption checks, applied adjustments, effect sizes, and clinical interpretation of findings. This approach ensured valid and rigorous results while maintaining scientific and clinical relevance.

The study respected ethical principles of beneficence, confidentiality, and biosafety. Its purpose was to contribute useful scientific information without posing risks to participants or researchers. As an *in vitro* study, it did not require informed consent and did not involve people or vulnerable populations. Samples were donated teeth, coded to preserve confidentiality. Biosafety standards were followed throughout the procedure, ensuring ethical and responsible handling of biological material.

Results

Initial distribution of dental color before bleaching

Baseline evaluation of dental color is essential to understand the magnitude of change produced by bleaching and by subsequent exposure to chromogenic substances. In this first color measurement, the L, a, and b coordinates were obtained for the four experimental groups, all measured under the same conditions. Descriptive analysis makes it possible to identify initial patterns of lightness, hue, and yellow saturation that serve as a reference for interpreting later changes.

Baseline color characterization is particularly relevant for this experimental design because groups were assigned before any intervention. Therefore, similarity (or differences) among groups at this stage determines whether later changes can be attributed more confidently to bleaching and beverages rather than to significant baseline differences. Descriptive statistics are presented below.

In terms of lightness (L), the four groups showed relatively high initial values, consistent with light or moderately light tooth shades. The control group presented a mean $L = 73.6$, while the groups later exposed to Coca-Cola, red wine, and coffee had mean values of 76.6, 74.8, and 76.4, respectively. These differences were small and within comparable ranges, suggesting homogeneous baseline conditions.

For the a component (tendency toward red), averages ranged from 5.8 to 6.5 with no striking differences among groups. The Coca-Cola group showed the greatest dispersion due to an isolated case with a high a value (14.8). For the b component (yellow hue), the control and Coca-Cola groups showed the highest values (≈ 40), while the red wine group had a somewhat lower mean (36.9) but the greatest variability.

For L, the four groups show similar medians and widely overlapping ranges, confirming that baseline lightness was comparable across groups. The red wine group shows greater dispersion, consistent with the extreme values reported.

For coordinate a (reddish tendency), boxplots again show overall similarity among groups, with close medians. The Coca-Cola group shows an outlier (value 14.8).

Finally, for coordinate b (yellow tone), groups show greater variability. The Coca-Cola group presents relatively high

values, while the red wine group includes low values that widen its range. These baseline differences are relevant when interpreting the magnitude of later staining changes.

Overall, descriptive results and graphical representation show that all four groups start from comparable baseline conditions in terms of lightness and chromatic components. This provides a solid basis for attributing changes observed in subsequent phases to bleaching and exposure to staining solutions.

With baseline data established, the next step is to evaluate the bleaching effect after 10 days of continuous application. For this, Measurement 1 (baseline) is compared with Measurement 2 (post-bleaching), ΔE_{BLEACH} is calculated per tooth and per group, and inferential analyses are conducted to determine whether bleaching was effective and whether differences existed among groups.

Bleaching analysis

The second step of statistical analysis focused on quantifying the effect of the bleaching protocol applied for 10 days, 6 hours per day. L, a, and b coordinates obtained in Measurement 1 (baseline) and Measurement 2 (post-bleaching) were compared for each tooth. Total color change was calculated using ΔE_{BLEACH} with the classic ΔE_{ab} formula, which integrates differences in lightness (L), red-green component (a), and yellow-blue component (b). Higher ΔE values indicate a more noticeable color change after bleaching.

This analysis was performed at two levels. First, ΔE_{BLEACH} was calculated tooth by tooth, allowing observation of within-group variability. Second, values were summarized by group (Control, Coca-Cola, Red Wine, and Coffee) to compare average behavior and dispersion among groups. Additionally, one-way ANOVA was applied to explore whether statistically significant differences in ΔE_{BLEACH} existed among the four groups.

ΔE_{BLEACH} per tooth: ΔE_{BLEACH} was calculated for each of the 60 specimens using their L, a, and b values before (Measurement 1) and after bleaching (Measurement 2). For example, in the control group, tooth ED001 showed a ΔE_{BLEACH} of 13.76 units, while ED003 showed a smaller change ($\Delta E = 3.14$), highlighting that even under the same protocol, response to bleaching is not homogeneous across teeth. Conversely, teeth such as ED004 (control) and ED058 (coffee) showed changes greater than 19 units, corresponding to clinically very evident color modifications. In all groups, most specimens had ΔE_{BLEACH} values above the commonly accepted perceptibility threshold (≈ 2 –3 units), suggesting a generalized bleaching effect, although with varying magnitude among teeth.

ΔE_{BLEACH} by group: Based on individual values, descriptive statistics were calculated per group. On average, all four groups showed a very similar color change, with mean ΔE_{BLEACH} between 11.7 and 13 units, indicating clearly perceptible whitening. The control group had the slightly highest mean (12.95), followed by Coca-Cola (12.56) and red wine (12.04), while coffee had the lowest mean (11.71); however, between-group differences were small. The minimum–maximum ranges show that each group included specimens with moderate changes and others with very marked changes.

Group medians were very close, with a slight tendency toward higher values in the control group. Dispersion was relatively similar across groups, though the control and red

wine groups showed some higher values extending toward the upper end of the scale. No strong visual differences suggest a markedly different bleaching behavior among groups at this stage.

Inferential analysis (ANOVA): One-way ANOVA comparing ΔE_{BLEACH} means among the four groups yielded $F = 0.23$ and $p = 0.88$, indicating no statistically significant differences in color change after bleaching among groups. In other words, the degree of whitening was comparable for specimens later assigned to control, Coca-Cola, red wine, and coffee.

In summary, bleaching produced a clinically evident color change in virtually all specimens, with mean ΔE_{BLEACH} values close to 12 units across groups. The absence of statistically significant differences indicates the bleaching protocol was equally effective across groups, regardless of the beverage to which specimens would later be exposed. This is relevant because it ensures groups begin the staining phase with a similar level of whitening.

After confirming that bleaching was effective and comparable across groups, the next step is to analyze color behavior after the beverage exposure phase. Point 3 presents post-staining descriptives (Measurement 3), describing final L, a, and b coordinates by group. This will allow comparison of the final color after staining with the post-bleaching status and prepare for the specific analysis of chromogenic impact.

Final color evaluation after exposure to chromogenic beverages

The third measurement (Measurement 3) corresponds to the final color state of the teeth after 4 days of exposure to the assigned beverages (Coca-Cola, red wine, and coffee) following a protocol of 2 hours of daily immersion. This section describes the resulting L, a, and b coordinates after staining, allowing observation of general trends in darkening, reddening, or increased yellowing generated by each beverage. The control group allows comparison of color evolution without external pigments.

L, a, and b coordinates were analyzed by group using descriptive statistics and panel-style boxplots, allowing visualization of differences in lightness (L), red-green component (a), and yellow-blue component (b) among groups.

Table 1: Pigmentation analysis

Group	L mean	L SD	a mean	a SD	b mean	b SD
Control	65.28	5.34	2.60	0.91	25.36	2.86
Coca-Cola	38.88	6.61	15.27	2.88	38.09	4.74
Red wine	45.15	8.63	12.52	2.41	27.74	2.48
Coffee	59.79	6.94	4.76	1.23	28.97	2.07

Regarding lightness (L), the Coca-Cola group showed the lowest post-staining lightness (38.88), followed by red wine. Coffee showed higher values, and the control group maintained the highest lightness, as expected due to no pigment exposure. For the red-green axis (a), the Coca-Cola group shifted most strongly toward positive (red) values, followed by red wine. Coffee showed a slight increase, while the control group showed virtually no reddish staining. For the yellow-blue axis (b), the highest values were observed in the Coca-Cola group (≈ 38), indicating a

marked tendency toward yellowing. Red wine and coffee showed moderate increases, while the control group maintained lower values. These descriptives confirm that each beverage generates a characteristic staining pattern.

Lightness (L): • Coca-Cola shows the greatest loss of lightness, with medians well below the other groups. • Red wine also darkens but with greater variability. • Coffee darkens less than Coca-Cola and red wine. • The control group retains the highest lightness.

Green-Red axis (a): • Coca-Cola and red wine generate notably higher red values. • Coffee slightly increases this component. • The control group shows no significant changes.

Yellow-Blue axis (b): • Coca-Cola reaches the highest yellowing values. • Red wine and coffee show moderate increases. • The control group maintains the baseline.

These patterns suggest that beverages affect each color component differently, and staining is not uniform: Coca-Cola stands out for darkening and yellowing, red wine contributes a shift toward red, and coffee produces a more moderate darkening.

Post-staining descriptives show clear differences among groups exposed to chromogenic beverages. Overall, Coca-Cola produced the greatest decrease in lightness and greatest increase in yellowing, while red wine generated a notable increase in the red component (a) and coffee produced moderate changes in all directions. The control group remained stable, confirming that observed changes in other groups are due to staining.

With final color values characterized, the next step (Point 4) is to directly compare Measurement 2 vs Measurement 3 by calculating ΔE_{STAIN} to determine which beverage stained the most after bleaching. This analysis—combining descriptives, plots, and inferential statistics—identifies the substance with the greatest overall chromatic impact.

ANOVA analysis — Measurement 3

ANOVA for lightness (L) showed statistically significant differences among groups after staining ($F = 46.74$; $p \approx 2.91 \times 10^{-15}$), confirming that beverages altered enamel clarity differently, producing distinct levels of darkening beyond random variation. The a component (red-green axis) showed even more pronounced differences ($F = 134.44$; $p \approx 1.46 \times 10^{-25}$), indicating that some beverages—especially red wine and Coca-Cola—increased the tendency toward reddish hues. The b component (yellowing) also showed highly significant differences ($F = 45.29$; $p \approx 5.44 \times 10^{-15}$), evidencing beverage-specific effects on the yellow-blue dimension. Together, the three CIELAB components (L, a, b) varied significantly, confirming that staining acts differently on enamel depending on the beverage.

Overall, these results support the descriptive and graphical trends: Coca-Cola produced marked darkening and increased yellowing; red wine strongly increased the red component; coffee generated moderate but consistent staining; and the control group remained practically stable. With evidence of significant differences in final chromatic parameters, the next step is to analyze total color change between Measurement 2 and Measurement 3 using ΔE_{STAIN} , which will precisely identify which beverage produced the greatest chromatic alteration after bleaching.

Comparison Measurement 2 vs Measurement 3 (ΔE_{STAIN})

After bleaching (Measurement 2), specimens were exposed for 4 days to the assigned beverages. The goal of this section is to quantify how much color changed between Measurement 2 and Measurement 3—i.e., how much each substance actually stained. ΔE_{STAIN} represents the total color change between these measurements using the ΔE_{ab} formula, integrating differences in L, a, and b.

The control group showed minimal changes in ΔE_{STAIN} , attributable to natural material fluctuation or instrument variability, confirming that in the absence of chromogenic agents, bleached enamel tends to maintain its color without clinically relevant alterations. In contrast, groups exposed to chromogenic beverages showed clearly differentiated chromatic modifications.

Coca-Cola produced the greatest post-bleaching staining, with a mean ΔE_{STAIN} of 35.3—far higher than the other groups—indicating an extremely evident color change. Red wine ranked second with a mean of 24.8 and a wider dispersion reflecting variability among specimens. Coffee produced moderate changes around 13, representing perceptible but less intense staining than Coca-Cola and red wine.

Clinically, ΔE values greater than ~ 3 are typically detectable by the naked eye, and values greater than 10 represent markedly noticeable changes. Under this criterion, Coca-Cola and red wine induced intense and easily observable staining, while coffee produced moderate but clinically perceptible darkening. The control group's stability confirms these alterations are due to beverage contact rather than typical enamel behavior.

Graphically and numerically, the Coca-Cola group shows the highest medians and ranges of staining, evidencing an aggressive, generalized, and consistent chromatic alteration. The red wine group also shows marked staining but with greater variability, suggesting that while wine has considerable chromogenic potential, its effect may vary depending on specimen characteristics or pigment absorption. Coffee produces moderate staining, perceptible but significantly lower than Coca-Cola and red wine, positioning it as an intermediate chromogenic agent in this experimental context. The control group maintains very low staining, confirming that changes in other groups are directly due to beverage contact.

ANOVA — ΔE_{STAIN}

One-way ANOVA comparing staining among the four groups yielded $F = 54.80$ with $p = 1.148 \times 10^{-16}$, providing extremely strong statistical evidence that differences in ΔE_{STAIN} are not due to chance but reflect true differences in the chromogenic capacity of the evaluated beverages after bleaching.

The ranking of groups by staining intensity was clear: Coca-Cola showed the highest ΔE_{STAIN} (around 35), representing a highly perceptible and clinically evident change; red wine ranked second (around 25), also indicating marked staining; coffee showed moderate staining (around 13); and the control group remained at the lowest levels (around 9), consistent with minor variation attributable to natural behavior or instrument variability.

In summary, ΔE_{STAIN} values demonstrate that staining induced by chromogenic beverages differs significantly among groups. Coca-Cola produced the greatest total color

change, followed by red wine, while coffee caused lower-magnitude staining. The control group confirms that unstained variation is minimal. These findings quantify staining intensity and support integration of differences in the overall color-trajectory analysis across the experiment.

Global comparison of the three measurements (Measurement 1 vs Measurement 2 vs Measurement 3)

The purpose of this section is to analyze the full evolution of color throughout the experiment, simultaneously considering baseline measurement (Measurement 1), post-bleaching measurement (Measurement 2), and final measurement after exposure to chromogenic beverages (Measurement 3). This global comparison identifies the chromatic trajectory of each group and shows how each substance interacts with the changes initiated by bleaching. Results show that all groups experienced an initial decrease in L after bleaching, consistent with progressive reduction of yellow components and increased perceived brightness. However, this trend changed notably after beverage exposure. The Coca-Cola group showed an abrupt drop in lightness at Measurement 3, reaching values near 38 that reflect deep darkening. Red wine also produced a substantial decrease, though less pronounced than Coca-Cola. Coffee showed a moderate decrease, while the control group maintained the expected gradual reduction trend.

For axis a, behavior differed markedly among groups. After staining, Coca-Cola and red wine showed significant increases, indicating a shift toward reddish/ochre tonalities. In contrast, coffee and control remained at low and relatively stable values. For axis b, Coca-Cola showed a significant increase after staining, producing a combination of darkening and yellow saturation that yields a very noticeable visual change. Red wine and coffee tended to decrease progressively, while the control group maintained a continuous reduction without abrupt fluctuations.

Overall, these results suggest each group follows a distinct chromatic trajectory over time, again highlighting Coca-Cola as the beverage producing the most intense change across the three CIELAB dimensions.

Trajectory plots for L, a, b

The trajectory plots clearly show color evolution by group. For L, the control, coffee, and to a lesser extent red wine show a moderate reduction across the three measurements, whereas Coca-Cola shows an abrupt decrease after staining, confirming strong darkening. For a, Coca-Cola and red wine show marked increases at Measurement 3, indicating movement toward reddish tones typical of strong pigments; coffee and control remain more stable with minimal variation.

For b, the analysis again shows divergence: Coca-Cola reverses the post-bleaching decrease and registers a relevant increase at Measurement 3, generating a characteristic dark yellowish tone of intense pigmentation. Red wine and coffee show progressive decreases, while the control group maintains a stable pattern. The combination of these trends confirms that Coca-Cola produces simultaneous modifications in lightness and chromaticity that clearly distinguish it from other beverages.

The global comparison of the three measurements demonstrates that groups present differentiated color trajectories throughout the experiment. The control group maintains a stable pattern, confirming methodological

consistency. Coffee causes moderate changes, while red wine produces important changes mainly in chromatic axes. In contrast, Coca-Cola shows intense and simultaneous alterations in L, a, and b, explaining why it is the group with the greatest accumulated ΔE during the process.

With all measurements integrated and beverage-specific chromatic patterns identified, the next step is to synthesize the numerical findings in the conclusions section.

Conclusions

All groups began the study with similar dental color, which ensured homogeneous baseline conditions and allowed subsequent changes to be attributed to the experimental protocol. Bleaching produced a perceptible and uniform color change in all groups, with no statistically significant differences, confirming that its effect was similar regardless of the beverage evaluated.

After exposure to chromogenic beverages, significant differences in the degree of staining were observed. The control group showed minimal changes, while coffee and red wine showed progressive increases in color change; Coca-Cola produced the greatest chromatic alteration. The global analysis showed that beverage type and time were determining factors in final dental color variability, concluding that although bleaching was effective and homogeneous, subsequent exposure to chromogenic beverages significantly affected color stability.

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