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RESEARCH ARTICLE



# Investigating the role of chlorogenic acids and coffee type in coffee-induced teeth discoloration

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**ABSTRACT**

**Objective:** Coffee is one of the most popular beverages in the world, with millions of people consuming it every day. The effect of coffee on teeth discoloration has long been a concern for both coffee drinkers and dental professionals. To address this concern, this study aimed to investigate the role of chlorogenic acids (CGAs) and the type of coffee in coffee-induced teeth discoloration.

**Materials and Methods:** High-performance liquid chromatography with a photodiode array detector was used to determine the CGA contents of instant coffee produced by five manufacturers (Starbucks, Dunkin' Donuts, Kanu, Ediya, Coffee Bean). A total of 180 bovine tooth specimens were immersed in the coffee samples for varying durations (3, 9, 24, 48, and 72 h), and the discoloration levels were measured using a spectrophotometer. A linear mixed-effects model analysis was used to determine the significance of L*, a*, and b* values in relation to the duration of coffee immersion and coffee type.

**Results:** Both immersion time and coffee type had significant effects on tooth discoloration ($p < 0.001$), with some types of coffee being more strongly associated with tooth discoloration than others. The amount of CGAs present in coffee was found to be positively correlated with the degree of discoloration ($p = 0.030$).

**Conclusions:** Prolonged exposure to coffee can exacerbate teeth staining, and different types of coffee can cause varying degrees of discoloration. Furthermore, coffee with higher levels of CGAs may lead to greater tooth discoloration.

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## Introduction

Coffee is a popular beverage consumed globally for its unique flavor and stimulating effects. With origins tracing back more than 1,000 years to Ethiopia, coffee has become a global commodity and an integral part of daily life for people all around the world [1]. According to the United States Department of Agriculture's most recent report on the coffee industry, the United States is the largest consumer and importer of coffee, while Finland has the highest per capita coffee consumption [2,3].

In addition to its popularity as a beverage, coffee has also been the subject of numerous studies exploring its potential health benefits. Some research suggests that moderate coffee consumption might be associated with a reduced risk of certain diseases, including type 2 diabetes, and Parkinson's disease [4–7]. However, one potential downside to coffee consumption is teeth discoloration. Teeth discoloration resulting from coffee consumption is a common concern of many coffee drinkers. The discoloration process is not a result of a single component but is rather complex and involves various chemical reactions. One of the processes involves pigmented compounds called chromogens, which can stick to tooth enamel, resulting in visible stains over time. Furthermore, coffee also contains acids that can erode tooth enamel, making it more susceptible to staining.

Chlorogenic acids (CGAs) are a group of phenolic compounds found in coffee that contribute to its bitter taste and are also among the culprits in coffee that cause teeth discoloration [8,9]. However, studies have also reported that CGAs are associated with several potential health benefits. These acids have been found to possess antioxidative and anti-inflammatory properties that may be important for preventing heart diseases [10,11]. In addition, CGAs are known to regulate blood glucose and blood pressure levels, which might be beneficial to patients with type 2 diabetes [12]. Despite their health benefits, attention should be paid to the quantity of these compounds with regard to their possible role in teeth discoloration, considering the richness of CGAs in coffee [10].

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The purpose of this study was to determine whether a certain type of coffee brand causes extrinsic dental stains more than others do and whether the amount of CGA in the coffee is related to the degree of staining. The null hypotheses state that there is no difference among different coffee brands and that there is no association between the amount of CGAs and discoloration. By exploring these hypotheses, we aim to shed light on the potential relationship between coffee consumption and teeth discoloration and to provide information that may help individuals make more informed decisions about their coffee consumption habits.

## Materials and methods

### HPLC chlorogenic acid determination

To determine the CGA contents in coffee, we targeted the three most abundant isomers, 3-CQA, 5-CQA, and 4-CQA [13]. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). CGA standards were prepared by first making a stock solution of 3-CQA, 5-CQA, and 4-CQA in distilled water and making dilutions (25, 12.5, and 6.25 ppm). The standards were run with a high-performance liquid chromatography (HPLC) photodiode array detector (HPLC YL 9100-PDA; Youngin Chromass, Kyeonggi, Korea) and the software YL-Clarity (Youngin Chromass) for analysis. We set the conditions according to the suggested setting of DIN 10767 [14]. Acetonitrile (Sigma-Aldrich) and 1% phosphoric acid (Sigma-Aldrich, MO, USA) were used as mobile phases. After running the standards, calibration curves ($R^2$ = 0.999) were drawn for the quantification analyses. We prepared the coffee samples by dissolving 0.9 g of powder in 100 mL of water. The coffee mixtures were heated until the powders were completely dissolved. Using a pipette, we transferred the coffee mixtures to vials for centrifuging. The samples were centrifuged for 10 min at 10,000 rpm, 4 °C. Only the supernatant was collected and filtered through a 0.2-μm filter (Advantec, Tokyo, Japan). The samples were then transferred to HPLC vials and loaded on the autosampler for test runs. Following the test runs, the sample data, and standard data were superimposed for match analysis. This procedure was repeated four times in between immersion, as the same coffee mixtures were used for HPLC analysis as well as the immersion experiment. A total of five measurements per coffee group were recorded.

### Specimen preparation

Bovine teeth was purchased from the Korean Traditional Market located in Seoul and stored in a freezer at −20 °C until usage (no ethical approval was required according to the ethics committee of Seoul National University). Only the central and second incisors were extracted from the dissected mandibular arch, and any teeth with cracks or caries were discarded. The teeth were then allowed to thaw at room temperature before modification. A bench drilling machine (YDM-13mm; Yongsoo Precision, Daegu, Korea) fitted with a cylindrical diamond core (⌀10 × ⌀/8 mm) was used to drill holes with a diameter of 8 mm through the center of the teeth while supplying water. To prevent damage to the teeth, care was taken to avoid excessive heat generation during the drilling process. The drilled teeth were fixated on to acrylic rings that were custom made (⌀30 × ⌀/12 × 4 mm thickness) in advance. Resin (Vertex; Vertex-Dental, Soesterberg, The Netherlands) was used to fixate the tooth with an acrylic ring, as shown in Figure 1. Specimens were then polished with a grinding/polishing machine (LaboPol-5; Struers, Copenhagen, Denmark) with silicon carbide papers (#220, #600, and #1200 SiC paper; R&B, Daejeon, Korea). The specimens were polished gently to preserve the enamel layer and prevent exposure to dentin. In addition, the specimen thickness was measured at four points with a digital micrometer (CD67-S15PM; Mitutoyo, Kawasaki, Japan) to ensure consistent thickness.

### Specimen standardization

To ensure standardization, all specimens were selected based on a predefined criterion and distributed evenly among the different groups by considering two parameters: hardness and lightness. Specimens with a mean hardness exceeding 250 were chosen prior to immersion to ensure they consisted primarily of enamel rather than dentin. The hardness of the samples was measured using a Vickers hardness tester (HM-220; Mitutoyo, Tokyo, Japan), with samples having a Vickers hardness of 250 or higher confirmed as enamel.

To evaluate the discoloration caused by coffee, spectrophotometry was employed. Prior to immersion in coffee, the colors of the pre-immersion specimens were assessed using a spectrophotometer (Ci7600, X-rite Pantone, Grand Rapids, MI, USA) in reflectance mode. After calibration, the L*, a*, and b* values of each specimen were recorded. In order to maintain consistency, the samples were distributed evenly among the different coffee groups by ensuring the mean L* value of 75—78 and a mean hardness value of 300.

### Coffee immersion

In this study, we selected five different brands of coffee—Starbucks, Dunkin' Donuts, Kanu, Ediya, and Coffee Bean—based on popularity and availability at the location of the study. To create the coffee solutions, 1.8 g of coffee powder was measured and dissolved in 200 mL of hot water (serving size is about 0.9 g/100 mL). Once the coffee dissolved

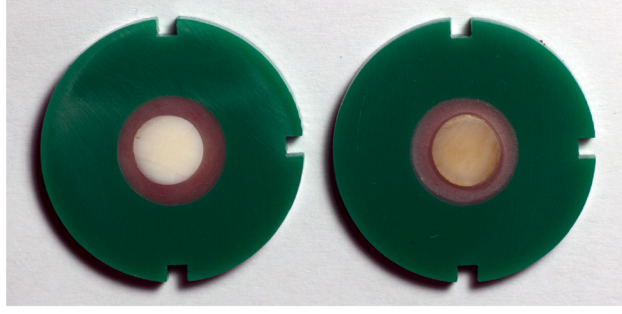

Figure 1. Tooth specimens before and after immersion in coffee. Right: before immersion; left: after 72 h of immersion.

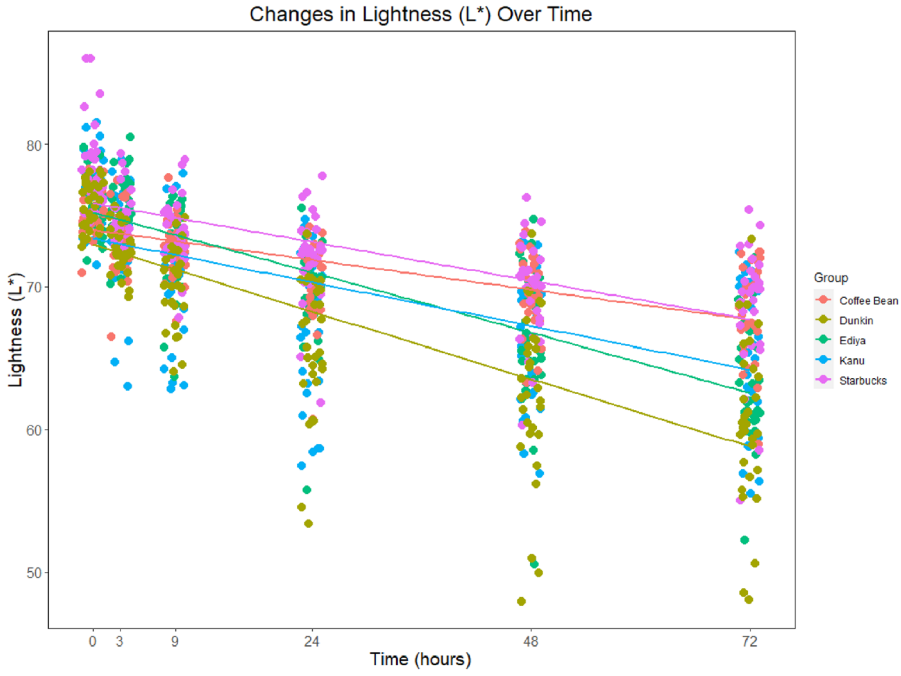


**Figure 2.** Changes in spectrophotometric measurements (*L) over time. Decrease in *L indicates that specimens became darker over time.

**Table 1.** List of instant coffee used and the amount of CGAs and caffeine determined by liquid chromatography.

| Manufacturer | Product name | pH | 3-CQA (mg/0.1L) | 5-CQA (mg/0.1L) | 4-CQA (mg/0.1L) | Caffeine (mg/0.1L) |
|---|---|---|---|---|---|---|
| Starbucks | House Blend | 5.39 ± 0.1 | 4.75 ± 0.1 | 7.11 ± 0.2 | 6.21 ± 0.1 | 45.94 ± 0.3 |
| Kanu | Mild Roast | 5.30 ± 0.1 | 5.31 ± 0.6 | 8.39 ± 1.1 | 7.16 ± 0.9 | 42.85 ± 0.4 |
| Dunkin' | Stick Coffee | 5.02 ± 0.1 | 6.18 ± 0.6 | 10.06 ± 1.1 | 8.49 ± 0.8 | 31.44 ± 0.4 |
| Ediya | Original Americano | 4.95 ± 0.1 | 4.79 ± 0.1 | 7.58 ± 0.2 | 7.19 ± 0.2 | 26.33 ± 0.7 |
| Coffee bean | Captain Americano | 5.21 ± 0.1 | 4.37 ± 0.1 | 7.66 ± 0.2 | 6.12 ± 0.3 | 20.91 ± 0.5 |

completely and the temperature of the mixture reached 40 °C, 30 specimens were immersed in the beaker containing the coffee solution. We determined the sample size ($n = 30$ per group; 150 total) considering a 90% statistical power and 95% confidence level using G*Power 3.1.9.7 software. The beakers were placed in a water bath set at 40 °C, and the specimens were left to soak for 3, 9, 24, 48, and 72 h. After each immersion, the specimens were rinsed with water and stored in artificial saliva until the next immersion.

### *Color measurements*

Color measurements were performed using the CIELAB color space to ensure precise and consistent evaluation across all specimens. The CIELAB comprises three components: L*, a*, and b*. The L* component indicates the lightness of the specimen, with higher values indicating lighter objects (100 for pure white and 0 for perfect black). The a* and b* coordinates describe the color characteristics, where positive a* values correspond to red hues and negative values indicate green hues. The b* values represent colors in the blue (negative) to yellow (positive) range. The midpoint of both the a* and b* axes represents a neutral gray color. To assess color changes of the specimens, spectrophotometer measurements were taken at intervals during the immersion processes. The differences were calculated as ΔL*, Δa*, and Δb*, obtained by subtracting the baseline values from those measured after a certain number of hours of immersion. To accurately reflect human visual perception, the color difference ($\Delta E_{00}$) was calculated, taking into account factors such as lightness, chromaticity, and hue differences:

$$\Delta E_{00} = \sqrt{\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_C S_C}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + R_T \left(\frac{\Delta C'}{k_C S_C}\right)\left(\frac{\Delta H'}{k_H S_H}\right)}$$

### *Statistical analysis*

We performed a linear mixed-effects model analysis to investigate the relationship between immersion time and the coffee tested while accounting for individual variation in the specimen color. We conducted a correlation analysis to reveal any association between the amount of CGAs and caffeine.

## Results

Within 7.5 min of chromatography, the major isomers of CGAs in coffee, 3-CQA, 5-CQA, and 4 CQA, were detected. Dunkin' Donuts coffee contained the highest amount of all three isomers of CGAs, followed by Kanu, Ediya, Starbucks, and Coffee Bean (Table 1). The specimens exhibited noticeable teeth discoloration both visually and numerically, indicating a strong

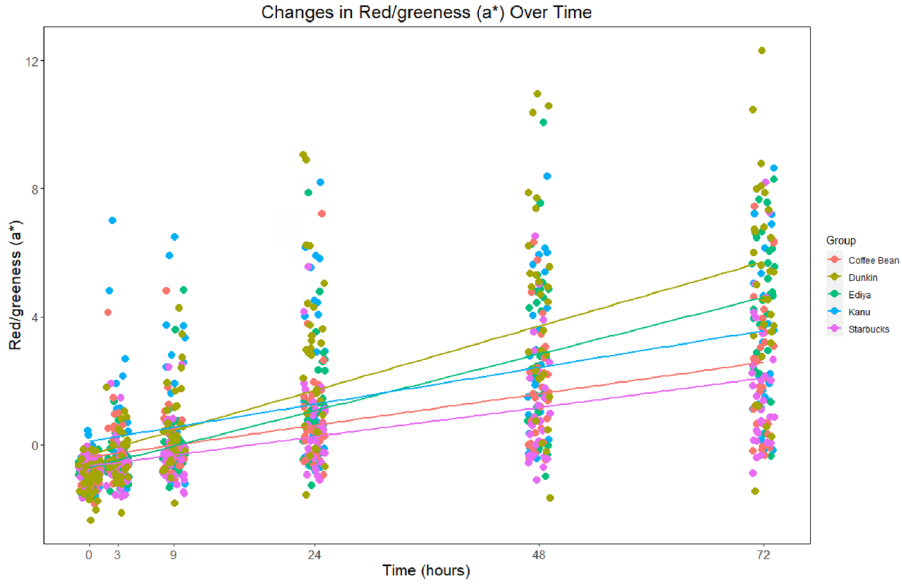


**Figure 3.** Changes in spectrophotometric measurements (*a) over time. Increase in *a indicates that redness in the specimens increased over time.

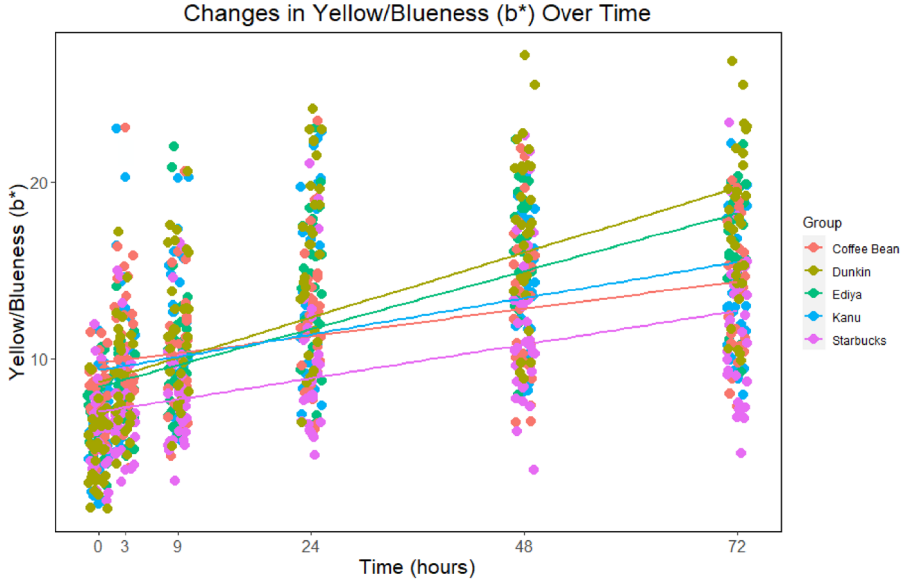


**Figure 4.** Changes in spectrophotometric measurements (*b) over time. Increase in *b indicates that yellowness in the specimens increased over time.

association between immersion time and the extent of discoloration (Figures 1–5). We calculated ΔL*, Δa*, Δb*, and $\Delta E_{00}$, and their values are summarized in Table 2. At 72 h of immersion, the mean $\Delta E_{00}$ was greatest in specimens immersed in Dunkin Donuts, followed by Ediya, Kanu, Starbucks, and Coffee Bean (Figure 5). To further confirm these observations and determine the effects of immersion time and coffee group on the colors of the specimens, we analyzed the data using a linear mixed-effects model. The results showed significant effects of both coffee type and immersion time on color changes in the specimens, as evidenced by significant changes in L*, a*, and b* (Table 3).

The effects of the coffee group and immersion time on L*, a*, and b* were all significant ($p < 0.001$) (Table 3). Immersion time had a significant effect on L*, a*, b* ($p < 0.001$). The type of coffee also had a significant effect on L*, a*, and b*, with Dunkin' Donuts coffee having the most significant effect on all three color spaces ($p < 0.050$). In addition, the interaction between the coffee group and immersion time was significant on L*, a*, and b* (Table 3). Finally, there was a strong correlation between the amount of CGAs and the discoloration of the specimens ($r = 0.913$, $p = 0.030$). However, there was no correlation between the amount of CGAs and caffeine ($p > 0.890$).

## Discussion

The results of this study showed that immersion time was strongly associated with color changes in all three color spaces, indicating that prolonged immersion in coffee led to greater discoloration. Furthermore, we found a significant

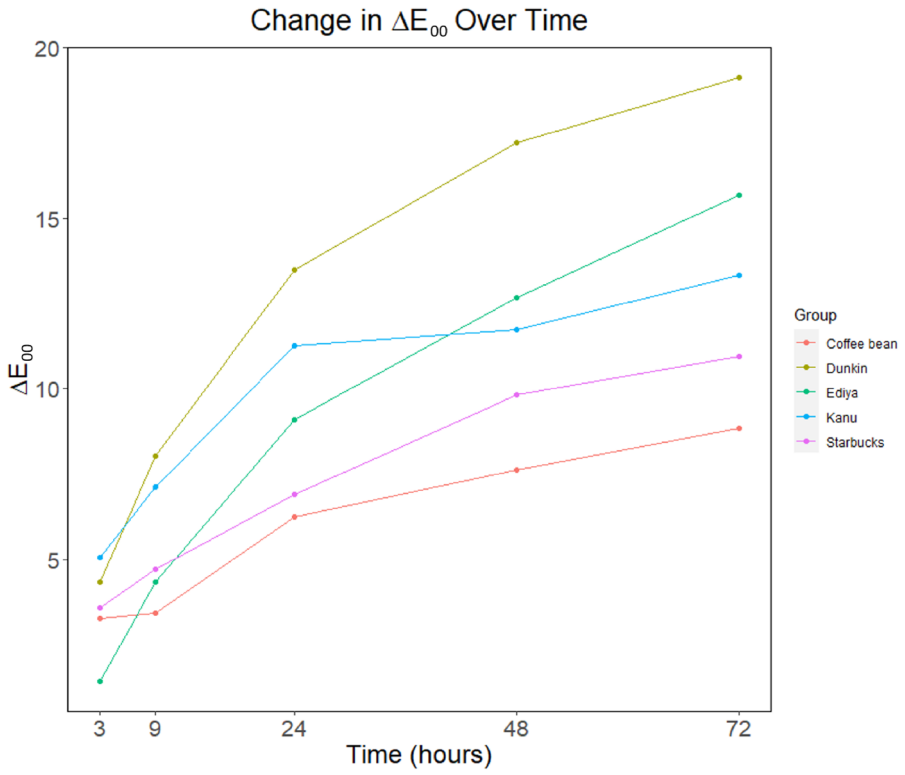


**Figure 5.** Change in $\Delta E_{00}$ over time. Specimens immersed in Dunkin coffee exhibited highest degree of discoloration, while specimens immersed in coffee bean exhibited least discoloration.

**Table 2.** Summary of mean and standard deviations of ΔL*, Δa*, Δb*, and ΔE values after 3, 9, 24, 48, and 72 h of immersion in five different coffee groups.

| | Hours of immersion | ΔL* | Δa* | Δb* | $\Delta E_{00}$ |
|---|---|---|---|---|---|
| Dunkin' | 3 | −2.9(1.76) | 0.75(0.63) | 3.86(2.52) | 4.36 |
| | 9 | −5.58(3.24) | 1.66(1.2) | 6.83(3.07) | 8.05 |
| | 24 | −9.59(5.44) | 3.71(2.52) | 10.84(4.16) | 13.48 |
| | 48 | −13.12(6.8) | 5.22(3.14) | 12.4(3.92) | 17.22 |
| | 72 | −15.18(6.53) | 6.11(2.99) | 12.62(3.91) | 19.12 |
| Kanu | 3 | −3.71(4.35) | 1.16(1.81) | 4.18(4.87) | 5.08 |
| | 9 | −5.56(4.66) | 1.78(1.99) | 5.29(5.39) | 7.14 |
| | 24 | −8.98(6.02) | 2.74(2.49) | 8.08(5.95) | 11.28 |
| | 48 | −9.49(5.75) | 3.42(2.28) | 7.91(4.73) | 11.75 |
| | 72 | −11.13(6.26) | 3.99(2.46) | 8.2(5.12) | 13.34 |
| Ediya | 3 | −0.88(1.48) | 0.47(0.4) | 1.35(1.88) | 1.44 |
| | 9 | −3.24(2.82) | 1.05(1.13) | 3.56(3.6) | 4.36 |
| | 24 | −6.82(3.86) | 2.38(1.72) | 7.36(3.75) | 9.12 |
| | 48 | −9.89(5.24) | 4.04(2.35) | 9.25(3.61) | 12.67 |
| | 72 | −12.86(4.59) | 5.36(2.11) | 10.04(3.44) | 15.68 |
| Starbucks | 3 | −3.47(2.5) | 0.09(0.6) | 1.16(1.64) | 3.59 |
| | 9 | −4.42(3.79) | 0.51(0.73) | 2.04(1.98) | 4.73 |
| | 24 | −6.11(4.91) | 1.33(1.26) | 3.81(2.76) | 6.92 |
| | 48 | −8.41(5.43) | 2.00(1.62) | 6.01(3.49) | 9.83 |
| | 72 | −9.69(6.23) | 2.73(2.03) | 5.7(3.21) | 10.96 |
| Coffee Bean | 3 | −2.09(2.32) | 0.85(0.87) | 3.26(2.89) | 3.29 |
| | 9 | −2.72(2.83) | 0.95(1.28) | 2.6(4.18) | 3.45 |
| | 24 | −4.85(3.07) | 1.86(1.48) | 4.85(3.01) | 6.27 |
| | 48 | −5.87(3.26) | 2.64(1.64) | 5.78(3.56) | 7.63 |
| | 72 | −7.23(4.02) | 3.25(1.87) | 5.75(3.2) | 8.86 |

**Table 3.** The effects of immersion time and coffee group on L*, a*, b* and the interaction between immersion time and coffee group on L*, a*, b*.

| CIELAB color space | Immersion time | Coffee group | Interaction |
|---|---|---|---|
| L* | $\beta^a$ = −0.04, $p < 0.001$ | **Coffee bean** β = −2.64, $p = 0.024$ | **Coffee Bean** β = 0.02, $p = 0.015$ |
| | | Dunkin β = −4.67, $p = 0.001$ | **Dunkin'** β = −0.03, $p = 0.007$ |
| | | **Ediya** β = −2.71, $p = 0.041$ | **Ediya** β = −0.02, $p = 0.038$ |
| | | **Kanu** β = −2.62, $p = 0.025$ | |
| a* | $\beta = 0.02$, $p < 0.001$ | **Dunkin'** β = 1.32, $p = 0.026$ | **Dunkin'** β = 0.01, $p = 0.029$ |
| b* | $\beta = 0.08$, $p < 0.001$ | **Dunkin'** β = 1.68, $p = 0.046$ | **Dunkin'** β = 0.07, $p < 0.001$ |
| | | | **Ediya** β = 0.06, $p < 0.001$ |

*$\beta^a$: estimated effect sizes.*

effect of coffee type on color change, suggesting that different types of coffee had varying degrees of staining properties. As a result, the first hypothesis that coffee type had no effect on discoloration was rejected. In addition, we found a strong positive correlation between the amount of CGA present in the coffee and the degree of staining. This led to the rejection of the second hypothesis, which stated that changes in CGA content did not affect staining properties.

Based on the findings, it can be concluded that the high concentration of CGAs in Dunkin' coffee resulted in a significant level of discoloration in the specimens, as indicated by $\Delta E_{00}$. On the other hand, specimens exposed to Coffee bean coffee, which had the lowest CGA content, exhibited the least amount of discoloration. However, it is important to note that there were inconsistencies in the extent of discoloration among individual specimens, as observed from the spectrophotometer measurements after each immersion.

For instance, the level of discoloration in the specimens immersed in Ediya and Kanu coffee reversed after 24 h of immersion. This variability could be attributed to inherent variations in the specimens, despite efforts to ensure uniform hardness and absence of cracks. Such variations are inevitable when attempting to mimic natural teeth as closely as possible. Additionally, the use of artificial saliva for specimen storage was implemented to simulate real-life conditions. Future studies that seek to determine the level of discoloration may benefit from the use of a standardized model, especially if the purpose is only to compare the staining properties of different types of coffee.

To ensure a standardized approach and minimize variability, we considered various factors when designing the study protocol. For example, we chose instant coffee over freshly brewed coffee for the quantification of CGA contents, which can be influenced by factors such as preparation method or brewing temperature. In fact, the quantities of these components vary significantly, even when the coffee is from different branches of the same manufacturer. Therefore, we opted to use instant coffee for the present study, as it is the closest to a standardized coffee and readily available. In addition, we used bovine teeth instead of human teeth or other alternatives to meet the demands of this study. Acquiring human teeth can be challenging and may raise ethical concerns, and bovine teeth have frequently been used in other studies due to their compositional and physical similarities to human teeth [15–19].

Teeth discoloration caused by coffee is referred to as 'extrinsic staining', which can be further classified based on the classification system developed by Nathoo [20]. The Nathoo categorization system categorizes extrinsic teeth discoloration into three types: N1 type, which involves chromogens binding to the surface; N2 type, which causes chromogens to change color after binding due to denaturation or erosion by acids; and N3 type, which refers to staining caused by colorless matter that undergoes chemical reactions to create chromogens [20–22]. N1-type stains are known to progress into N2-type stains, and N2-type stains tend to be more difficult to remove [20].

During the bean-roasting process, sugars are broken down into monosaccharides, which further disintegrate into brown caramel substances [23]. These caramel substances contribute to the flavor and aroma of coffee as well as react with CGAs to produce brown–black pigments or chromogens [23–25]. Therefore, the discoloration process caused by coffee can involve all three types of extrinsic staining based on the Nathoo system. Specifically, discoloration due to CGA falls under the N2 and N3 categories. When the pH of the mouth becomes more acidic, it creates an environment that is more favorable for the growth of certain bacteria (acidophilic bacteria) [26]. Some of these bacteria can produce pigments that can stain the teeth over time [27]. The acidity of the mouth can be increased by consuming an acidic beverage such as coffee, which can lower the pH of the mouth and promote the growth of these bacteria. Although higher levels of CGAs may lower the overall pH of coffee and have an indirect effect on increasing the bacteria responsible for producing pigments, whether CGAs play a significant role in this particular process remains unknown and requires more evidence [28,29].

Previous studies have shown that various factors such as the type or origin of the Coffee Bean, roasting temperature, and brewing method affect the level of teeth staining [28]. CGAs, which are one culprit of teeth discoloration, are highly concentrated in coffee, with levels varying depending on the type of bean and degree of roasting [30]. In general, lighter roasts tend to have higher CGA content than darker roasts do [31]. Therefore, the more intense the coffee roast, the lower the CGA content and, potentially, the lower the risk of teeth discoloration. Roast levels can also affect pigmentation. Darker roasts tend to contain higher concentrations of chromogens. However, CGAs are known to be lost during intense roasting conditions [31–33]. A previous study revealed that the amount of CGA was higher in drip coffee than in espresso, because of the extended extraction time and method [9,34]. Unlike the process of espresso, in which water passes through the coffee grounds at high pressure and speed, some water is absorbed by coffee grounds. This water–coffee ground contact causes extraction of the remaining phenolic compounds, resulting in the higher CGA content in coffee.

Although the potential health benefits of CGAs found in coffee have been extensively studied, their role in teeth discoloration is less clear. Thus, the focus of this study was on the direct relationship between CGAs and teeth discoloration. Although other studies have also conducted research on teeth discoloration in association with CGAs, these studies did not determine the exact amounts of CGAs [9,35]. Moreover, the mechanisms by which tannins and chromogens cause teeth discoloration are relatively well understood, but the effect of CGA on discoloration is not as clear [36]. As mentioned earlier, CGA-induced discoloration can be quite complex due to the involvement of multiple reactions. Hence, there is a need for research examining the direct association between CGAs and teeth discoloration.

When examining the technical analysis of color in relation to coffee drinking habits, it is crucial to take into account the perceivable difference in discolored tooth specimens. It is widely known that individuals have varying perceptions of color, and their personal standards for what they consider as 'discoloration' can differ significantly. Consequently, the color differences observed in the tooth specimens may not be deemed substantial by the average person's perception. However, it is worth noting that the authors of this study acknowledge the existence of a perceived distinction, particularly between teeth specimens immersed in Dunkin' and Coffee Bean coffee. To further enhance the understanding of these perceptual differences, it may be beneficial to establish a specific threshold or criteria that indicates a noticeable difference in tooth discoloration. This would provide valuable insights into how individuals perceive and interpret changes in tooth color, contributing to a more comprehensive understanding of the impact of coffee consumption on dental aesthetics.

In addition to exploring the relationship between CGAs and teeth discoloration, the authors have also examined the caffeine content of each coffee and whether a correlation existed between the CGA levels and caffeine. Because many

coffee drinkers consume coffee for its stimulating effects, this is an important consideration. The lack of a correlation between CGAs and caffeine suggests that these two components should be evaluated independently, and consumers should make informed decisions based on their individual preferences.

This study yielded three main findings. The duration of coffee-to-teeth contact as well as the type of coffee has an impact on teeth discoloration. The results also suggest that the CGA content in coffee is strongly correlated with the level of teeth discoloration. These findings suggest that people who are concerned about their dental appearance may need to change their coffee-drinking habits and carefully choose the type of coffee they drink. Furthermore, future research could investigate the effects of coffee additives such as sugar or milk, as well as decaffeinated coffee, on teeth discoloration. This would provide a deeper understanding of the role and impact of CGAs on teeth discoloration.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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