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Relationship between Different Coffee Types and Tooth Discoloration in the Presence or Absence of Fluoride Varnish: An In Vitro Study

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Abstract

Purpose: To examine the effects of various commercially available coffee products on the dental enamel surface staining, as well as the effect of sodium fluoride varnish (%5) NaF applied before the coffee immersion cycle.

Methods: The study used seventy-two bovine permanent incisors. Coffee, coffee milk, ice, chocolate, coffee creamer, and control groups were the main groups into which enamel blocks were separated. Two subgroups were formed from each of the main groups: subgroups A samples were not exposed to NaF prior to the coffee immersion cycle without fluoride varnish (WOFV). Subgroups B samples were given NaF prior to the coffee immersion cycle with fluoride varnish (WFV). To assess the tooth discoloration a spectrophotometer was used.

Result: After pure coffee immersion cycles, there was a high statistically significant increase in color change ΔE for A subgroups WOFV; after ice coffee and coffee creamer immersion, there was a statistically significant increase in color change ΔE (less than that of pure coffee); and after coffee milk and chocolate coffee immersion, there was no statistically significant increase color change ΔE. For B subgroups WFV, following pure coffee immersion, there was a statistically significant increase in color change ΔE for B subgroups WFV. However, following ice coffee, coffee creamer, coffee milk, and chocolate coffee immersion, there was no statistically significant increase in color change ΔE.

Conclusion: Simply relying on the current study's color change ΔE mean results. Coffee milk and chocolate coffee had very little color change increase, followed by ice creamer, coffee creamer and coffee in that order.

Introduction:

Tooth discoloration is a prevalent issue with frequent coffee consumption. The discoloration, shift in opacity, and tint of teeth are all signs of staining (1). One of the dentition's significant aesthetic features is shade. Lighter hues are preferred by most people since they enhance their appearance and have a good impact on how others perceive their personalities in general (2). Tooth discoloration significantly affects the perceived color of a person's teeth. Extrinsic staining, a type of tooth discoloration, is influenced by dietary and oral hygiene habits. It causes a superficial color change and gradual enamel wear that reveals the darker dentin underneath; it can happen inside the tooth due to chemical interactions or on top of the enamel in the pellicle layer (3). The colored particles in the pellicle layer precipitate to cause dental discoloration. The tooth's surface then undergoes a chemical reaction with these particles. Although the exact mechanism of this reaction is unknown, some ideas have proposed that stains typically result in a brownish tint (4). They can also be brought on by smoking, growing older, and drinking wine, tea, and coffee (5). Coffee-induced discoloration is more challenging to get rid of than other extrinsic stains, such as smoking stains (6). Coffee is the dried seed of the fruit originated from a tree of the Coffea genus, Rubiaceae family (7). It is a complex chemical mixture containing thousands of different chemicals such as carbohydrates, lipids, nitrogen compounds, vitamins, minerals, alkaloids, and phenolic compounds (8). Coffee can be served in various ways, and it has a big impact on the oral environment. It was found to have an anti-periodontitis effect (9). It also significantly protects against alveolar bone resorption, and it inhibits the formation of caries (10, 11). Adversely, when consumed frequently coffee can cause a variety of dental health issues, It's may be a risk factor for periodontitis (12). Furthermore, a coffee solution with a pH of 4.9-5.2 can aggravate tooth discoloration, a low pH of less than 5.5 can cause the oral cavity to become acidic, resulting in the demineralization of the enamel (13). Also, coffee contains tannins and chlorogenic acid, which are able to cause tooth discoloration (6).

The aims of this study to assess the effect of various coffee types (pure coffee, coffee + milk, ice coffee, chocolate coffee and coffee creamer) on dental staining with or without fluoride varnish protection and identify the correlation between coffee temperature and degree of staining.

Materials and Methods

This an in vitro study was carried out in the Department of P.O.P (Pedodontic, Orthodontic and Preventive Dentistry Department). College of Dentistry, University of Mosul for the academic year 2023-2024 from 5 /Dec /2023 to 5 /Nov /2024. The study submitted and approved by the Local Ethics Committee (UoM.Dent.24/1001) The University of Mosul, College of Dentistry's Research Ethics Committee in Nineveh, Iraq.

Gathering of Teeth Sample

Newly extracted bovine incisors were taken and given away from Hawija City's slaughterhouses. After being cleansed and rinsed with tap water, the teeth were placed in a 0.1% thymol solution at 4 °C in the refrigerator until they were employed according to the suggested procedure by (14, 15). Out of all the teeth that were collected, only 72 were selected because they had healthy crowns and were free of stains and anomalies.

Criteria of Samples Gathering

Inclusion criteria: newly removed teeth with intact enamel surfaces that have not been damaged during the extraction process, healthy crowns, and no stains or abnormalities.

Exclusion criteria: the study rejected teeth with fluorosis, hypoplasia, wear, cracks, fractures, discolouration, and developmental anomalies of the enamel, as shown in Figure (1).

Teeth Sample Preparation: Firstly, some of the sample roots were cut by using high-speed straight fissure turbine bur with continuous water cooling, in order to prevent enamel damage. The remaining

sample roots were removed using a straight diamond sectioning disc bur with a low-speed motor and continually running water cooling, non-fluoridated pumice was then used to delicately clean and polish the samples (16, 17), as shown in Figer (2).

Following that, the crowns of bovine incisors were inserted within an auto polymerized cold cure acrylic resin blocks (18, 19). Employing a cylindrical plastic tubes 15mm in height and 32mm in diameter with smooth, parallel top and lower borders, the teeth's outer labial side was faced upward (20, 21). To produce a flat and consistent enamel surface specimen for surface roughness testing, the labial surface of each tooth crown was ground polished 10 times in a single direction utilizing grit paper (grit 400, 600) (18, 22). The same method was used to sand each sample employing afresh carbide silicon abrasive paper (22). A caliper was used to mark the middle third of the labial surface of the crown by measuring the distance between the incisal edge, cementoenamel junction, and mesiodistal dimension. An adhesive tape measuring 10 x 7 mm^2 was placed in the center of the crowns on the labial surface of the samples(18). After that, all the crowns—aside from a 10 x 7 mm2 window of exposed enamel—were coated with acid-resistant nail varnish. The tapes were removed to expose the enamel surface once the samples had dried (18), as shown in Figure (3).

Immersion cycle procedure:

Half of the specimens per coffee group were treated with a fluoride varnish (WFV) while other half did not receive fluoride coating. All prepared samples were kept in deionized water throughout the experiment (23). At the beginning, all groups were subjected to color (ΔE) analyses that were performed at the baseline. After baseline color analyses, WFV subgroups were treated with fluoride varnish, the specimens were maintained in deionized water after a thin layer of varnish was applied using micro brush, as shown in Figure (4).

A scalpel blade was used to carefully remove the varnish after six hours, being cautious not to disturb the enamel surface (24). After that, these fluoride varnish subgroups were subjected to color analyses prior to immersion in coffee. Following that all the groups WFV and WOFV were immersed in different coffee types and distill water (control group) 30 min per day for 14 days (25). For every immersion, a fresh beverage was prepared to guarantee the coffee's freshness, as shown in Figure (5). The temperature of each coffee type was measured using a digital thermometer. Determine the pH of each coffee type by using pH meter, as shown in Figure (6).

Color Analysis (ΔE, L*, a*, b*)

The tooth's color was measured by using a spectrophotometer (Vita Easyshade 5, Germany), as shown in Figure (7) depending on the (CIE-L*a*b*) color system. The system recorded the tooth's color and three-dimensionally analyzed it into three values: L*, which represents brightness (0-100), a* which represents a chromaticity of red-green color (-150 to +100), and b*, which represents a chromaticity of yellow-blue color (-100 to + 150) (26, 27). The spectrophotometer's 5 mm aperture is consistent across all samples; a calibration block located on the device's charging base was used to calibrate the instrument both before and after each measurement (18).

The samples were gently dried with a drying paper without dehydration after the spectrophotometer's hole was placed on the exposed enamel window at a right angle, touching the tooth surface. (28). Figer (8) shows two measurements that were done for each sample in A subgroups (at baseline and after coffee treatment) and three measurements were done for each sample in B subgroups (at baseline, after fluoride varnish application and after coffee treatment).

The following formula was used to evaluate the color changes (ΔE) following fluoride application and coffee immersion to compare the differences in tooth color at different time points with the baseline: $[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2} = \Delta E$, where the variations in the L*, a*, and b* values are denoted by ΔL, Δa, and Δb, correspondingly. Thus,

$\Delta E_{after\ fluoride\ varnish} = [(L_{fluoride\ varnish} - L_{baseline})^2 + (B_{fluoride\ varnish} - B_{Baseline})^2 + (A_{fluoride\ varnish} - A_{Baseline})^2]^{1/2}$, and $\Delta E_{after\ coffee\ treatment} = [(L_{after\ coffee\ treatment} - L_{baseline})^2 + (B_{after\ coffee\ treatment} - B_{baseline})^2 + (A_{after\ coffee\ treatment} - A_{baseline})^2]^{1/2}$ (29).

Subgroups (A) underwent a color test both before and after the coffee immersion cycles. Additionally, color tests for subgroups (B) were conducted concurrently at baseline, following fluoride varnish application and following coffee immersion cycles.

Result

Prior to conducting any tests, finding out if the data were routinely dispersed or not was the first step. For this, the Shapiro-Wilk test was employed, and parametric tests and two-way ANOVA were selected due to the regularly distributed nature of the data, see Table (1), (2) and (3). The two-way analysis of variance (ANOVA) test was used to compare the mean values of (ΔE1, ΔE2, and ΔE3) in each group, study groups, and the interaction between ΔE and study groups. The results revealed highly statistically significant differences ($p \leq 0.000$) between ΔE, study groups, and the interaction between ΔE and study groups for all treatment types, see Table (4). By using the standard deviation for each of the study groups' subgroups and the mean values for each group (ΔE1, ΔE2, and ΔE3) for Duncan's multiple range test (24). In all subgroups, the ΔE1 ΔE2 value increased after coffee treatment for both without fluoride varnish (WOFV) and with fluoride varnish (WFV) subgroups, but the ΔE3 value decreased after FV application, see Table (5). The results of the Independent Sample T-test comparing the mean values of (ΔE1 and ΔE2) in each group revealed statistically significant differences ($p < 0.05$) between the coffee subgroups between ΔE1 and ΔE2, but no statistically significant differences ($p \geq 0.05$) between the remaining study groups between ΔE1 and ΔE2, see Table (6). Figure (6) indicate the ΔE results for all study groups since the greater color changes (ΔE) were in A subgroup WOFV with the least color changes in B subgroups WFV after FV application with an increase in color changes after coffee treatment. The most significant color change was observed with coffee, followed by coffee creamer, iced coffee, chocolate coffee, and coffee milk.

Discussion

A VITA Easy Shade ® spectrophotometer was used in this work to measure color; instrumental color analysis may be superior to other techniques. This apparatus generates spectral color space readings that are not only precise but also rapid and repeatable. In addition, compared to colorimeters and laboratory spectrophotometers, which require a level surface and are therefore not advised for determining the spectral color of teeth, the VITA Easyshade® spectrophotometer was preferred for this purpose. Furthermore, a translucency of genuine teeth cannot be measured by laboratory spectrophotometers (30, 31).

The objects in the current study were located in a 3D color space using the CIE Lab color system dimensions (L*, a*, b*), which also quantify the variations in brightness and hue (ΔL*, Δa*, Δb*) for the computation of the overall color difference (ΔE). The statistical analysis results were comparable with the parameters L*, a*, and b* alone as well as the total color difference (ΔE) (32). The CIELAB color space was employed for color representation and analysis. Lightness (L*), green to red(a*), and blue to yellow (b*) were measured using a spectrophotometer.

All groups in this examination displayed a considerable change in ΔE values, depending on the current study after coffee treatment for both without fluoride varnish(WOFV) and with fluoride varnish(WFV) subgroups. Changes in ΔE values for WOFV subgroups are more than that for WFV subgroups after coffee treatment, this related to some protection action of FV by decreasing the surface roughness (SR) of tooth enamel that result in decreasing of tooth discoloration. Coffee immersion was associated with a decrease in the L* component and an increment in the b* and a* components,

suggesting a darker, yellowish, and reddish tooth color that confirmed the coffee discoloration effect. A variety of compounds included in coffee might cause discoloration. Coffee's tannic, chlorogenic and chromogen acids have been connected to tooth discoloration. These substances exhibit distinct staining mechanisms-chromogens attach to enamel surface pellicles, while acids degrade the enamel surface (6, 33). Coffee contains high levels of chromogens and melanoidins, both formed during roasting process. Melanoidins and chlorogenic acids (CGAs) in coffee cause significant tooth discoloration by binding to the enamel and potentially eroding its surface, leading to extrinsic stains that range from yellow to dark brown. Furthermore, the variety of beans, roasting level, frequency and mode of ingestion, and other variables can all affect how much staining occurs (34).

Tooth discolouration is also influenced by the acidity of beverages. Acidic conditions weaken enamel, making it more susceptible to chromogen stains (35). Coffee contains chromogenic substances that are tannin in which they are as binder and color provider. Another ingredient that acts as a color provider in coffee drinks is chlorogenic acid. The primary phenolic component found in coffee is chlorogenic acid. Coffee's pH may drop below the threshold range of less than 5.5 due to the elevated chlorogenic acid component. The acidic pH of beverages causes teeth to become discolored because calcium hydroxyapatite dissolves in dental enamel creating tooth holes that allow dyes to deposit more easily especially after prolonged exposure (36, 37).

The greater change of ΔE was associated with pure coffee (alameed coffee) for both WOFV subgroups and WFV subgroups with greater mean value of ΔE change for WOFV subgroups (29.9184) more than that for WFV subgroups (17.0407) this difference in the mean value of ΔE for both subgroups of the same main group(alameed coffee) was related to the role of FV as protective barrier against discoloration by decreasing the enamel SR.

As mentioned above the greater change of ΔE was associated with pure coffee (alameed coffee) followed by coffee creamer, ice coffee, chocolate coffee and coffee milk respectively. Previous research has demonstrated that the degree of teeth staining is influenced by several variables including the kind or origin of the coffee bean, roasting temperature, brewing technique, chemical makeup, acidity value (pH), and additions (6, 38, 39). The greater change of ΔE associated with pure coffee (alameed coffee) was due to the acidic nature (pH 4.4) of this type of coffee used in the study, low level of pH of this coffee type means that it contains a high level of (CGAs), since in coffee there is an inverse relationship between pH value and (CGAs) content (40). Additionally, the acidic action raises the SR of enamel teeth, giving them a sensitive site for the adsorption of dyes. On the other hand in the present study ΔE changed the value of ice coffee to less than that of hot pure coffee, since there is a direct proportional relation between teeth staining due to coffee consumption and its temperature (41). With respect to coffee milk and chocolate, coffee had the least ΔE value change among study groups. This belonged to its pH values which were near to neutrality (6.4, 6.2) respectively, and its additives (milk, chocolate) which are considered as good remineralizing agent that will facilitate decreasing enamel tooth SR and make it less susceptible for staining, see Table (7).

A recent study compared the tooth-staining effects of coffee with dairy products against coffee without milk (42). It reached the conclusion that adding milk to coffee reduced stains and advised drinking coffee that contains milk to limit the chance of discoloration (43). These suggestions corroborate the findings of the current investigation. In contrast to the pure coffee groups, the coffee milk group's teeth showed less discoloration. This discovery could potentially be attributed to the reduced concentration of coffee in the coffee milk group, that in turn leads to decrease staining.

When assessing how temperature affected teeth staining, the current study discovered that hot coffee had higher ΔE values than cold coffee. Recent research supports the idea that heating might enhance

pigmentation. By immersing composite material in distilled water, another study examined this phenomena and showed that more stains were created at 60 °C than at 37 °C (44, 45). This also explains that the teeth immersed in pure coffee (at 80°C) showed a larger degree of discoloration than teeth immersed in ice coffee (10 °C) in the current investigation.

Conclusion

The maximum increase in mean ΔE value was shown in the pure coffee (alameed coffee) group for both (without FV) and (with FV) subgroups, with the presence of a high statistically significant difference between the two subgroups of the same main group followed by coffee creamer, ice coffee, chocolate coffee, and coffee milk. In relation to effect of temperature on tooth discoloration ΔE, it was found ice coffee (10 °C) had less effect than hot pure coffee (80 °C) of this study.

Author contribution: Study conception and design by Aisha Akram Qasim, data collection Hussein Ibrahim Hamada, analysis and interpretation of results by Aisha Akram Qasim and and Hussein Ibrahim Hamada, and manuscript preparation by Hussein Ibrahim Hamada.

Author declaration: We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed

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Figure (1): Teeth with enamel developmental abnormalities, discoloration, cracks and fractures were excluded from the study.

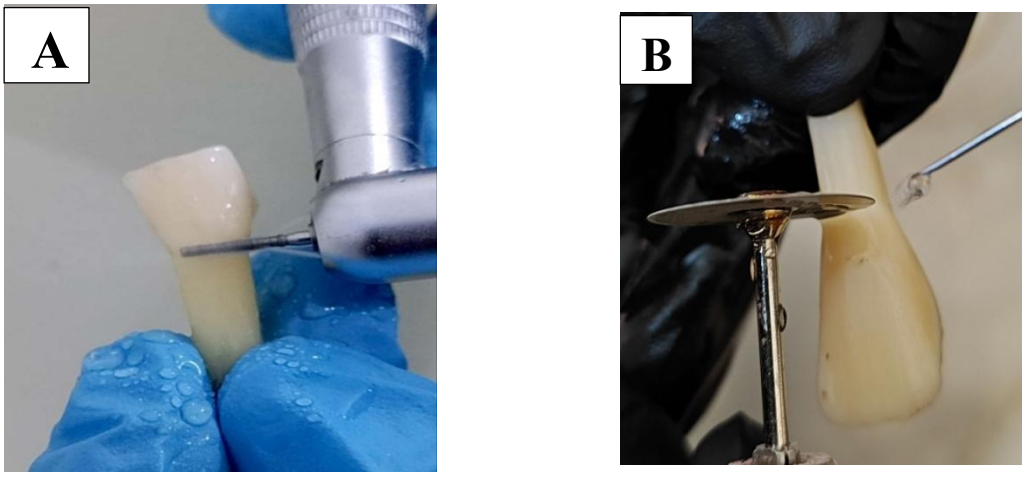


Figure (2): **(A)** the specimen roots were removed utilizing a high-speed straight turbine fissure bur and continuous water cooling. **(B)** A straight diamond splitting disc bur with a low speed motor and constant water cooling was employed for removing the roots.

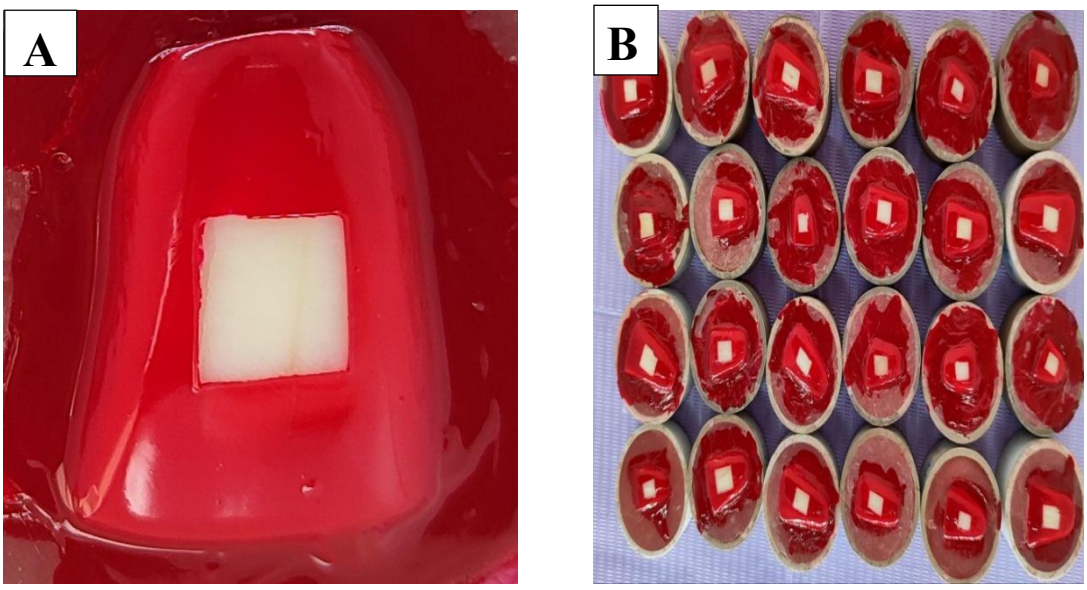


Figure (3): (A) and (B) A window revealing the enamel surface following the removal of the adhesive tape and hardening of the nail polish.

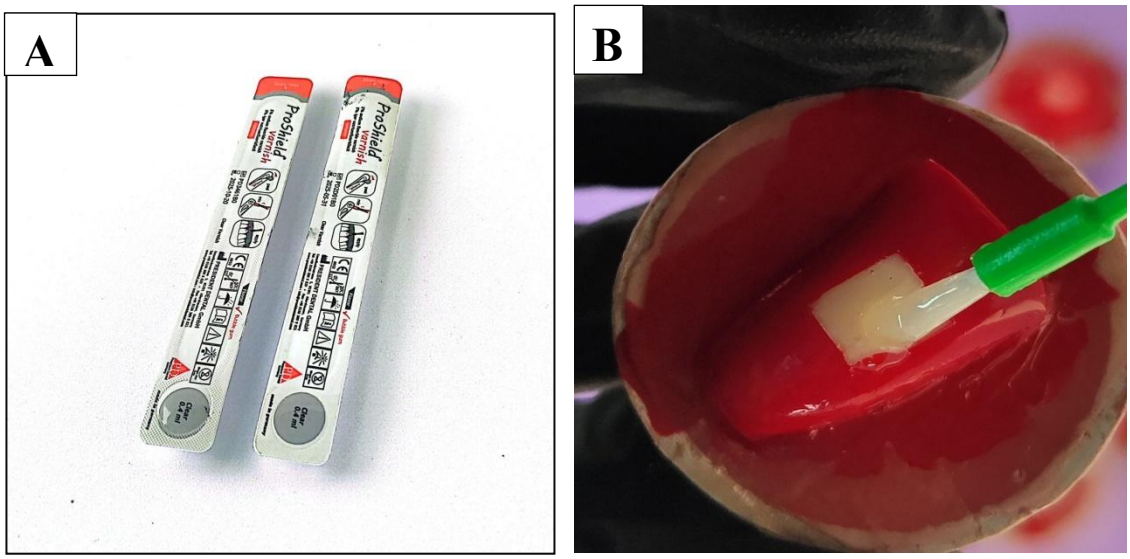


Figure (4): (A**)** 5% Proshield sodium fluoride varnish (President Dental, Germany), **(B)** Samples were coated with a thin layer of varnish using a fine brush for 6 hours.

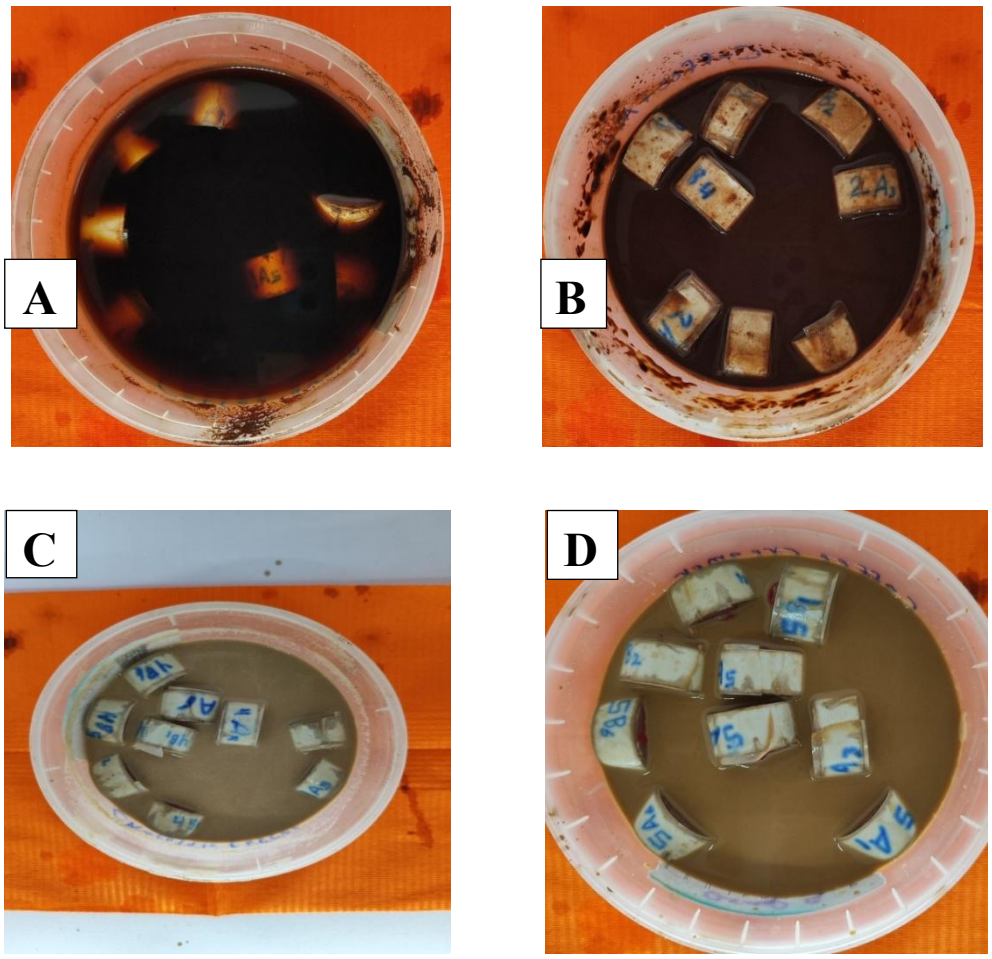


Figure (5): samples were immersed in different plastic containers that contained (A) pure coffee, (B) coffee milk, (C) chocolate coffee, and (D) coffee creamer.

Figure (6): (A) PH meter device pen type, (B) digital thermometer device

Figure (7): Spectrophotometer Vita Easyshade 5, Germany.

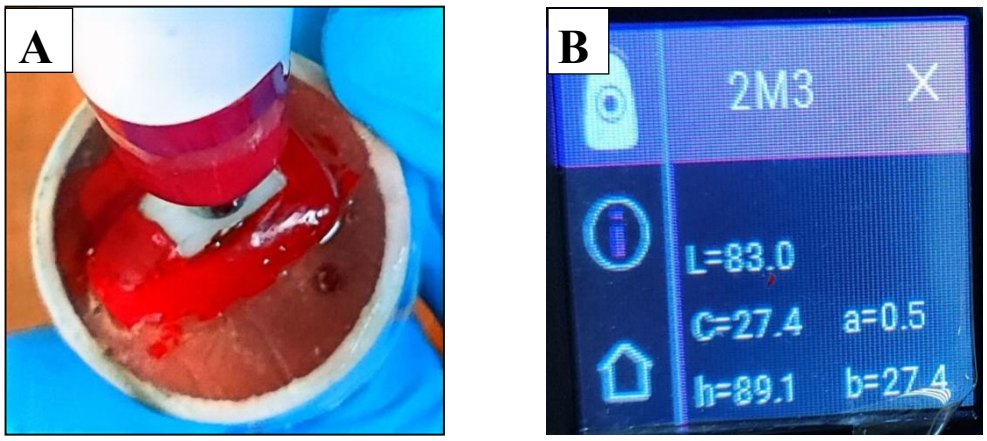
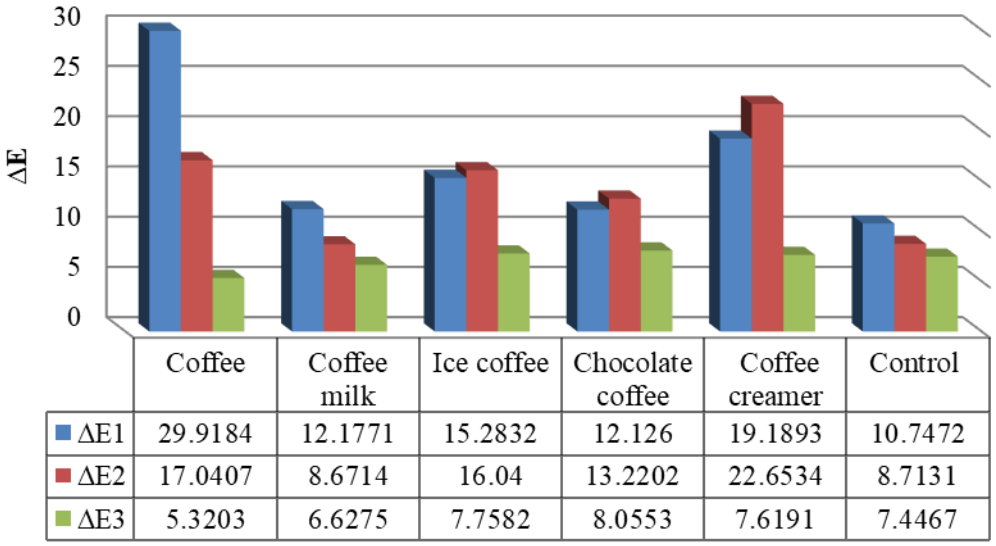


Figure (8): **(A)** The aperture was positioned perpendicular to the surface of the tooth, **(B)** Vita Easyshade screen with CIE LAB readings.



	Coffee	Coffee milk	Ice coffee	Chocolate coffee	Coffee creamer	Control
ΔE1	29.9184	12.1771	15.2832	12.126	19.1893	10.7472
ΔE2	17.0407	8.6714	16.04	13.2202	22.6534	8.7131
ΔE3	5.3203	6.6275	7.7582	8.0553	7.6191	7.4467

Figure (9): Indicates the ΔE results for all study groups since ΔE1 = $\Delta E_{\text{after coffee}}$ - $\Delta E_{\text{baseline}}$ for A study subgroups (WOFV), ΔE2 = $\Delta E_{\text{after coffee}}$- $\Delta E_{\text{baseline}}$ for B study subgroups (WVF) and ΔE3 = $\Delta E_{\text{after fluoride}}$ - $\Delta E_{\text{baseline}}$ for B study subgroups (WFV).

Table (1): Results of Shapiro-Wilk test for ΔE1

ΔE1	Shapiro-Wilk		
	Statistic	df	Sig.
Coffee	.982	6	.963
Coffee milk	.919	6	.497
Ice coffee	.881	6	.276
Chocolate coffee	.938	6	.646
Coffee creamer	.700	6	.006
control	.990	6	.988

Table (2): Results of Shapiro-Wilk test for ΔE2

ΔE2	Shapiro-Wilk		
	Statistic	df	Sig.
Coffee	.954	6	.770
Coffee milk	.964	6	.849
Ice coffee	.937	6	.636
Chocolate coffee	.940	6	.660
Coffee creamer	.917	6	.483
Control	.925	6	.543

Table (3): Results of Shapiro-Wilk test for ΔE3

ΔE3	Shapiro-Wilk		
	Statistic	df	Sig.
Coffee	.860	6	.189
Coffee milk	.971	6	.900
Ice coffee	.920	6	.504
Chocolate coffee	.904	6	.401
Coffee creamer	.934	6	.615
Control	.897	6	.357

Table (4): Result of Two-Way ANOVA test between ΔE1, ΔE2, and ΔE3

	Type III Sum of Squares	df	Mean Square	F	Sig.
ΔE(ΔE1,ΔE2 and ΔE3)	1756.692	2	878.346	58.914	.000
Study groups	1182.678	5	236.536	15.865	.000
ΔE * study groups	1271.903	10	127.190	8.531	.000

Table (5): Result of standard deviation, mean values and Duncan's Multiple Range test

		ΔE1	ΔE2	ΔE3
Coffee	Mean	29.9184 a	17.0407 cd	5.3203 i
	N	6	6	6
	Std. Deviation	8.66971	8.44847	3.05563
Coffee milk	Mean	12.1771 d-g	8.6714 f-i	6.6275 hi
	N	6	6	6
	Std. Deviation	2.28019	3.18191	2.12915
Ice coffee	Mean	15.2832 cde	16.0400 cd	7.7582 ghi
	N	6	6	6
	Std. Deviation	3.24607	3.77729	2.02895
Chocolate coffee	Mean	12.1260 d-g	13.2202 def	8.0553 ghi
	N	6	6	6
	Std. Deviation	4.00084	2.25708	1.15142
Coffee creamer	Mean	19.1893 bc	22.6534 b	7.6191 ghi
	N	6	6	6

	Std. Deviation	4.10627	1.85657	2.29676
Control	Mean	10.7472 e-h	8.7131 f-i	7.4467 ghi
	N	6	6	6
	Std. Deviation	1.84727	2.78870	2.12226

Statistically significant differences within the same column (Vertically) represented by Different small letters N: Number of the specimens.

Table (6): Result of independent samples t-test of ΔE for all study groups after coffee treatment

	Color Change	N	Mean	t-value	sig	Std. Deviation	Std. Error Mean
Coffee	ΔE1	6	29.9184	2.606	.026	8.66971	3.53940
	ΔE2	6	17.0407	2.606	.026	8.44847	3.44907
Coffee milk	ΔE1	6	12.1771	2.194	.053	2.28019	.93089
	ΔE2	6	8.6714	2.194	.056	3.18191	1.29901
Ice coffee	ΔE1	6	18.7462	.586	.571	10.66712	4.35484
	ΔE2	6	16.0400	.586	.579	3.77729	1.54207
Chocolate coffee	ΔE1	6	12.9761	-.148-	.885	3.35381	1.36919
	ΔE2	6	13.2202	-.148-	.886	2.25708	.92145
Coffee creamer	ΔE1	6	19.1893	-1.883-	.089	4.10627	1.67638
	ΔE2	6	22.6534	-1.883-	.102	1.85657	.75794
Control	ΔE1	6	10.7472	1.490	.167	1.84727	.75414
	ΔE2	6	8.7131	1.490	.172	2.78870	1.13848

Table (7): Determination of pH and temperature values of different study solutions during immersion cycles

Type of immersion solution	pH	Temperature at starting of immersion	Temperature at ending of immersion (after 30 min)
Coffee (alameed coffee)	4.4	80 °C	40 °C
Coffee + milk	6.4	80 °C	40 °C
Ice coffee	6.09	10 °C	20 °C
Chocolate coffee	6.2	80 °C	40 °C
Coffee creamer	5.7	80 °C	40 °C
Distil water	7	36 °C	40 °C

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