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ORIGINAL RESEARCH ARTICLE

The Effect of a Microencapsulated Iron Supplement, Hydrogen Peroxide Superior and Professional Dental Prophylaxis on the Color and Whiteness of Teeth

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Abstract

Purpose: To investigate the effect of a microencapsulated liposomal nano-iron supplement, a Fe^{+2} supplement, black tea, a bleaching agent with a new composition and professional dental prophylaxis (PDP) on tooth color and whiteness change.
Materials and Methods: 84 extracted teeth were immersed in liposomal iron, Fe^{+2} and black tea solutions by special apparatus. Then different applications were applied: (1) no treatment, (2) water polishing, (3) PDP, (4) bleaching with 25% hydrogen peroxide superior (HPS). Measurements were taken at baseline, the 2nd, 4th, and 8th weeks, as well as after the applications. Statistical analysis of color and whiteness changes was performed by ANOVA and Bonferroni tests (α=0.05).
Results: The effects of time, application, beverage and their interactions on color and whiteness were statistically significant ($p<0.05$). At week 8 and after application, the highest color change was observed in the black tea-HPS pair (ΔE_{00}=3.38, ΔE_{00}=2.21, respectively) ($p<0.001$). At week 8, the highest darkening (ΔWI_D=-4.67) and at after application, the highest whitening (ΔWI_D=3.01) was caused by the black tea-HPS pair ($p<0.001$).
Conclusions: Black tea, Fe^{+2} and liposomal iron supplements increased the color change of the teeth towards black over time. Black tea caused the highest darkening of the teeth. The order of magnitude was black tea>Fe^{+2}>liposomal iron ($p<0.001$). Hydrogen peroxide superior and PDP provided an effect above the clinical perceptibility threshold selected in this study in terms of color recovery of darkened teeth. The order of magnitude was hydrogen peroxide superior>PDP.

Keywords: Color; Hydrogen peroxide superior; Iron supplements; Professional dental prophylaxis; Whiteness

Introduction

Tooth esthetics and color are crucial for many individuals. However, tooth color may change over time due to internal or external factors. Beverages or iron supplements (ISs) may lead to external discoloration by forming colored regions in the pellicle on the enamel surface.[1]

Iron deficiency (ID) is the most common micronutrient deficiency found worldwide, with at least 20 percent of women experiencing this deficiency during their reproductive life. Approximately 75% of anemias are due to ID. The World Health Organisation (WHO) has targeted a 50% reduction of anemia in women in reproductive years as one of its global nutrition goals for 2025.[2] ID can be overcome by taking ISs. Fe^{+2} and Fe^{+3} salts are often used for this purpose. However, ISs can cause change in tooth color. Due to the fact that iron causes a color change towards black, ISs which have different technologies have been tried to be produced.[3]

The logical way to prevent iron-caused color change in teeth is to prevent direct contact of iron with tooth surface. Encapsulation technology that provides controlled release of the content may help in this regard. Various materials can be used for capsule production. Liposomes are the materials recommended for this purpose. Liposomal phospholipids are microscopic vesicles consisting of a double layer and can be encapsulated to increase the efficacy of drugs.[4] Studies on the effect of iron particles converted into nanoparticles by the micronization process and encapsulated in capsules on the color of teeth are quite insufficient.[5]

Bleaching is one of the most commonly used methods to recover the color of teethchanged by beverages and is usually performed by applying 30-35% hydrogen peroxide (HP) by the dentist in the

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clinic or 10-16% carbamide peroxide (CP) by the patient at home.[6] Hydrogen Peroxide Superior (HPS) gel is a new gel which is being used today. The manufacturer reports that this product, which contains 25% HP, has a unique composition that has the beneficial properties of carbamide peroxide (CP) or polyvinyl pyrrolidone peroxide and thus bleaching can be performed in a short time. It is also claimed that the power of bleaching is higher due to the viscosity controlling poloxamer in the content. A special pen brush has been developed for the use of this product and it is claimed that this is the only product that bleaches using a pen.[7,8] However, no study was found that examined the effect of 25% HPS pen on teeth discolored by beverages or ISs.

Another method for restoring the color of teeth is regularly polishing the teeth by dentists. For this procedure, which is defined as professional dental prophylaxis (PDP), special prophylaxis pastes are used. Various PDP pastes have been introduced to the market in recent years and current research is needed on these new generation pastes having low relative dentin abrasiveness (RDA).[9]

The color values of teeth can be described by L*, a*, b* color space parameters of the International Commission on Illumination (CIE). The CIEDE2000 color difference formula (ΔE_{00}), calculated by using these parameters, is the current recommendation of the CIE.[10] The whiteness index formula (WI_D) is a new formula proposed for the study of tooth bleaching and is based on the distance from a nominal white point in the L*, a* ,b* color space, represented as L*=100, a*=0 and b*=0. ΔE_{00} provides information on the magnitude of the color change, but not on the direction in which the change diverges from the nominal white point. Therefore, in this study, the color change caused by beverages and applications was evaluated by using ΔE_{00} and ΔWI_D formulas combined.[11]

For the aforementioned reasons, the aim of this study was to investigate the effect of Fe^{+2} and liposomal iron supplements and black tea on the color and whiteness change of teeth and a fine-grained PDP paste and an HPS bleaching pen on the recovery of color and whiteness in discolored teeth. The null hypotheses were that (1) beverages and (2) applications would not cause changes in tooth color and whiteness.

Material and Methods

The study protocol including human research specimens was approved by Ethics Committee of the Faculty of Dentistry, Ankara University (Number: 36290600/13/2024, Date: 11.03.2024). A power analysis (G*Power ver. 3.1.9.7; Heinrich-Heine-Universität Düsseldorf, Germany) was performed to ensure a sampling size that produced a significant group effect. Using a significance level (alpha) of 0.05, power of 0.80 and a moderate group effect size (partial eta squared) of 0.06, the minimum sampling size was calculated as 5 specimens in each application group and 60 specimens in total. Considering potential sample loss during the research process, the minimum sample size was set at 7and the total sampling size at 84.[12,13]

The 84 teeth were randomly allocated into 3 groups (n=28) according to the beverages in which they would be kept: (1) Fe^{+2} solution (Bestiron Plus; Valens İlaç, Turkey) (IR2), (2) liposomal iron solution (Newvit Iron Syruo; RC Farma, Turkey) (LPI), (3) black tea (Lipton Earl Grey Tea Bag; Unilever, Turkey) (TEA). TEA was set as a positive control for comparison with iron solutions. The pH levels of the beverages were measured using a pH meter (SevenExcellence, Mettler-Toledo; Oregon, USA). Each group was divided into groups of 4 (n=7) according to the applications to be applied after the waiting period in the beverages: (1) Waiting in distilled water without treatment (negative control) (NOT), (2) Polishing with water (positive control) (PWW), (3) Professional dental prophylaxis (PDP), (4) Hydrogen Peroxide Superior (HPS). The materials used in the study are shown in Table 1 and the design of the study is shown in Figure 1.

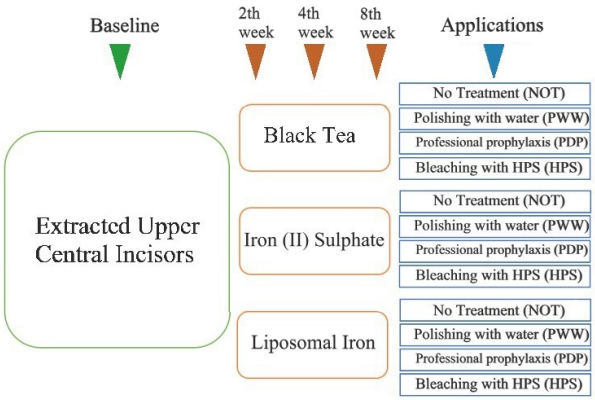

Figure 1. The study design

Extracted teeth that had been stored in distilled water at 4°C for a maximum of 1 month were included in the study. Human maxillary anterior incisors, extracted for periodontal reasons and free of hypocalcification and caries, were carefully cleaned of calculus and other debris. A quantitative light source digital camera (Stemi305; ZEISS, Germany) was used to select suitable teeth.

The root of each tooth was cut 2 mm below the enamel-cement border under water cooling. The pulp chambers of the teeth were sealed apically with a flowable resin composite (Aelite Flo; Bisco, Schaumburg, USA) after abrasion and rinsing to prevent the solution from entering through the roots. The teeth were attached to specially prepared retainers. The spindle at the top of the retainer had a feature that pointed to the center of the enamel-cement border of the tooth. The containers had a volume of 5 mL. To provide a close-to-natural color measurement, black colored immersion cups were used.[12] The immersion cup of each specimen was coded with the label of that specimen.

Previous studies have reported that the recommended daily dose for adults with anemia is between 15-20 mg. A dosing period of at least 6 months is recommended to overcome ID and to fully replenish existing stores with iron.[14] The daily dose required for IR2 and LPI is approximately 4-6 mL. Specimens were immersed in the containers for 1 min, 3 times a day, at 8 h intervals[15] (Figure 2). After each immersion, the specimens were washed and dried and placed in an artificial saliva solution (Artificial Saliva Solution; Colin Kimya, Turkey) at 37 °C to simulate the oral environment. This solution has a pH of 6.8 and meets the standard DN 53160-1, which is required for studies examining the effect of dental materials in saliva. The process was continued for 2 months to simulate 6-month iron intake.[16] The specimens in the TEA group were kept in the beverage for 2.5 hours per day. The specimens were washed and dried after each immersion and kept in artificial saliva at 37°C. To prepare the TEA, 3.2 g of tea bags were added to 250 mL of boiled water and allowed to infuse for 10 min. The beverage was replaced daily. The process continued for 2 months. Taking into account that a 24-hour immersion procedure simulates a consumption of approximately 1 month, it can be reported that a 2-month intermittent immersion procedure simulates a 6-month consumption.[17]

After the immersion process was completed, the application process was initiated. The specimens in the NOT group were not treated but were kept in distilled water to prevent the teeth from drying out. Specimens in the PWT group were polished under distilled water at 2500 rpm using a dental contra-angle handpiece (CrossPro; Anthogyr SA, France) with a latex-free rubber brush (Pro-Cup light blue; Kerr, Germany) attached. The PDP procedure is performed up to 4 times a year and takes approximately 20 s to polish one surface of the tooth. Therefore, the application for each specimen in the PDP group lasted for 1.4 min to simulate 1 year.[18] The PDP paste was used in a circular motion with a drop of distilled

Table 1. Materials used in the study

Name	Code	Type	Composition	Manufacturer
Bestiron Plus	LPI	Micro-encapsulated iron	16 mg iron (2.5 mL), deionised water, ferric pyrphosphate	Valens İlaç, İstanbul, Turkey
Newvit Iron Syrup	IR2	+2 valence ferrous bisglycinate	16 mg Fe^{+2} sulphate (5 mL)	RC Farma, İstanbul, Turkey
Lipton Earl Grey Tea Bag	TEA	Black tea	Black tea	Unilever, İstanbul, Turkey
Cavex Bite&White In-Office	HPS	Office bleaching gel	25% hydrogen peroxide superior, thickeners, pH regulators,reversible poloxamer, glycerine	Cavex Holland BV, Haarlem, Netherlands
Proxyt Fine	PDP	Fine-grain professional prophylaxis paste	Silica (RDA 7 size particles), water, glycerine, sorbite, xylit, anorganic fillers, natriumfluoride, flavor and pigments	Ivoclar Vivadent, Schaan, Liechtenstein

Figure 2. Immersion of specimens in LPI

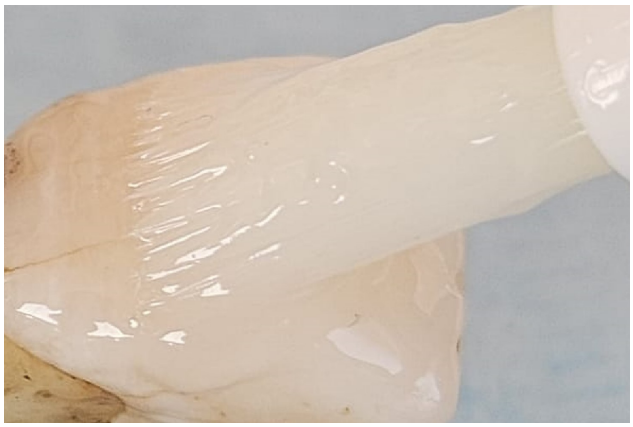

Figure 3. Application of the HPS agent

water. In the HPS group, the gel was applied to the middle region of the teeth using the bleaching pen provided by the manufacturer and the entire labial surface was covered with a thickness of approximately 2 mm (Figure 3). The gel was left on the teeth for 45 minutes, the maximum holding time specified by the manufacturer. [19]

Color change measurements were calculated for a total of 4 time points (TP): TP 1: between BAS-2th week; TP 2: between BAS-4th week; TP 3: between BAS-8th week; TP 4: between 8th week and after application. Color measurements were performed using a spectrophotometer (Vita Easyshade V; VITA Zahnfabrik, Germany). Three measurements were taken from the center of the buccal surface of each specimen with the spindle tip as a reference, the average of which was calculated and recorded. The color change was calculated by the following formula including the parameters L*, a*, and b* [20]:

$$\Delta E_{00}=[(\frac{\Delta L'}{K_L S_L})^2+(\frac{\Delta C'}{K_C S_C})^2+(\frac{\Delta H'}{K_H S_H})^2+R_T(\frac{\Delta C'}{K_C S_C})(\frac{\Delta H'}{K_H S_H})]^{1/2}$$

The whiteness index was calculated by the following formula: WI_D=0.511L-2.324a-1.100b. Then, whiteness index changes were calculated with the following formula [11]: $\Delta WI_D=WID_{treatment}-WID_{baseline}$. Whiteness change measurements were calculated for a total of 3 TPs: TP 1: between BAS-2th week; TP 3: between BAS-8th week; TP 4: between 8th week and after application.

Statistical analyses of TPs were performed using a statistical software package (IBM SPSS Statistics 29.0.2.0; IBM Corp, Chicago, USA). The effects of time (TIME), beverage (BEV) and application (APP) variables on ΔE_{00} and ΔWI_D were analyzed using 3-way ANOVA and Bonferroni tests. The statistical significance level was set at p<0.05.

Results

The ANOVA results of ΔE_{00} are shown in Table 2. The effect of TIME, APP, BEV, TIME-APP, TIME-BEV, APP-BEV and TIME-APP-BEV on ΔE_{00} was statistically significant (p<0.001).

Bonferroni results for ΔE_{00} are shown in Table 3. In TP 1, the lowest ΔE_{00} was in the specimens immersed in LPI in the PWW group (p=0.031). In TP 2, the lowest ΔE_{00} was also in the specimens immersed in LPI in the PWW group (p<0.001). In TP 3, the highest ΔE_{00} was in the specimens immersed in TEA in the PWW group (p<0.001) and the lowest was in the specimens immersed in LPI in the PDP group (p<0.001). In TP 4, the lowest ΔE_{00} was in the specimens immersed in LPI and in the NOT group (p=0.003), and the highest was in the specimens immersed in TEA and in the HPS group (p<0.001).

When analyzing the APP-BEV pair, the highest color change occurred in the following order: HPS>PDP> PWW > NOT for applications and TEA > IR2 > LPI for beverages (p<0.001) (Figure 4). When the TIME-BEV pair were examined, the highest color change for BEV was TEA>IR2>LPI at the TP 3 (p<0.001) (Figure 5). The ΔE_{00} value exceeded the 2 level at TP 3 but dropped below this level at TP 4 (p<0.001). Analyzing the TIME-APP pair, the order of magnitude of ΔE_{00} provided by the APPs was HPS>PDP>PWW>NOT (p<0.001). HPS provided a color change above 1.5, while PWW and NOT provided a color change below 1.0 (p<0.001) (Figure 6).

The ANOVA results of ΔWI_D are shown in Table 4. The effect of TIME, APP, BEV, TIME-APP, TIME-BEV, APP-BEV and TIME-APP-BEV on ΔWI_D was statistically significant (p<0.001).

Bonferroni results for ΔWI_D are shown in Table 5. In TP 1, the highest ΔWI_D was in specimens immersed in LPI in the PWW group

Table 2. Results of 3-way ANOVA of ΔE_{00}

Source	Type III Sum of Squares	df	Mean Square		p-value	Partial Eta Squared
Corrected Model	362.135[a]	47	7.705	593.780	<0.001	0.990
Intercept	563.688	1	563.688	43440.147	<0.001	0.993
TIME	300.708	3	100.236	7724.606	<0.001	0.988
APP	7.239	3	2.413	185.945	<0.001	0.660
BEV	21.781	2	10.890	839.259	<0.001	0.854
TIME-APP	18.215	9	2.024	155.973	<0.001	0.830
TIME-BEV	9.722	6	1.620	124.874	<0.001	0.722
APP-BEV	0.288	6	0.048	3.700	0.001	0.072
TIME-APP-BEV	4.182	18	0.232	17.905	<0.001	0.528
Error	3.737	288	0.013			
Total	929.560	336				
Corrected Total	365,872	335				

[a] R Squared = 0.990 (Adjusted R Squared = 0.988)

Table 3. Bonferroni results of ΔE_{00}

ΔE_{00}		TEA	IR2	LPI	Total
TP 1	NOT	0,17 (0,07)	0,11 (0,06)	0,21 (0,06)	0,16 (0,07)
	PWW	0,24 (0,09)^{A}	0,20 (0,05)^{B}	0,09 (0,06)^{A}	0,17 (0,09)
	PDP	0,22 (0,07)	0,17(0.07)	0.11 (0.09)	0.17 (0.09)
	HPS	0.15 (0.05)	0.11 (0.06)	0.10(0.05)	0.12 (0.06)
	Total	0.20 (0.81)	0.15 (0.07)	0.12 (0.85)	0.15 (0.08)
TP 2	NOT	1.50 (0.08)^{A,a}	1.38 (0.06)^{A,a}	0.58 (0.15)^{B,a}	1.15 (0.42)^{a}
	PWW	1.50 (0.12)^{A,b}	1.32 (0.09)^{A,ab}	0.75 (0.12)^{B,b}	0.12 (0.29)^{a}
	PDP	1.50 (0.08)^{A,a}	1.20 (0.08)^{B,b}	1.08 (0.13)^{B,c}	1.26 (0.20)^{b}
	HPS	1.38 (0.06)^{A,ab}	1.17 (0.34)^{B,b}	1.21 (0.06)^{B,c}	1.25 (0.21)^{b}
	Total	1.42 (0.12)^{A}	1.27 (0.19)^{B}	0.91 (0.28)^{C}	1.2 (0.30)
TP 3	NOT	3.42 (0.11)^{A,ab}	2.78 (0.10)^{B,ab}	2.05 (0.09)^{C,a}	2.75 (0.58)
	PWW	3.50 (0.14)^{A,a}	2.65 (0.09)^{B,a}	2.31 (0.03)^{C,b}	2.82 (0.51)
	PDP	3.30 (0.16)^{A,b}	2.84 (0.18)^{B,b}	2.17 (0.07)^{C,ab}	2.77 (0.49)
	HPS	3.38 (0.10)^{A,ab}	2.75 (0.09)^{B,ab}	2.20 (0.10)^{C,ab}	2.20 (0.10)
	Total	3.40 (0.14)^{A}	2.76 (0.13)^{B}	2.18 (0.12)^{C}	2.78 (0.50)
TP 4	NOT	0.38 (0.14)^{A,a}	0.40 (0.08)^{A,a}	0.18 (0.06)^{B,a}	0.18 (0.06)^{B,a}
	PWW	0.88 (0.19)^{A,b}	0.78 (0.15)^{A,b}	0.41 (0.09)^{B,b}	0.41 (0.09)^{B,b}
	PDP	1.92 (0.09)^{A,c}	1.57 (0.11)^{B,c}	0.95 (0.07)^{C,c}	0.95 (0.07)^{C,c}
	HPS	2.21 (0.06)^{A,d}	1.61 (0.10)^{B,c}	1.10 (0.11)^{C,c}	1.10 (0.11)^{C,d}
	Total	1.35 (0.77)^{A}	1.09 (0.53)^{B}	0.66 (0.39)^{C}	1.03 (0.64)
Total	NOT	1.37 (1.31)^{A,a}	1.17 (1.06)^{B,a}	0.76 (0.78)^{C,a}	1.10 (1.09)^{a}
	PWW	1.48 (1.25)^{A,b}	1.24 (0.93)^{B,b}	0.89 (0.87)^{C,b}	1.20 (1.04)^{b}
	PDP	1.73 (1.12)^{A,c}	1.44 (0.97)^{B,b}	1.08 (0.75)^{C,c}	1.42 (0.98)^{c}
	HPS	1.78 (1.12)^{A,c}	1.41 (0.98)^{B,c}	1.15 (0.76)^{C,c}	1.45 (1.02)^{c}
	Total	1.59 (1.22)^{A}	1.31 (0.98)^{B}	0.97 (0.79)^{C}	1.29 (1.04)

Different letters in upper case in row and lower case in column indicate statistically significant difference ($p<0.05$).

Table 4. Results of 3-way ANOVA of ΔWI_D

Source	Type III Sum of Squares	df	Mean Square	F	p-value	Partial Eta Squared
Corrected Model	1154.962[a]	35	32.999	304.283	<0.001	0.980
Intercept	179.765	1	179.765	1657.616	<0.001	0.885
TIME	1012.586	2	506.293	4668.528	<0.001	0.977
APP	16.872	3	5.624	51.858	<0.001	0.419
BEV	8.126	2	4.063	37.463	<0.001	0.258
TIME-APP	35.480	6	5.913	54.527	<0.001	0.602
TIME-BEV	73.090	4	18.272	168.490	<0.001	0.757
APP-BEV	1.989	6	0.331	3.056	0.007	0.078
TIME-APP-BEV	6.820	12	0.568	5.241	<0.001	0.225
Error	23.425	216	0.108			
Total	1358.153	252				
Corrected Total	1178.387	251				

[a] R Squared = 0.980 (Adjusted R Squared = 0.977)

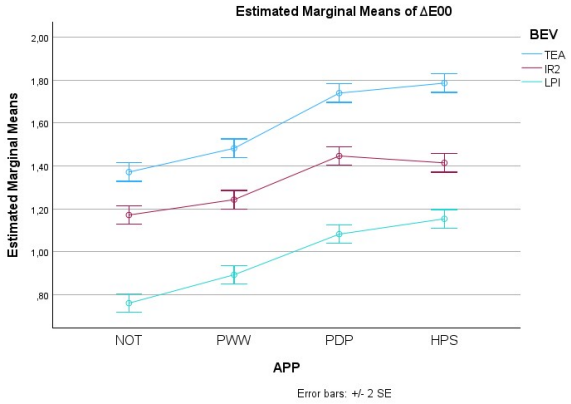

Figure 4. ΔE_{00} results for APP-BEV pair

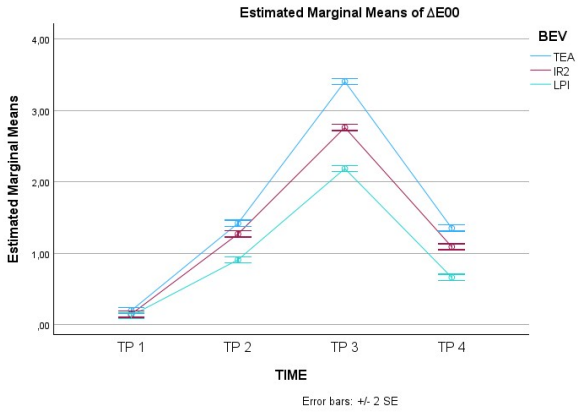

Figure 5. ΔE_{00} results for TIME-BEV pair

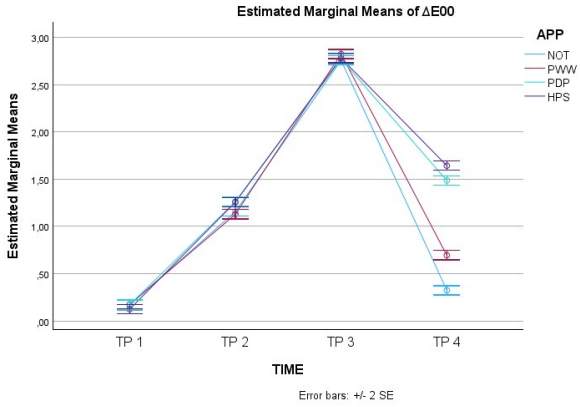

Figure 6. ΔE_{00} results for TIME-APP pair

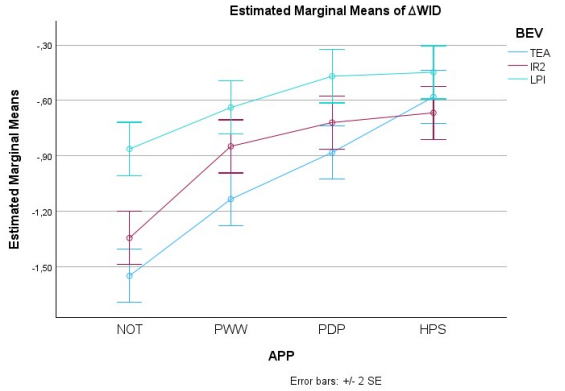

Figure 7. ΔWI_D results for APP-BEV pair

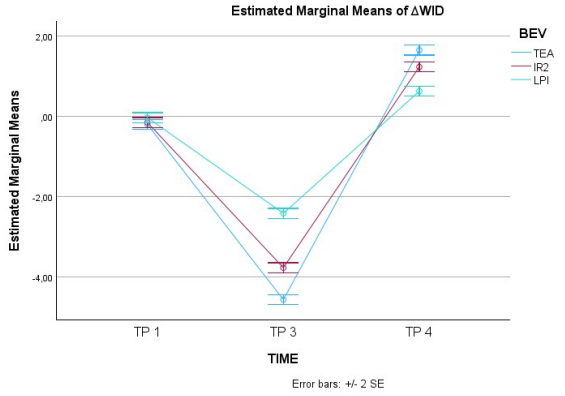

Figure 8. ΔWI_D results for TIME-BEV pair

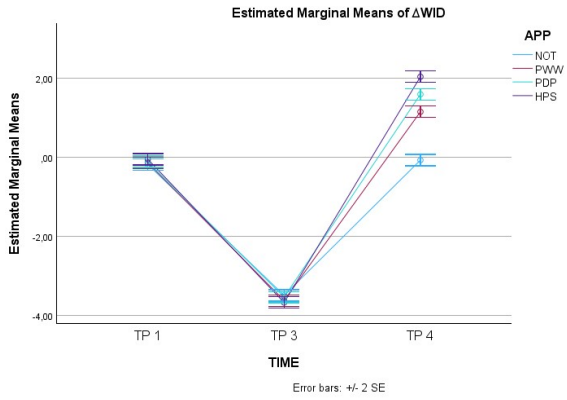

Figure 9. ΔWI_D results for TIME-APP pair

(p>0.05). The values were negative in the other specimens and the highest negative value was in the specimens immersed in TEA in the PWW group (p>0.05). In TP 3, the highest negative value was in the specimens immersed in TEA in the PWW group and the nearest to positive value was in the specimens immersed in LPI in the PDP group (p<0.001). In TP 4, the lowest value was in specimens immersed in TEA in the NOT group (p>0.05) and the highest value was in specimens immersed in TEA in the HPS group (p<0.001).

When the APP-BEV pair was analyzed, the magnitude of the increase from negative to positive values after APP was LPI>TEA>IR2 (p<0.001) (Figure 7). When the TIME-BEV pair was analyzed, ΔWI_D in the specimens immersed in TEA decreased to the lowest negative value at TP 3 and increased to the highest positive value at TP 4 in the opposite direction (p<0.001) (Figure 8). The order of magnitude at TP 4 was TEA>IRO2>LPI (p<0.001). When the TIME-APP pair was analyzed, ΔWI_D decreased to below -2.0 at TP 3 and increased again at TP 4 (p<0.001) (Figure 9). With NOT, the ΔWI_D was as high as 0.0, while with HPS the ΔWI_D was as high as 2.0 (p<0.001).

Discussion

The International Organization for Standardization (ISO) recommended the use of 50:50 perceptibility and acceptability thresholds (PT and AT) for the interpretation of color results. In this study, PT/AT was 0.8/1.8 for ΔE_{00} and 0.72/2.60 for ΔWI_D.[11] According

Table 5. Bonferroni results of ΔWI_D

ΔWI_D		TEA	IR2	LPI	Total
TP 1	NOT	-0.25 (0.97)	-0.18 (0.69)	-0.11 (0.69)	-0.11 (0.69)
	PWW	-0.27 (0.75)	-0.20 (0.05)	0.04 (0.06)	0.04 (0.06)
	PDP	-0.17 (0.75)	-0.10 (0.12)	-0.08 (0.10)	-0.08 (0.10)
	HPS	-0,08 (0.06)	-0.11 (0.10)	-0.04 (0.09)	-0.04 (0.09)
	Total	-0.19 (0,10)	-0.15 (0.10)	-0.02 (0.11)	-0.12 (0.12)
TP 3	NOT	$-4.27\ (1.67)^{A}$	$-3.75\ (0.15)^{B}$	$-2.44\ (0.09)^{C}$	$-2.44\ (0.09)^{C}$
	PWW	$-4.70\ (0.20)^{A}$	$-3.75\ (0.09)^{B}$	$-3.78\ (0.08)^{C}$	$-3.78\ (0.08)^{C}$
	PDP	$-4.60\ (0.08)^{A}$	$-3.78\ (0.14)^{B}$	$-2.22\ (0.87)^{C}$	$-2.22\ (0.87)^{C}$
	HPS	$-4.67\ (0.07)^{A}$	$-3.75\ (0.09)^{B}$	$-2.58\ (0.10)^{C}$	$-2.58\ (0.10)^{C}$
	Total	$-4.56\ (0.81)^{A}$	$-3.76\ (0.11)^{B}$	$-2.41\ (0.43)^{C}$	-3.57 (1.03)
TP 4	NOT	$-0.11\ (0.10)^{a}$	$-0.08\ (0.06)^{a}$	$-0.02\ (0.09)^{a}$	$-0.02\ (0.09)^{a}$
	PWW	$1.57\ (0.09)^{A,b}$	$1.41\ (0.10)^{A,b}$	$0.44\ (0.05)^{B,b}$	$0.44\ (0.05)^{B,b}$
	PDP	$2.12\ (0.09)^{A,c}$	$1.72\ (0.11)^{A,b}$	$0.90\ (0.08)^{B,c}$	$0.90\ (0.08)^{B,c}$
	HPS	$3.01\ (0.08)^{A,d}$	$1.87\ (0.09)^{B,b}$	$1.20\ (0.08)^{C,c}$	$1.20\ (0.08)^{C,d}$
	Total	$1.65\ (1.16)^{A}$	$1.23\ (0.79)^{B}$	$0.62\ (0.47)^{C}$	1.17 (0.94)
Total	NOT	$-1.15\ (2.17)^{A}$	$-1.34\ (1.75)^{A}$	$-0.86\ (1.14)^{B}$	$-1.25\ (1.74)^{a}$
	PWW	$-1.13\ (2.70)^{A}$	$-0.84\ (2.21)^{B}$	$-0.63\ (1.28)^{B}$	$-0.87\ (2.12)^{b}$
	PDP	$-0.88\ (2.86)^{A}$	$-0.71\ (2.35)^{A}$	$-0.46\ (1.41)^{B}$	$-0.68\ (2.26)^{b}$
	HPS	-0.58 (3.23)	-0.66 (2.39)	-0.44 (1.62)	$-0.56\ (2.46)^{c}$
	Total	$-1.03\ (2.74)^{A}$	$-0.89\ (2.16)^{B}$	$-0.60\ (1.36)^{C}$	-0.84 (2.16)

Different letters in upper case in row and lower case in column indicate statistically significant difference ($p<0.05$).

to the findings, the first null hypothesis that beverages would not cause color and whiteness changes in teeth was rejected. In TP 1, ΔE_{00} did not exceed the PT for any APP-BEV pair. In TP 2, ΔE_{00} was between the PT and AT for all pairs except NOT-LPI and PWW-LPI. In TP 3, ΔE_{00} exceeded AT for all time pairs. In TP 4, the AT was exceeded for PDP-TEA and HPS-TEA pairs, but not for NOT-TEA, NOT-IR 2, NOT-LPI, PWW-IR2, and PWW-LPI pairs. For PWW-TEA, PDP-IR2, PDP-LPI, HPS-IR2, and HPS-LPI pairs, ΔE_{00} was between PT and AT.

When the results were examined regardless of time point, the highest color change was for the TEA-HPS pair (ΔE_{00}=1.78) and the lowest for the LPI-NOT pair (ΔE_{00}=0.76). When the results were examined regardless of time point and application, the highest color change was for TEA (ΔE_{00}=1.59) and the lowest for LPI (ΔE_{00}=0.97). When the results were examined regardless of beverage, the highest color change was for the TP3-PWW pair (ΔE_{00}=2.82) and the lowest for the TP1-HPS and TP2-PWW pair (ΔE_{00}=0.12). When the results were examined regardless of application and beverage, the highest color change was for TP3 (ΔE_{00}=2.78) and the lowest for TP1 (ΔE_{00}=0.15).

Spectrophotometers are the most commonly used devices for color measurement today. These devices are highly accurate and can be used for the long-term without sensitivity to object metamerism. [21] For these reasons, the Vita Easyshade V was used in this study. However, to eliminate limiting environmental conditions and to ensure standardization between multiple measurements, the tooth specimens were placed in custom-made holders.

The majority of studies investigating the effect of beverages on the color of teeth have been for beverages such as tea, coffee and wine. [22] There are limited studies on the effect of ISs on tooth color. However, iron is one of the essential elements required by the human body and ID is the most common micronutrient deficiency affecting the world population. Food supplements taken to remedy ID can lead to deeply penetrating, permanent color changes in teeth. [3]

Two types of ISs were used in this study. The first was a Fe^{+2} sulfate supplement reported to contain no additional colorant. [23] Nowadays, new ISs have been developed in which iron particles reduced to nanoparticles are encapsulated in liposomal microcapsules. [4] It is claimed that these supplements do not change the color of teeth to a clinically perceptible magnitude. [5] However, the number of studies for LPI supplements was very limited; therefore, this study included an LPI supplement reported to contain no additional colorants as a second IS. [24]

Since the mechanism by which iron causes tooth color change has not yet been fully explained, and since the hypothesis that iron binds to the pellicle was still the most widely accepted theory, [13] the teeth were immersed in artificial saliva for the time intervals between immersion in iron solutions, and were not polished, even though they were rinsed when removed from the dishes.

Some previous studies [4,25,26] have produced results for ISs that are consistent with this study. In a study [4] in which LPI caused a considerable magnitude of color change than IR2, it was reported that the consumption time and acidity of the beverage caused color change. In this study, LPI and IR2 were kept in 5 mL containers for equal periods of time. It can be reported that citric acid increases the magnitude of the color change.This acid may lead to the chelation of calcium, an increase in the solubility of hydroxyapatite and thus, surface degradation. [27] However, acidity alone cannot explain why LPI produces less color change than IR2. In conventional iron supplements, the amount of iron circulating freely in the mouth is higher than in LPIs. [13] The prevention of direct contact of LPI particles to the tooth by microencapsulation technology and the presence of particles smaller than 100 nm in size reduces the possibility of tooth color change. In conventional ISs, free circulating particles bind to compounds such as tannins, leading to the formation of larger coloring compounds.

In this study, even though IR2 caused less color change than TEA, the magnitude of the color change was higher than that of AT, as in a previous study. [26] TEA has low polarity yellow pigments and tannins that bond more strongly to the tooth than IR2 and LPI. Furthermore, in a study, [28] with results consistent with this study, it was reported that the polyphenols of TEA reacted with the cationic salivary membrane to form thick layers of coloring particles.

The ΔE_{00} formula, which is widely used today, gives the magnitude of the color change, but not its direction. The ΔWI_D formula, which is currently used to calculate the direction in which the color changes away from the nominal point L*=100, a*=0, b*=0, has emerged as a new formula suggested by the CIE. [11] In this study, it was found that the ΔWI_D findings were compatible with the ΔE_{00} findings. According to the findings, the second null hypothesis that the applications would not lead to color and whiteness changes in teeth was rejected. In TP 1, ΔWI_D did not exceed the PTfor any APP-BEV pair. In TP 3, ΔWI_D for NOT-LPI, PDP-LPI, and HPS-LPI was between the PT and AT with a negative value. For the other pairs, it exceeded the AT threshold with negative value. In TP 4, for

the other pairs except for the HPS-TEA pair, values between PT and AT with positive values were detected, and for the HPS-TEA pair, values above the AT with positive values were detected.

When the results were examined regardless of time point, the highest whiteness change was for the IR2-NOT pair (ΔWI_D=-1.34) and the lowest for the LPI-HPS pair (ΔWI_D=-0.44). When the results were examined regardless of time point and application, the highest whiteness change was for TEA (ΔWI_D=-1.03) and the lowest for LPI (ΔWI_D=-0.60). When the results were examined regardless of beverage, the highest whiteness change was for TP3-PWW (ΔWI_D=-3.78) and the lowest for TP1-HPS pair (ΔWI_D=-0.04) and TP1-PWW pair (ΔWI_D=0.04). When the results were examined regardless of application and beverage, the highest whiteness change was for TP3 (ΔWI_D=-3.57) and the lowest for TP1 (ΔWI_D=-0.12).

PDP is a polishing procedure used by most dentists to remove color change and plaque deposits after prosthodontic application. Nowadays, new generation PDP pastes with low dentin abrasiveness have been developed and their effects on dental materials should be investigated. [9] However, no study was found on the recovery of the color of teeth discolored by ISs using PDP or alternative polishing/bleaching methods.

In a study using multivitamins, [29] it was reported that the pH level decreased due to the oral environment, resulting in deterioration of the tooth surface and facilitating the adhesion of yellow coloring pigments to the tooth surface. The pH levels of the beverages used for this study were not specified by the manufacturer; therefore, pH levels were measured using a pH meter and the values obtained were 5.5 for TEA, 2.92 for IR2 and 4.6 for LPI. The results showed that the recovery of color by PDP was naturally higher in teeth that discolored more with beverages (TEA>IR2>LPI). Few studies [8,11] were found on the effect of new PDP pastes on color and whiteness change, and the findings of this study were consistent with the mentioned studies. Further research on the new generation of pastes which has a lower RDA than conventional pastes, is necessary for an in-depth study of the subject.

HP, the active component of bleaching agents, is a strong oxidizing agent that generates reactive oxygen species and HP anions. The reactive molecules attack the long-chain dark colored molecules and break them down into smaller, less colored molecules that diffuse to the outside of the tooth. In this study, a new HP component called HPS was used. A limited number of studies [30,31] have previously been carried out on the 6% formulation of HPS and, in agreement with the findings of the present study, this agent was found to be effective for color change. The fact that 25% HPS produced a color and whiteness change between PT and AT for IR2 and LPI, higher than AT for TEA, may be due to its composition, which is different from conventional bleaching agents. In conventional bleaching agents, HP or polyvinyl pyrrolidone peroxide is used. HPS has a new composition that combines the properties of these two agents. Since it is a material with high viscosity, it can stay on the tooth surface for a longer time without flowing, and this is provided by the poloxamer contained in the content. Due to the high temperature and saliva, a reaction begins (water breaks down into oxygen and radicals). Following this, energy is released. The process releases a new energy that repeats the whole process. Thus, a rapid, large bleaching effect occurs. [7,30]

In a previous study, [31] which showed results consistent with this study, it was shown that the color deteriorated by TEA could be recovered by HP. Since TEA has polar colorants, it is easy for HP to react with TEA pigments deposited on the teeth. In addition, bleaching does not only oxidize coloring pigments; it may also affect the inner layers of enamel and dentin. [32] Previous studies [33,34] on the effect of bleaching agents on teeth discolored by beverages have reported that the magnitude of the effect of these agents depends on the application method, agent concentration, and application time. Future research involving ISs is critically needed, as the available evidence is only on commonly consumed beverages such as tea, coffee and wine.

It is expected that the color of teeth with a nice appearance should be preserved its color as much as possible over time. Therefore, it is recommended to consume as little as possible of beverages that have the potential to cause discoloration. However, ISs are important supplements that should be taken in case of ID. Liposomal ISs can cause less color change than conventional Fe^{+2} supplements because of their technology, which reduces contact with the teeth. Therefore, it could be reported as a good alternative for the protection of the appearance of teeth. On the other hand, the findings from this study showed that the HPS with new technology and the fine-grained PDP paste were effective in recovering the discoloration of the teeth. Therefore, HPS and PDP could be the preferred methods for recovering the color of teeth discolored by black tea or ISs.

A limitation of this study is that only two types of IS were used. The relationship betweenpH and the polishing method may also affect the result. In addition, it is not possible to exactly replicate the intraoral condition in a laboratory environment. Therefore, more research is needed on the effect of ISs, new generation PDP paste and new bleaching agents on the color and whiteness of teeth.

Conclusion

The results obtained within the limitations of this study are as follows:

(1) Tooth discoloration after 2 months exceeded the AT, regardless of the beverage in which they were immersed. The whiteness change exceeded the AT for all beverages except liposomal iron supplementation. The order of color and whiteness change was TEA>IR2>LPI2.

(2) PDP and HPS caused a color change in the specimens in TEA that exceeded AT. The change in whiteness exceeding AT in magnitude was only due to HPS. Regardless of the applications, the order of color and whiteness change in the beverage groups was TEA>IR2>LPI, and regardless of the beverages, the order of color and whiteness change in the application groups was HPS>PDP>PWW>NOT.

Ethical Approval

This study was reviewed and approved by the Ethics Committee of the Faculty of Dentistry, Ankara University (Decision No: 36290600/13/2024). Informed consent was obtained from all participants.

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Validation, Writing the References : F.G.
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Conflict of Interest

The authors declare no conflicts of interest.

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