ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

DOI: 10.5336/dentalsci.2021-84418

Color Stability of Preheated Novel Composite Resins Immersed in Different Beverages: *In Vitro* Study

Farklı İçeceklerde Bekletilen Ön-Isıtma Uygulanmış Güncel Kompozit Rezinlerin Renk Stabilitesi: *İn Vitro* Çalışma

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ABSTRACT Objective: To examine the color stability of 3 preheated composite resins immersed in different beverages for 1, 7, and 30 days. **Material and Methods:** Ninety disc-shaped specimens were prepared, 30 specimens from each of three different restorative materials [GrandioSO (GSO), GrandioSO x-tra (GSX), VisCalor bulk (VCB)], using 8-mm diameter and 4-mm thickness molds. Each group was divided into 3 subgroups (n=10) to be immersed into beverages (red wine, coffee, and distilled water). The color change values [CIEDE2000 (ΔE_{00})] were calculated at the end of the 1st, 7th, and 30th days. Generalized linear models and multiple comparison tests with Bonferroni correction at a significance level of $p<0.05$ were performed for the comparisons of ΔE_{00} values. **Results:** Among all composite and immersion media combinations, at the end of 30 days, the most prominent color change values were observed in the GSX group immersed in coffee and red wine and the VCB group immersed in red wine. For all composite groups, the lowest color change values were measured after immersion in distilled water in all evaluation periods. All composite groups immersed in red wine showed the color change from the 1st day, while those exposed to coffee from the 7th day, above the clinically acceptable values. **Conclusion:** The color change values of preheated resin-based composites kept in coffee and red wine were affected by the properties of the immersion solution and storage times rather than the type of the material.

Keywords: Composite resin; CIEDE2000; color stability; dental materials; preheating

ÖZET Amaç: Bu çalışma, 1, 7 ve 30 gün boyunca farklı içeceklerde bekletilen ön-ısıtma uygulanmış 3 farklı kompozit rezinin renk stabilitelerini incelemektedir. **Gereç ve Yöntemler:** Üç farklı restoratif materyalin [GrandioSO (GSO), GrandioSO x-tra (GSX), VisCalor bulk (VCB)] her birinden 30 örnek olmak üzere, 8×4 mm kalıplar kullanılarak 90 adet disk şeklinde örnek hazırlandı. Her bir grup, 3 farklı içecekte (kırmızı şarap, kahve ve distile su) bekletilmek üzere alt gruplara ayrıldı (n=10). Renk değişim değerleri [CIEDE2000 (ΔE_{00})] 1, 7 ve 30. günlerin sonunda hesaplandı. ΔE_{00} değerlerinin karşılaştırılmasında $p<0,05$ anlamlılık düzeyinde genelleştirilmiş lineer modeller yöntemi ve çoklu karşılaştırmalar için Bonferroni düzeltmesi kullanıldı. **Bulgular:** Otuz günün sonunda, tüm kompozit ve bekletme ortamı kombinasyonları arasında en belirgin renk değişim değerleri, kahve ve kırmızı şarapta bekletilen GSX ile kırmızı şarapta bekletilen VCB gruplarında gözlemlendi. Tüm kompozit grupları için en düşük renk değişim değerleri, tüm değerlendirme periyotlarında distile suda bekletme sonrasında ölçüldü. Tüm kompozit grupları için kırmızı şarapta bekletme 1. günden itibaren klinik olarak kabul edilebilir düzeyin üzerinde renk değişimine neden olurken, bu durum kahveye maruz kalan örneklerde 7. günden itibaren geçerliydi. **Sonuç:** Kahve ve kırmızı şarapta bekletilen ön-ısıtma uygulanmış rezin bazlı kompozitlerin renk değişim değerleri, materyalin tipinden çok bekletilen solüsyonun özellikleri ve bekletilme sürelerinden etkilenmiştir.

Anahtar Kelimeler: Kompozit rezin; CIEDE2000; renk stabilitesi; dental materyaller; ön-ısıtma

Developments in composite resin materials with the basis of restorative dentistry include strengthening the quality of physical and mechanical characteristics and improving the esthetic properties.[1] Since the concept of esthetics as "the art of the imperceptible", the optical properties of dental materials have progressed in a way of mimicking the natural teeth appearance. Despite the continual evolution of restorative materials, discoloration is still a factor that compromises the functional lifetime of restorations.[2]

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Peer review under responsibility of Turkiye Klinikleri Journal of Dental Sciences.

Received: 11 May 2021 ***Received in revised form:*** 07 Jan 2022 ***Accepted:*** 03 Feb 2022 ***Available online:*** 18 Feb 2022

Hence the importance of esthetics in dentistry which affects the patient's quality of life and self-confidence substantially cannot be denied. It is important to know the behavior of dental materials in the oral environment and their possible interplays with colorants to mention long-term esthetic performance.[3]

Resin-based restorative materials tend to stain due to several intrinsic and extrinsic reasons in the oral environment. Intrinsic factors which contain the degree of conversion, organic matrix content, inorganic particle size and hardness, type of initiator system, material polishability, and extrinsic factors such as water sorption, adsorption of colorant agents, smoking habits, deficient oral hygiene, and contact time with coloring mediums can be pointed out among the main reasons for staining of restorative materials.[4-8] Consumption of some beverages with intense colors, such as red wine, coffee, tea, the juice is admitted as a major risk factor to stain tooth and resin-based materials.[9,10] The interaction between color stability of resin composite systems in several commercial brands and different immersion media has been searched in the studies.[10,11]

Nowadays, the focus in modern dentistry is on the bulk-fill formulations which allow clinicians to place up to 4-5 mm layers in deep cavities without an incremental technique.[5] One of the most recent evolutions in restorative dentistry is a new bulk-fill resin composite with "thermo-viscous technology" specifically designed for use with preheating/heating procedures.[12] In dental literature, it is stated that preheating procedure of resin-based composites induces to optimize the handling properties of dental materials, improve the degree of conversion, advance marginal adaptation of restoration through decreasing the viscosity, and decline microleakage and gap formation.[13,14] Nevertheless, to the best of the authors' knowledge, no study has been published until now evaluating the stainability of recently introduced thermo-viscous bulk-fill composite.

A thorough understanding of how current restorative materials may be affected by the consumption habits of patients can greatly influence clinicians' choices by being a determining factor in material selection. Thus, the aim of this study was to comparatively investigate the color stability of recently developed resin composites immersed in distilled water, coffee, and red wine for up to 30 days. The null hypothesis was that the staining susceptibility of tested composite resins would not differ according to the properties of restorative materials, the type of staining solution, and the duration of storage in beverages.

MATERIAL AND METHODS

SPECIMEN PREPARATION

The materials selected for this study contained a universal nanohybrid composite [GrandioSO (GSO), VOCO, Cuxhaven, Germany], a nanohybrid bulk-fill composite [GrandioSO x-tra (GSX), VOCO, Cuxhaven, Germany] and a thermoviscous bulk-fill composite [VisCalor bulk (VCB), VOCO, Cuxhaven, Germany] (Table 1). Sample size calculation was performed with the G*Power 3.1 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany),

TABLE 1: Specifications of tested composite resins.

Material	Type	Composition	Filler content %	Lot number	Manufacturer
GrandioSO	Universal nanohybrid composite	Matrix: Bis-GMA, BisEMA, TEGDMA Filler: Glass ceramic, silicon dioxide	89 (w/w)	2409075	VOCO, Cuxhaven, Germany
GrandioSO x-tra	Nanohybrid bulk-fill composite	Matrix: Bis-GMA, BisEMA, aliphatic dimethacrylate Filler: Inorganic filler, organically modified silica	86 (w/w)	2026139	VOCO, Cuxhaven, Germany
VisCalor bulk	Thermoviscous, nanohybrid bulk-fill composite	Matrix: Bis-GMA, aliphatic dimethacrylate Filler: Inorganic filler	83 (w/w)	2048318	VOCO, Cuxhaven, Germany

Bis-GMA: Bisphenol A glycidyl methacrylate; Bis-EMA: Bisphenol A diglycidyl methacrylate ethoxylated; TEGDMA: Triethylene glycol dimethacrylate.

following these input conditions: alpha-type error of 0.05, a beta power of 0.95, an effect size of 0.54, and sample size was calculated 10 per group.[15] A total of 90 disc-shaped specimens (8 mm in diameter and 4 mm in thickness) were prepared from three different composite resins of shade A2 (n=30).

Besides a bulk-fill composite designed with thermo-viscous technology (VisCalor), all compules containing resin composite were preheated using a Caps Warmer (VOCO, Cuxhaven, Germany) in T3 mode at 68 °C for 20 min. Specimens were prepared by applying in one layer (4 mm) for GSX and VCB groups, and in layers at 2 mm thickness for the GSO group in accordance with the instructions of the manufacturer. After inserting the composite material into a mold, a Mylar strip (Hawe Stopstrip; Kerr Hawe, Bioggio, Switzerland) was pressed onto the mold surface with a glass slide to obtain a smooth surface without porosity. Resin composite specimens were then photoactivated for 20 sec as recommended by the manufacturer, using an LED curing unit (Bluephase PowerCure, Ivoclar Vivadent AG, Schaan, Liechtenstein) with a mean output of 1,200 mW/cm^2 (high power mode). To provide surface standardization; coarse, medium, fine, and super-fine grains of aluminum oxide discs (Sof-Lex™ XT, 3M ESPE, St Paul, MN, USA) were sequentially applied in the same direction for 10 sec, without water cooling, following the manufacturer's instructions. To ensure complete polymerization, specimens were kept in an incubator (EN055, Nüve, Ankara, Türkiye) at 37 °C for 24 h before initial color measurements.

BEVERAGE IMMERSION PROCEDURES

The specimens of each restorative material were randomly divided into 3 subgroups, which were immersed in one of the staining solutions: coffee (Nescafé Classic®, Nestlé, Sweden), red wine (Clarendelle Saint-Émilion, Clarence Dillon, France), and distilled water as a control group (n=10). The subgroups in each beverage were numbered from 1 to 10 for ensuing measurements. In accordance with the manufacturer's instructions, the coffee solution was prepared by dissolving 2 g coffee granule in 200 mL boiled water. In a previous study, it is stated that 24 hours of immersion to the coffee solution simulates nearly 1 month of consumption.[16] Hence, 30 days- immersion to coffee, red wine, and distilled water solutions in this study simulates approximately 2.5 years of consumption. For standardization, the same immersion period was applied in all beverages. Subsequently, specimens were stored in the incubator at 37 °C for 30 days. All solutions were refreshed every 2 days to avoid bacteria or yeast contamination.

COLOR MEASUREMENTS

Before the immersion procedure, the baseline color values were measured by a dental spectrophotometer (VITA Easyshade V, Vita Zahnfabrik, Bad Säckingen, Germany) over a gray card used as neutral background (Munsell N7 neutral gray color).[17] Three measurements were taken from the center of each specimen, and the arithmetic means of readings were recorded. The calibration of the spectrophotometer was performed before each measurement session. For the determination of the color change on the 1st (T1), 7th (T2), and 30th days (T3) owing to the immersion procedure, the measurements were repeated as mentioned above, and the following CIEDE2000 formulation was applied:[18]

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

Where ΔL', ΔC' and ΔH' are the differences in lightness, chroma and hue for a pair of samples in CIEDE2000. S_L, S_C, and S_H are the weighting functions for the color difference adjustment made necessary by the varied locations of the L^*, a^*, and b^* coordinates. R_T (rotation function) represents the interaction between chroma and hue differences in the blue region. K_L, K_C, and K_H, which are the parametric factors, were set to values of 2:1:1.[19]

STATISTICAL ANALYSIS

Data were analyzed with IBM SPSS version 23 (IBM Software Group, Chicago, IL, USA). The distribution normality was examined using Shapiro-Wilk test. Generalized linear models were used considering the following factors: material type, immersion media, and measures repeated in time and multiple comparisons were made with the Bonferroni test. The significance level was taken as $p<0.05$.

RESULTS

A statistically significant effect of "material"*"immersion media"*"time" interaction on the color change values was found (p<0.001) (Table 2). Results of color change values are shown in Table 3. For all composite groups, the lowest color change values were observed when the specimens were immersed in distilled water and there was no significant difference between the evaluation periods (1st, 7th, and 30th days). In addition, when the immersion media was distilled water, there was no statistical difference between the color change values in terms of composite groups. The highest color change values were in the GSX group immersed in coffee and red wine for 30 days, and in the VCB group immersed in red wine for 30 days.

For the GSO group, the color change values of the specimens immersed in coffee increased significantly at T3 compared to T1 (p<0.001), while the values at T2 were close to the values at T1. While the color change value of the GSO group in red wine was close at T1 and T2, it increased statistically at T3 (p<0.001). For both GSX and VCB groups, the time-dependent color change values of the specimens when immersed in both coffee and red wine were as follows: T3>T2>T1 (p<0.001; excluding the p value of 0.043 comparing the T2 and T1 periods of the VCB group in coffee).

Figure 1 presents the color change values of composite groups at different immersion media after 30 days. While all composite groups in distilled water showed similar color change values, the values in coffee were as follows: GSX>VCB>GSO (p<0.001). In terms of red wine, values of the group VCB was similar to the group GSX in every evaluation period, but both groups exhibited significantly higher color change values compared to the group GSO at T2 and T3 periods (p<0.001).

TABLE 2: Effect of immersion media and time on the color change values (ΔE_{00}) of the materials.

	Test statistics*	df	p value
Materials	263.197	2	**<0.001**
Immersion media	2053.994	2	**<0.001**
Time	771.290	2	**<0.001**
Materials*immersion media	198.859	4	**<0.001**
Materials*time	78.154	4	**<0.001**
Immersion media*time	298.001	4	**<0.001**
Materials*immersion media*time	78.291	8	**<0.001**

*Wald chi-square; df: Degree of freedom.

DISCUSSION

In the era of tooth-colored dental restorations, the color parameter has the greatest share in satisfying the expectations of patients and achieving optimum esthetics.[8] Regrettably, unpredictable color alteration in consequence of exposure to the food and beverage colorants in the oral environment is still one of the most prominent issues in clinical practice. In the present study, analyzing the staining susceptibility of novel composite resins exposed to

TABLE 3: Mean ΔE_{00} values±standard deviations at T1, T2 and T3.

		Evaluation periods		
Materials		**T1**	**T2**	**T3**
GrandioSO	Distilled water	0.234 ± 0.090^{E}	0.372 ± 0.140^{E}	0.768 ± 0.340^{E}
	Coffee	1.544 ± 0.417^{DG}	1.980 ± 0.306^{CD}	2.686 ± 0.228^{CF}
	Red wine	1.895 ± 0.516^{CD}	2.203 ± 0.523^{CD}	3.333 ± 0.555^{BF}
GrandioSO x-tra	Distilled water	0.328 ± 0.143^{E}	0.436 ± 0.160^{E}	0.768 ± 0.236^{E}
	Coffee	2.657 ± 0.399^{CF}	3.995 ± 0.628^{B}	5.513 ± 0.769^{A}
	Red wine	2.033 ± 0.315^{CD}	3.382 ± 0.541^{BF}	6.314 ± 0.794^{A}
VisCalor bulk	Distilled water	0.227 ± 0.077^{E}	0.307 ± 0.069^{E}	0.397 ± 0.130^{E}
	Coffee	1.650 ± 0.306^{DG}	2.463 ± 0.558^{C}	3.847 ± 0.828^{B}
	Red wine	2.039 ± 0.471^{CD}	3.394 ± 0.680^{BF}	6.252 ± 0.873^{A}

A-G: Different capital letters indicate statistically significant differences between material*immersion media*time interactions. p<0.05

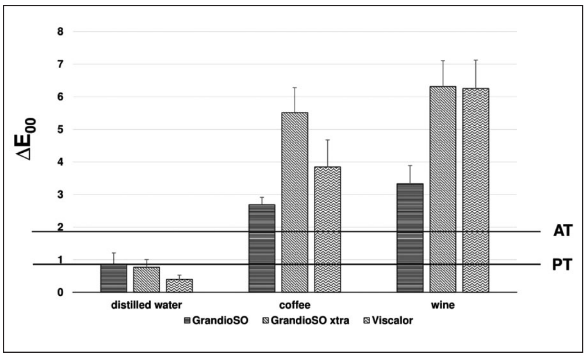

FIGURE 1: Color change values of resin-based composites (ΔE_{00}) according to immersion media at the end of the 30th day. AT=1.8; PT=0.8. AT: Acceptability threshold; PT: Perceptibility threshold.

different beverages, the null hypothesis was rejected, as the degree of discoloration differed concerning the properties of restorative materials, the type of staining solution, and duration of storage in beverages.

Within dentistry, the color difference of dental materials is mostly calculated by the CIELab (ΔE^*_{ab}) and CIEDE2000 (ΔE_{00}) formulas.[20] The CIEDE2000 color difference formula is proposed in up-to-date dental research because this formula corrects the nonuniformity of the CIELab color space, especially with slight color differences.[20-22] Color stability is evaluated based on 50:50% acceptability (AT) and 50:50% perceptibility (PT) thresholds after some procedures that may affect color parameters.[23,24] In this study, color stability after 1-, 7- and 30-day immersion periods was appraised with respect to the threshold values of AT (ΔE_{00}=1.8) and PT (ΔE_{00}=0.8), which have been accepted as the ISO/TR 28642:2016 standard.[20] Additionally, it is worth mentioning that a spectrophotometric device was utilized in the present study to perform color evaluation quantitatively without the connatural subjectivity of the clinician's decision-making process.[25]

Discoloration of composite resins may be the result of several intrinsic or extrinsic factors. The magnitude of discoloration varies due to several intrinsic factors including the composition of the resin materials (filler particles, organic matrix, activators, and photoinitiators), and chemical properties of resins such as hydrophilicity/hydrophobicity of monomers, water sorption, and polymerization degree.[4,7,9] Additionally, extrinsic contributing factors for staining comprise dietary habits, smoking, and inadequate oral hygiene.[4-6] Lago et al. have accentuated that staining by extrinsic factors is cumulative and occurs in synergy with the deterioration of materials.[26] In the current study, the possible impact of beverages that are commonly consumed by the general population on the staining susceptibility of the different resin composite materials is focalized. The control immersion media was represented by distilled water. Coffee has a lot of chromogens with a low polarity that appears to be accountable for discoloration owing to their affinity to the polymer network.[4,9] Ertaş et al. have declared that discoloration of composite with coffee pigments happens not only on the surface but also in deep layers of the material, because of the dye adsorption and absorption in the composite resins.[16] Furthermore, it is mentioned that the high temperature of coffee can facilitate the staining process of restorations.[11] On the other hand, red wine which is counted among the anthocyanins-rich beverages, contains alcohol, acid, chromogen, and tannins.[4] The intensive staining of composite materials caused by the red wine can be imputed to matrix degradation by acids and alcohol, penetration of pigment molecules deep into the resin matrix as well as adsorption of colorant molecules.[6] In the literature, it has not been clarified whether the discoloration caused by red wine is substantially due to alcohol or the presence of pigments in the wine.[27] It is reported that different degrees of discoloration could also occur as a consequence of the type of beverage, amount of colorant, and pH value.[1] In the present research, considering the staining potential for the long term, the solutions can be put in the following order: red wine>coffee>distilled water. Additionally, these results were similar to previous studies regarding the staining capacity.[7,15,28] Based on the study by Ertaş et al., which mentioned that immersion in beverages for 24 hours corresponds to one month *in vivo*, the process of immersing the samples into the solutions for 30 days in this study simulates a 2.5-year clinical lifespan.[16] Some researchers have pointed out that the immersion time of restorative materials into the beverages may affect the level of discoloration.[4,7] This could be ascribed to the greater penetration of colorants into the resin as a result of the more protracted interplay between the staining solutions and the resin.[8,29,30] Given the results obtained in this study, it has been observed that the staining degree of tested materials in all solutions increased with time. Our findings coincide with findings from other studies reporting that immersion in distilled water does not visibly change the color of composite resins.[7,26] On the other hand, Erdemir et al. had notified that immersion in distilled water for a month concluded visually perceptible color alterations (ΔE^*_{ab}=1.30-1.63) in all studied composites [Clearfil Majesty Posterior (Kuraray, Okayama, Japan), Filtek Supreme (3M ESPE, St. Paul, MN, USA), Clearfil APX (Kuraray, Osaka, Japan), Filtek Z250 (3M ESPE, St. Paul, MN, USA)], and also ΔE values of some composites (Filtek Supreme, Clearfil APX, Filtek Z250) exceeded the acceptable threshold (ΔE^*_{ab}>3.3) after 6-month immersion period.[8] This difference may have been due to the CIELab formulation that the researchers used, and the 6-month period that was not evaluated in the present study. This increased color changes due to 6-month immersion in distilled water with no colorants might be justified by water sorption of the organic matrix, and elution/oxidation of the initiation system components over time.[26,31]

It is also worth highlighting that the tendency of discoloration in resin composites is under the influence of conversion degree. In previous investigations, it is mentioned that the degree of conversion may affect the discoloration of restorative materials in long term.[32,33] A study investigating the color stability of a bulk-fill composite [Filtek One Bulk Fill (3M ESPE, St. Paul, MN, USA)] light-cured at different distances revealed that regardless of the immersion media, bulk-fill composite applying in 2 mm thickness presented lower ΔE^*_{ab} and ΔE_{00} values than conventional composite [Filtek Z350 XT (3M ESPE, St. Paul, MN, USA)] at all light-activation distances.[9] This could be related to the fact that the bulk-fill composite exhibited a higher conversion rate and color stability due to modifications in its structure, as a result of the application in 2 mm thickness like the conventional composite resin. In this study, considering the manufacturer's recommendations in the placement of the materials, GSX and VCB were inserted in a single increment (4 mm), while GSO was placed in 2 increments and polymerized throughout 20 sec. Unlike the study of Backes et al., the methodology difference in material placement may explain the better color stability achieved in the GSO group which is the conventional one in this investigation.[9] The preheating procedure, which enhances the monomer mobility and collision frequency of the reactive species, also leads to an improvement in the degree of conversion.[12-14] Schneider et al. specified that utilization of preheated composite could improve color stability, but the proof is limited.[34] In a previous study that investigated the color stability, opacity, and degree of conversion of pre-heated composites, it has been declared that preheating application at 60 °C increases the conversion rate, but this improvement does not increase optical features, which behaves similarly in all experimental conditions.[35] The preheating device used in this study submits three different temperatures (37 °C, 54 °C, or 68 °C) as disclosed by the manufacturer. All studied materials were preheated at 68 °C (T3 mode), which may increase the conversion rate, for 20 min in accordance with the operating instructions. Though other composites except VCB are not specifically designed for this procedure, and preheating has been applied to all groups in order not to make a difference in the color stability by affecting the conversion degree of the materials. Therefore, discrepancies between the findings of the current study and other investigations may have resulted from the fact that the conversion rate of materials can be affected by the preheating procedure as well as by the application of different incremental thicknesses, and the light application distance. However, it should be emphasized that more research is needed to entirely elucidate the effect of preheating technique on the restorative materials because of the lack of consensus in the literature. Outside of the factors aforementioned above, the staining susceptibility of composite resins may also vary according to distinction in the chemical compounds of restorative materials, qualification of filler-resin silanization, and affinity level of colorants to certain resin matrix components. In this regard, it is necessary to keep in mind that it is difficult to attribute the outcomes to the material structure, as manufacturers do not report the contents of materials in detail.

Patients constantly inquire dentist how long a restoration should preserve the esthetic semblance, and whether their dietary habitude may impress the longevity and quality of restoration. Notwithstanding that the results were obtained from *in vitro* study, it makes *in vivo* estimations possible in terms of long-term clinical behavior. With the results obtained, it is intended to help clinicians to choose the most suitable material according to the patients' beverage consumptions containing dyes and to make recommendations to the patients to ensure the continuity of esthetics.

One of the limitations of this experiment was that the non-preheated composite pairs were not included as the control group, so the additive effect of preheating could not be evaluated. Besides this, it can be stated as another limitation that resin composites except VCB are not specifically designed for preheating application. As other limitations, it can be specified that no brushing was performed, and all materials were chosen from the same company. The outcomes of the current research should be corroborated with the *in situ* and/or *in vivo* studies since they simulate oral environment conditions entirely and prevent incorrect estimation from the results of *in vitro* methodologies.

CONCLUSION

It can be concluded that:

1. In terms of color change, all materials exposed to the distilled water were below the clinically perceptible threshold values, while the same materials immersed in coffee and red wine were above.

2. Each staining beverage, most notably red wine, caused an increase in color change in the composites tested over time.

Acknowledgements

The authors would like to thank VOCO for supplying the dental materials used in this study.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Özge Gizem Cabadağ, Tuğba Misilli;* ***Design:*** *Özge Gizem Cabadağ;* ***Control/Supervision:*** *Tuğba Misilli;* ***Data Collection and/or Processing:*** *Özge Gizem Cabadağ;* ***Analysis and/or Interpretation:*** *Tuğba Misilli;* ***Literature Review:*** *Özge Gizem Cabadağ, Tuğba Misilli;* ***Writing the Article:*** *Özge Gizem Cabadağ;* ***Critical Review:*** *Özge Gizem Cabadağ, Tuğba Misilli;* ***References and Fundings:*** *Özge Gizem Cabadağ.*

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