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Effect of Effervescent C Vitamins on the Surface Roughness and Color Stability of Composite Resins

Efervesan C Vitaminlerinin Kompozit Reçinelerin Yüzey Pürüzlülüğü ve Renk Stabilitesi Üzerine Etkisi

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This study has been presented orally at the 2nd International Dentistry Congress (22-24 September 2022, Sivas).

Abstract	
Aim	To investigate the effect of effervescent vitamins C on the color change and surface roughness of resin composites.
Material and Method	30 specimens (8 mm*2 mm) were prepared for each restorative material (G-ænial Posterior, Solidex). The specimens were polymerized with LED device for 20 seconds and were incubated in distilled water for 24 hours for post-polymerization. Initial color values were measured using a spectrophotometer (Vita Easyshade V, Vita Zahnfabrik, Bad Säckingen, Germany), and initial surface roughness was measured with roughness tester (Mitutoyo SJ-410 Surftest Analyzer). The specimens were randomly divided into 3 groups according to their immersion solution (Redoxon, Ocean efervit, and distilled water). During the 28-day test period, the specimens were immersed in the solutions for 2 minutes, twice a day. Color and surface roughness measurement procedures were repeated. Data were analysed using One-way ANOVA, Tukey test, and t-test (p=0.05).
Results	When the color change (Δ E00) results are examined statistically; the highest Δ E values were observed in the Redoxon group, regardless of the restorative material. G-ænial Posterior specimens from both study groups and Solidex specimens incubated in Redoxon showed clinically unacceptable discoloration. When the average roughness values (Ra) are analyzed statistically, roughness values above the threshold surface roughness (Ra=0.2 μ m) was detected in all groups. However, no statistically significant difference between groups (p>0.05).
Conclusion	Despite the fact that effervescent C vitamins significantly increased the discoloration of the tested composite resins, not cause a statistically significant difference in surface roughness.
Keywords	Vitamin C, effervescent tablet, composite resin, color stability, surface roughness
Özet	
Amaç	Bu çalışmanın amacı, efervesan C vitamin tabletlerinin kompozit rezinlerin renk değişimine ve yüzey pürüzlülüğüne olan etkisini araştırmaktır.
Gereç ve Yöntem	Kompozit rezin materyallerinin (G-ænial Posterior ve Solidex) her biri için 8 mmx2 mm boyutlarında 30 adet numune teflon kalıp kullanılarak hazırlandı ve LED ışık kaynağı ile 20 saniye polimerize edildi. Post-polimerizasyon için distile su içerisinde 24 saat inkübe edildi. Numunelerin başlangıç renk değerleri spektrofotometre cihazı (Vita Easyshade V, Vita Zahnfabrik, Almanya) ile, başlangıç yüzey pürüzlülükleri ise pürüzlülük test cihazı (Mitutoyo SJ-410 Surftest Analyzer) ile ölçüldü. Numuneler çalışmada değerlendirilen solüsyonlara (Redoxon, Ocean efervit ve distile su) göre rastgele üç gruba ayrıldı ve 28 günlük deney süresi boyunca günde iki kez 2 dakika boyunca çözeltilere daldırıldı. Deney süresi sonunda renk ve yüzey pürüzlülük ölçümleri tekrarlandı. Verilerin analizinde One-way ANOVA, Tukey testi ve t-testi kullanıldı (p=0,05).
Bulgular	Renk değişimin (ΔΕ00) sonuçları istatistiksel olarak incelendiğinde; restoratif materyalden bağımsız olarak en yüksek ΔE değerleri Redoxon grubunda görüldü. G-ænial Posterior numuneleri ve Re- doxon'da inkübe edilen Solidex numunelerinin klinik olarak kabul edilemez renk değişiklikleri gösterdiği saptandı. Ortalama pürüzlülük değerleri (Ra) istatistiksel olarak analiz edildiğinde, tüm gruplarda eşik yüzey pürüzlülüğünün (Ra=0,2 µm) üzerinde değerler tespit edildi. Ancak gruplar arasında a ik ve ikinci ölçümler arasında pürüzlülük açısından istatistiksel olarak anlamlı fark görülmedi (p>0,05).
Sonuç	Efervesan C vitaminleri, test edilen kompozit rezinlerde önemli ölçüde renklenmeye neden olurken, yüzey pürüzlülüğünde istatistiksel olarak anlamlı farklılığa yol açmamıştır.
Anahtar Kelimeler	C vitamini, efervesan tablet, rezin kompozit, renk stabilitesi, yüzey pürüzlülüğü.

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INTRODUCTION

Minimal interventional dentistry approaches have led to an enhancement in the use of resin-based composite materials to satisfy the increasing esthetic expectations of patients¹. In addition to the advantages of these materials such as bonding to dental tissues and less preparation requirement, some disadvantages may cause restoration failure in clinical conditions such as discoloration, deterioration in surface properties, and polymerization shrinkage that causes microleakage².

Restorations are exposed to several conditions that cause physical and mechanical changes such as wear and discoloration in the oral cavity³. The reason for the discoloration encountered due to these conditions can be internal (monomer content, particle size and filler ratio) and external origin (such as coffee, tea, orange juice, acidic beverages, and oral hygiene habits)⁴. Preservation of the initial color, called color stability, is a very important feature in dental restorations. It may be related to the surface characteristics of resin composites such as roughness and water absorption^{5,6}. The hydrophilic nature of the resin matrix of composite materials exposed to staining beverages affects their color stability and water absorption potential. In addition, the roughness of the restoration surfaces creates areas suitable for plaque accumulation and discoloration by the adhesion of the pigments to the plaque⁷.

immune system has increased. Vitamin C, one of them, reduces the pH below 2.0 in the oral environment when used in syrup, effervescent and chewable tablet forms⁸. Previous studies have shown that supplements such as vitamins and multivitamins with different forms change the surface properties of teeth and increase microhardness and tooth erosion due to their acid content and low pH potentials⁹⁻¹². The acids in the vitamins dissolve the organic matrix structure of the composite resins, the staining substances are absorbed, and thus the composite restorations exhibit discoloration¹³.

In the literature, there are currently few studies focused on investigating the surface roughness and color stability of dental materials treated with effervescent vitamin C tablets. Therefore, the purpose of the study is to investigate the effect of supplemental vitamin C tablets on the color change and surface roughness of resin composites. The definitions of the study's null hypotheses were as follows: [1] effervescent vitamin C tablets do not cause color change on resin composites, [2] effervescent vitamin C tablets do not affect the surface roughness of restorative materials.

MATERIAL and METHODS

A microfilled hybrid resin composite [G-ænial Posterior (GP)], and an indirect resin composite [Solidex (SDX)] were evaluated in the current study. And the chemical composition of these restorative materials is given in Table

Product	Lot No	Manufacturer	Shade	Classification	Composition	
G-ænial Posterior (GP)	1709223	Kuraray Noritake Dental Inc.; Okayama, Japan	A3	Microhybrid composite	UDMA, dimethacrylate co-monomers, stron- tium and lanthanoid, fluoroaluminosilicate glass, silica (Bis-GMA-free)	
Solidex (SDX)	1606112	Shofu Dental Corporation, Quioto, Japan	A3	Light-curing ceramic filled, micro- hybrid indirect com- posite	UDMA, Silicon dioxide, aluminum oxide (53% other fillers (25%), DMAE methacrylate (22%	

In recent years, the daily use of vitamins to strengthen the

1.

Specimen preparation

A schematic illustration of study design is presented in Figure 1. The sample size was calculated using G*Power program (version 3.1.9.4, Heinrich Heine, University of Düsseldorf, Düsseldorf, Germany). A significance level of 0.05 and an effect size of 0.25 were assumed in the calculations, and 10 specimens from each final subgroup were prepared to provide a total of 30 specimens. Teflon molds in size of 8x2 mm were used to prepare specimens for each resin composite (GP and SDX). A mylar strip band and a glass plate were used to obtain smooth surfaces on the specimens. All of the specimens were polymerized using a LED (D-Light Pro, GC, Japan) with irradiation of 1200 mW/cm2 for 20 sec. The SDX specimens were additionally cured in the Solidilite multifocal light-curing unit (Shofu Inc., Kyoto, Japan) for 3 more minutes. A polishing system (Super-Snap Rainbow Technique Kit, Shofu Inc., Kyoto, Japan) was applied to a single surface of the specimens in each group. Then the specimens were post-polymerized in distilled water at 37°C for 24 hours.

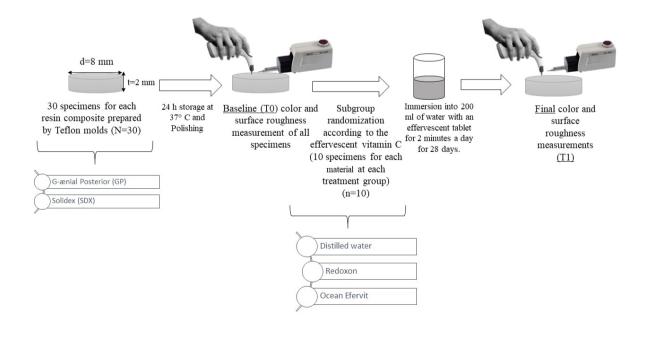
Figure 1. Schematic illustration of study design.

Color measurement

A digital spectrophotometer (Vita Easyshade V, Vita Zahnfabrik, Bad Säckingen, Germany) was used to measure the baseline colors (T0) of the specimens. The device's probe was positioned in the center of the specimens, which are on a white surface without reflection. "L*, C*, and H*" values were averaged after being measured three times for each specimen. The spectrophotometer was re-calibrated after every nine measurements according to the manufacturer's recommendations.

Surface roughness measurement

Initial surface roughness (Ra) measurements (T0) were determined with a contact profilometer (Mitutoyo, Surftest SJ-410, Japan) with a measuring distance of 4 mm and a cut-off value of 0.8 mm. Before each measurement, the calibration was performed with the aid of a reference plate with a Ra value of $3.05 \,\mu$ m. Three measurements obtained from the center of each specimen surface were averaged and Ra values (μ m) were recorded.



Staining procedure

The chemical compositions of vitamins C used in the present study are given in Table 2. Specimens in the study group were immersed in 200 ml of water with an effervescent tablet for 2 minutes a day for 28 days¹⁴. The staining solutions were re-prepared daily. For the vitamin groups, the specimens were kept in 20 ml of distilled water for the remaining time. Following this process, the specimens were rinsed with water for 10 s and gently dried. The specimens of the control group were immersed in distilled water throughout the experiment.

Table 2. Chemical compositions of vitamins C used in the study				
Product	Manufacturer	Erosion related ingredient	pН	
Redoxon Effervescent tablet	Bayer	L-ascorbic acid, Malic acid, Citric acid	4.32	
Ocean efervit Effervescent tablet	Orzax	Citric acid anhydrate, L-ascorbic acid, lactose anhydrate	4.69	

When the immersion period was completed, the final color and surface roughness measurements (T1) were performed. To evaluate the color differences between the baseline and final color measurements after staining, ΔE_{00} (T1- T0) values were calculated via the following formulation:

$$\Delta E_{00} = \left[\left(\frac{\Delta L}{k_L S_L} \right)^2 + \left(\frac{\Delta C}{k_C S_C} \right)^2 + \left(\frac{\Delta H}{k_H S_H} \right)^2 + R_T \left(\frac{\Delta C}{k_C S_C} \right) \left(\frac{\Delta H}{k_H S_H} \right) \right]^{1/2}$$

In this study, using the CIEDE2000 (1:1:1) formula, a 50:50% acceptability threshold (AT: ΔE_{00} =1.8) and 50:50% perceptibility threshold (PT: ΔE_{00} =0.8) were determined for all resin-based materials.

Surface topography evaluation by Scanning Electron Microscope (SEM)

Scanning electron microscope images were taken from randomly chosen specimens following the staining procedure. Specimens were sputter-coated with palladium in the ion plating unit (Polaron SC500 sputter coater, FISONS Instrument, UK) and were observed by SEM device (Zeiss GEMINI 500, Zeiss, Oberkochen, Germany) at the Erciyes University Technology Research and Application Center. The surface of the specimen was entirely scanned and representative areas were photographed at 1,500x magnification.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (version 25.0, SPSS Inc., Chicago, IL, USA). The normality of the ΔE_{00} and Ra (µm) data was determined using the Shapiro-Wilk test. One-way ANOVA was used for statistical analysis of the measured parameters. Tukey test for pairwise comparisons and Student t-test for dependent variables were performed. P=0.05 was set as the level of statistical significance.

RESULTS

The mean ΔE_{00} values (T1- T0) and standard deviations of the restorative materials after treatment with effervescent vitamin C are shown in Table 3. When the color change (ΔE_{00}) results were evaluated; the highest ΔE values were observed in the Redoxon group, regardless of the restorative material. G-ænial Posterior specimens from both vitamin C groups and SDX specimens incubated in Redoxon showed clinically unacceptable (AT>1.8) discoloration. Also, both materials in all solutions presented perceptible (PT>0.8) discoloration. In both materials, the specimens in the distilled water group exhibited the lowest color change. There were statistically significant differences between the solutions for both restorative materials (p^a=0.000). No significant differences was found between composite materials used in this study in each treatment group (p^b=0.204, p^b=0.351, and p^b=0.873, respectively).

The mean surface roughness (Ra) values and standard deviations of the restorative materials after treatment with effervescent vitamin C are summarized in Table 4. When the average roughness values (Ra) were evaluated, roughness above the threshold surface roughness (Ra= 0.2μ m) was detected in all groups. However, the difference was not statistically significant between solution groups in all restorative materials at each time-period (p^b>0.05). Solidex specimens in Ocean-efervit solution exhibited more roughness values (Ra1) and the difference between groups was statistically significant (p^a=0.041). After treatment with solutions, GP showed fewer roughness values than Solidex.

The difference in surface roughness between restorative materials in the same period is schematized in Figure 2. Regarding Ra0, no significant differences were detected between resin composites in each treatment group (p>0.05). However, regarding the Ra1, there were significant differ-

ences between resin composites due to the SDX exhibiting more surface roughness in Ocean efervit group (p=0.045).

Assessment of SEM images

Scanning electron microscope images of a specimen from each group of GP and SDX are shown in Figure 3 and Figure 4, respectively. SEM micrographs of the GP specimens treated with Redoxon and Ocean efervit revealed a more surface porosity compared to specimens immersed in distilled water (Figure 3). However, the SDX specimens immersed in Ocean efervit displayed more alterations than other groups (Figure 4).

Table 3. The mean ΔE_{00} (T1- T0) values±standard deviations after immersion in vitamins C.						
Materials		Solutions				
Materials	Distilled water	Redoxon	Ocean-efervit	Results		
$GP \Delta E_{00}$	1.30±0.41ª	*4.31±0.66 ^b	*3.69±1.45 ^b	p ^a =0.000		
SDX ΔE_{00}	1.38±0.9ª	*4.12±1.55 ^b	1.61 ± 0.77^{a}	p ^a =0.000		
Results	p ^b =0.204	p ^b =0.351	p ^b =0.873			

Lower letters indicate the difference between lines. capital letters indicate the differences between rows. AT: $\Delta E00=1.8$ and PT: $\Delta E00=0.8$ GP: G-ænial Posterior, SDX: Solidex.

pa values are based on One-way ANOVA test, bp values are based on The Student t-test. *p<0.05 is significant.

Table 4. Comparison of	of mean Ra values for material gr	oups and tested vitamins C.			
Materials	Solutions				
Materials	Distilled water	Redoxon	Ocean-efervit	Results	
GP					
Ra0	1.21±0.84	1.47±1.33	1.68±1.01	p ^a =0.616	
Ra1	1.21±0.86	1.36±1.01	1.73±1.1	p ^a =0.491	
Results	p ^b =0.771	p ^b =0.663	p ^b =0.965		
SDX					
Ra0	1.67±1.01	1.82±0.93	1.46±0.51	p ^a =0.318	
Ra1	2.20±1.57 ^b	2.04±1.01 ^b	3.03±1.66ª	pª=0.041*	
Results	p ^b =0.675	p ^b =0.840	p ^b =0.522		

Lower letters indicate the difference between lines and capital letters indicate the difference between columns

GP: G-ænial Posterior, SDX:Solidex.

^ap values are based on One-way ANOVA test, ^bp values are based on The Student t-test. *p<0.05 is significant. The acceptability threshold of surface roughness was considered as $0.2 \mu m$.

J Biotechnol and Strategic Health Res. 2023;7(1):43-53 AVUNDUK, DELİKAN, Effervescent C vitamins and composite resins

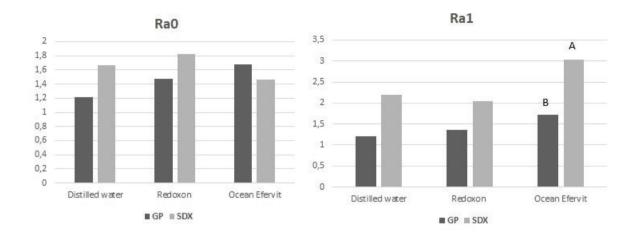


Figure 2. Avarage Ra values of resin composite materials before and after treatment procedure. GP: G-ænial Posterior, SDX: Solidex. Capital letters indicate statistical difference between resin composite materials.



Figure 3. SEM images of a G-ænial Posterior specimen. (a) control group, (b) Redoxon group, (c) Ocean efervit group.

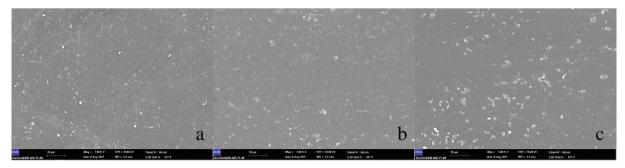


Figure 4. SEM images of a Solidex specimen. (a) control group, (b) Redoxon group, (c) Ocean efervit group.

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DISCUSSION

The maintenance of surface topography and color stability plays an important role in the clinical success of resin-based restorative materials. An ideal dental restoration should have as smooth a surface as possible to prevent plaque accumulation and discoloration¹⁵. Previous studies have reported that the use of the effervescent form of supplements can lead to dental erosion and have negative effects on dental hard tissue depending on the consumption frequency^{16,17}. Also, it has been reported that these supplements cause surface alterations on resin-based materials due to their low pH and high titratable acidity¹⁸. Therefore, the present study sought to answer the effects of effervescent vitamin C tablets on the color stability and surface roughness of resin-based composites.

Visual and instrumental methods can be used to investigate the color changes of dental materials. However, instrumental techniques have been widely recommended due to visual color assessment can create inconsistencies inter-observers in color perception¹⁹. The use of spectrophotometers for color matching has become popular as it provides objective, standardized and accurate measurements with numerical expressions of colors²⁰. In this study, the clinical spectrophotometer device was performed to measure the color changes of specimens.

In the current study, color differences were calculated with the CIEDE2000 formulation. Researchers reported that this formulation is more suitable for evaluating color differences²¹. In addition, it has been noted that the CIEDE2000 formula has improved visual data in dentistry compared to other formulas such as CIELAB and CMC $(1:1)^{22}$. Visual acceptability and perceptibility threshold values are significant in detecting color differences of dental tissues and materials in clinical dentistry²³. In the current study, the perceptibility (PT) and acceptability threshold (AT) in analyzing color changes were specified as ΔE_{00} =0.8 and ΔE_{00} =1.8 respectively, as reported by Paravina et al.²⁴ No color difference should be detected after exposure to the test environment for the material to achieve complete color stability ($\Delta E=0$)²⁵.

Factors such as the type of composite resin and immersion time may have contributed to the differences observed in the color stability of the restorative materials²⁶. Experimental immersion time is a critical factor to consider. The clinical detection of color variations of composite resins can be achieved with long-term simulated protocols. However, there is no previously stated standard protocol for composite resins treated with vitamin supplements in the literature. In the present study, the specimens were immersed daily for 2 minutes a day for 28 days to stimulate transient contact of restorations with supplements and to mimic minimum oral exposure time²⁷. In addition, distilled water was preferred as a control group to understand the intrinsic changes of resin-based materials and to compare the effect of effervescent vitamins C on color and roughness changes in restorative materials in the current study, as in most studies investigating color change. When the relevant literature was examined, it has been seen that artificial saliva is included in color change studies. However, it has been stated that artificial saliva has limitations in terms of exactly reflecting the oral environment due to it does not contain proteins and enzymes^{28,29}.

Indirect composite systems have been developed to eliminate the disadvantages of polymerization shrinkage and microleakage³⁰. The main purpose of these systems is to prevent polymerization shrinkage of the composite material, to increase the adhesion and to improve the mechanical properties of the restoration by reducing the amount of unreacted residual monomer³¹. In the present study; a micro-filled hybrid resin composite (GP) was compared with the indirect resin composite (SDX). The deterioration and reduced surface hardness of indirect composite resins over time, the chemical reactions of tertiary amines present in their structure, and the movement of filler particles to the surface cause changes in their optical properties and an unexpected color change³². Although no statistically significant difference was detected between restorative materials in all treatment groups regarding the color change findings in the current study, the discoloration was generally unacceptable in both materials. Not consistent with the results of the current research, Papadopoulos et al.³³ observed clinically acceptable color changes in indirect composite resins after accelerated aging and polymerization.

Organic acids (such as citric and phosphoric acids) reduce the oral pH, degrade the polymer matrix, and reduce the microhardness of composite resins³⁴. It has been stated that low pH (pH 2.7) affects the surface integrity of the material by causing the loss of structural ions such as calcium, aluminium, and silicon from the glass phase of composite resins and softening the matrix structure³⁵. The manufacturer did not report the pH information of the effervescent vitamin C supplements used in the current study. In this context, the pH of solutions was measured with a pH meter (Hanna instruments, Padova, Italy) before starting the study. The pH values recorded for Redoxon and Ocean efervite were below the critical pH value of 5.5 and were 4.32 and 4.69, respectively. The low-pH effervescent vitamins used in the current study affected the color stability and surface properties of the composite resins. Following the results of this study, a previous study reveals that acidic pH level affects the coloration of resin-based materials by affecting the surface integrity³⁶.

Based on the findings of the current study, GP specimens in both study groups (Redoxon and Ocean Efervit) and SDX specimens incubated in Redoxon showed clinically unacceptable discoloration. Therefore, the first null hypothesis of this study, which defended that "effervescent vitamin C tablets do not cause color change on resin composites" is rejected. Consistent with this finding, Sarialioğlu Güngör et al.³⁷ reported that Redoxon was associated with the most severe color change (4.86±0.63) compared to other solutions (Sambucol and distilled water) they used in their study, and there were statistically significant differences (p<0.001) between resin composite samples in terms of ΔE values. Consistent with the present study, Poggio et al.³⁸ also stated that the most discoloration was observed in GP specimens. On the other hand, the staining of resin composites has been stated to be closely related to the resin phase. UDMA-based resin composites exhibit lower surface hardness, degree of polymerization, and molecular hardness when compared to Bis-GMA-based resins³⁹. The difference in filler content per volume of resin composite can be cited as the reason of GP exhibited higher color change values. The incorporation of pre-polymerized fillers in the GP and the addition of fillers such as glass, ceramic, and zirconia may also be evidence for this finding.

In the current study, after the SDX specimens were immersed in different solutions, the Ocean efervit group exhibited significantly higher surface roughness than other solution groups in the present study. According to these findings, the second hypothesis is also rejected. Our findings supported a previous study investigating the effect of multivitamin syrups and effervescent on the surface roughness and microhardness of restorative materials¹⁴. Researchers stated that effervescent tablets may have adverse effects on the physical properties (surface roughness and microhardness) of restorative materials. This result may be due to the low pH of the supplements, which affects the surface properties of resin-based composites. Since vitamin supplements are usually used for a prolonged period, it should be taken into account that effervescent tablets can have an aggressive effect on the surface structures of restorative materials. When evaluated in terms of restorative material, the large particle size of SDX (43 to 56 μ m) may have caused the surface roughness to be higher. In a previous study investigating the effect of different polymerization systems on the surface roughness of indirect composite resins (Solidex, Signum, Sinfony, Resilab, Epricord, and Adoro) the situation was unexpected for Solidex and the surface roughness was the lowest⁴⁰. In addition, in agreement with the findings of this study, there are previous studies stating that the smaller the particle size in the organic matrix, the greater the smoothness and gloss of the surface of composite resins^{41,42}. Previous studies also stated that a perfectly smooth surface cannot be obtained after finishing-polishing methods in tooth-colored restorative materials.¹⁵ Although the threshold surface roughness was mentioned for bacterial plaque retention as 0.2 μ m, no significant difference was found in plaque on surfaces with Ra values between 0.7 and 1.4 μ m⁴³. Due to the presence of specimens showing roughness above these values in the current study, surface smoothness could be checked with a method such as light microscopy after finishing-polishing methods, and if the polishing was found to be insufficient, this process could be repeated with different finishing-polishing kits. This condition is the first limitation of the present study.

In dentistry, there are different roughness parameters to measure surface roughness, and the roughness average (Ra) is most frequently used. Ra is defined as the arithmetic mean of all deviations of the roughness profile from the centerline⁴⁴. Methods for measuring surface roughness include contact profilometry, laser profilometry, scanning electron microscope, and 3D optical profilometry. The contact profilometer provides more accurate results in measuring changes in surface height⁴⁵. A previous study involving a comparison between contact and non-contact profilometry reported that both methods provided reliable measurement⁴⁶. However, it has been reported that supporting profilometry findings with qualitative methods (such as SEM) increases the reliability of the findings due to these methods provide more detailed surface information than profilometry⁴⁷. Considering these findings, SEM imaging was performed to support and detail the profilometer findings in the current study (Figure 3 and 4).

Evaluation of the performance of restorative materials should be supported by clinical studies. Therefore, this in-vitro study has also some another limitations. Saliva, temperature changes, and pH levels in the oral environment can also affect the long-term color stability and surface structure of composite resin materials. Therefore, further clinical research is required to support the findings of the present study.

CONCLUSION

Despite the fact that effervescent C vitamins significantly increased the discoloration of the tested composite resins, not cause a significant difference in surface roughness. In the use of drugs with low pH, the surface properties of resin-based restorative materials may change. Patients may be advised to rinse their mouths with water following the consumption of such supplements. The vitamins should be used under the control of physicians and patients should pay attention to regular dentist controls.

Conflict of Interest

The authors declare that they have no conflict of interest

Author contributions

Conceptualization: Ayşe Tuğba Ertürk-Avunduk, Ebru Delikan; Methodology: Ayşe Tuğba Ertürk-Avunduk, Ebru Delikan; Software: Ayşe Tuğba Ertürk-Avunduk, Ebru Delikan; Validation: Ayşe Tuğba Ertürk-Avunduk, Ebru Delikan, Investigation: Ayşe Tuğba Ertürk-Avunduk, Ebru Delikan; Resources: Ayşe Tuğba Ertürk-Avunduk, Ebru Delikan; Data curation: Ebru Delikan, Ayşe Tuğba Ertürk-Avunduk; Writing – original draft preparation: Ebru Delikan, Ayşe Tuğba Ertürk-Avunduk; Writing – review and editing: Ebru Delikan, Ayşe Tuğba Ertürk-Avunduk; Visualization: Ebru Delikan, Ayşe Tuğba Ertürk-Avunduk.

Ethical Approval

This in-vitro study does not contain any studies with human participants or animals performed by any of the authors. All methods were performed in accordance with the relevant guidelines and regulations.

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None declared.

Informed Consent

For this type of in-vitro study, formal consent is not required.

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