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# Evaluation the Effect of Different Antioxidants Applied After Bleaching on Teeth Color Stability

Avaliação do efeito de diferentes antioxidantes aplicados após o clareamento na estabilidade da cor dos dentes

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## ABSTRACT

**Objective:** Vital bleaching is a popular treatment option for discolored teeth; but at post-treatment stage, loss of adhesion is highly reported. Literature focused on antioxidant application for the answer of this issue. The aim of this study was to compare the effects of six different antioxidants on color stability of bleached teeth. **Material and Methods:** This study included total of 84 extracted intact non-carious lower incisors. 35% hydrogen peroxide was applied on the labial surfaces of specimens in accordance with manufacturer's instructions. The bleached teeth were divided into 7 groups. No antioxidants were applied to the control group. For the experimental groups, the following antioxidants were applied for 10 minutes each: 5% proanthocyanidin, 5% sodium ascorbate, 5% lycopene, %5 green tea, %5 white tea and %5 α-tocopherol. CIE L*, a* and b* values of the teeth were measured by a spectrophotometer. One-way ANOVA was used to determine the differences among the groups. Multiple comparisons were examined with Tukey HSD. **Results:** The one-way ANOVA test revealed a statistically significant difference between the groups ($p<0.005$). Highest color change was observed in lycopene group and the lowest in green tea group. **Conclusion:** Proanthocyanidin, white tea and green tea could be considered as post-bleaching antioxidant alternatives based on their herbal nature.

## KEYWORDS

Green Tea; Lycopene; Proanthocyanidin; Alpha-tocopherol.

## RESUMO

**Objetivo:** O clareamento vital é uma opção popular de tratamento para dentes descoloridos, mas na fase pós-tratamento, a perda de adesão é altamente relatada. A literatura enfocou a aplicação de antioxidantes para a resposta desta questão. O objetivo deste estudo foi comparar os efeitos de seis diferentes antioxidantes na estabilidade da cor de dentes clareados. **Material e Métodos:** Este estudo incluiu um total de 84 incisivos inferiores extraídos, intactos e não cariados. Peróxido de hidrogênio a 35% foi aplicado nas superfícies labiais dos espécimes de acordo com as instruções do fabricante. Os dentes clareados foram divididos em 7 grupos. Nenhum antioxidante foi aplicado ao grupo controle. Para os grupos experimentais, os seguintes antioxidantes foram aplicados por 10 minutos cada: proantocianidina a 5%, ascorbato de sódio a 5%, licopeno a 5%, chá verde a 5%, chá branco a 5% e α-tocoferol a 5%. Os valores CIE L *, a * e b * dos dentes foram medidos por um espectrofotômetro. ANOVA um fator foi usada para determinar as diferenças entre os grupos. As comparações múltiplas foram examinadas com Tukey HSD. **Resultados:** O teste ANOVA revelou uma diferença estatisticamente significativa entre os grupos ($p <0,005$). A maior mudança de cor foi observada no grupo do licopeno e a menor no grupo do chá verde. **Conclusão:** Proantocianidina, chá branco e chá verde podem ser considerados como alternativas antioxidantes pós-clareamento com base em sua natureza fitoterápica.

## PALAVRAS-CHAVE

Chá verde; Licopeno; Proantocianidina; Alfa-tocoferol.

## INTRODUCTION

The most common treatment option in teeth coloration is vital bleaching [1]. This method is reliable and well-known and has conservative and acceptable results. The commonly preferred bleaching agents are the concentrations of carbamide peroxide (35–37%) and hydrogen peroxide (30–35%) [2]. The mechanism of bleaching agents is based on oxidation-reduction reactions. The chemical process converts organic material into carbon dioxide and water. This reaction releases oxygen, which is a highly reactive free radical. This reaction creates a bleaching effect by penetrating the porosities of the enamel and converting the high-molecular-weight organic molecules in enamel prisms into low-molecular-weight compounds [3].

However, dental bleaching agents have several side effects, including hypersensitivity, reduction of enamel micro-hardness, gingival irritation, micro-morphological defects in dental hard tissues, and loss of adhesion/retention in post-treatment restorations [2]. Fluoride application is recommended after bleaching to repair demineralized lesions in many studies [4, 5]. Another recent alternative option for eliminating these side effects is the use of antioxidant agents [6, 7]. Antioxidants absorb free oxygen radicals, thus increasing the oxidation/reduction reaction of the enamel surface [8]. The most commonly indicated antioxidants in dental researches include sodium ascorbate, ascorbic acid, catalase, and acetone [8, 9]. Sodium ascorbate is a neutral, non-toxic and biocompatible material [10]. For this reason, it is the most preferred antioxidant by dentists for the elimination of peroxide and oxygen radicals that inhibit post-bleaching composite polymerization [11]. α-tocopherol is an active component of the Vitamin E and this substance is a powerful antioxidant for the lipid phase of the human body [12]. It has been recently suggested for improving composite bonding following bleaching [13]. The beneficial effect of alpha-tocopherol solution is attributed, in addition to its antioxidant effect, to its alcoholic solvent [12]. With the inclusion of prominent medical antioxidants in dental literature, several new antioxidants have come into use in dental practice. Natural antioxidants, such as proanthocyanidin, white tea, and green tea with free radical scavenging ability that has been shown to be 50 times more potent than that of sodium ascorbate [14]. Proanthocyanidin (PAC) is a naturally occurring plant metabolite commonly found in fruits, vegetables, dried nuts, seeds, flowers, and tree barks. PACs, often used as natural antioxidants and free radical scavengers, have proven to be safe in various clinical applications and as dietary supplements [15]. Grape seed extract is a rich source of PAC and is reported to augment collagen-based tissues by improving collagen crosslinkers [16]. Proanthocyanidins obtained from grape seed extract were found to lower hyperglycemia and improve insulin levels in the blood while reducing pancreatic oxidative stress in diabetic rats [17]. Studies have reported the use of proanthocyanidins in the treatment of cardiovascular diseases and certain types of cancer including oral cancers [18]. PAC, obtained from cranberries, was found to inhibit surface-adsorbed glucosyltransferases and acid production of S. mutans [19]. White tea is obtained from an unfermented tea made from the new growth buds and young leaves of the plant [20]. Green tea is made from the Camellia sinensis plant [14]. Both white and green tea contains mainly flavanols or catechins, such as epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC) [21]. The catechins can donate hydrogens from the hydroxyl groups in their structure, they have excellent antioxidant activities [22]. White tea has been related to beneficial effects on several diseases such as neurodegenerative and cardiovascular diseases, diabetes, obesity and basically, to every pathology involving oxidative stress [23]. Green tea have an ability to prevent cardiovascular disease, and reduce dentin erosion and periodontal inflammation [24]. Lycopene is an antioxidant included in routine adjunctive therapy by medical doctors after the discovery of its accelerating effect in wound healing in 1959,

and also a carotenoid present in tomato extract [25]. Despite its known free radical scavenging ability (7), lycopene's efficiency after bleaching has never been evaluated by the researchers.

This study aims to compare the color change on teeth caused by six different antioxidants (sodium ascorbate, proanthocyanidin, lycopene, green tea, white tea, α-tocopherol) applied after laser-activated vital bleaching procedure with 35% hydrogen peroxide and to examine their safety in terms of color stability.

## MATERIAL AND METHODS

The study was approved by the Clinical Research Ethics Committee of Van Yuzuncu Yil University Faculty of Medicine (24.10.2017/04).

### Preparation of Specimens

In the study, $\Delta E_{002}$ is considered as main trait. From the previous studies [6], the standard deviation for $\Delta E_{002}$ varies between 0.1 and 1. Thus, standard deviation was taken as 0,55. For the 95% of confidence coefficient and approximately 80% power value, Type I error is 0,05 (Z value is 1,96 for the 5% type I error), the effect size was taken by the researcher as 0,3.

Based on this information, the necessary sample size was calculated by the equation

$$"n = Z^2 \times \sigma^2 / d^2"$$

According to this equation, minimum sample size in each group was found as 12 [$n = (1.96^2 \times 0.55^2 / 0.3^2 \cong 12$]. A total of 84 extracted intact non-carious mandibular incisor human teeth were included in the study. Fractures were detected with the transillumination method with the assistance of light-transmitting glass fibers, and cracked teeth were excluded from the study. Dental roots were removed from the cementoenamel junction with a diamond blade. The teeth were polished with pumice and separated from the residual tissues, and the remnants of pumice were washed away with clear water. The teeth were kept in an incubator at 37 °C for three days before bleaching. The specimens were embedded in silicone molds in groups of three.

### Bleaching Procedure

All the bleaching procedures were performed by a single operator. Bleaching agent, including 35% hydrogen peroxide, was applied on the labial surfaces of all 84 specimens according to manufacturer's instructions (Fotona Chairside Bleaching Gel, Fotona, Vilnius, Lithuania). A diode laser (Ezlase, Biolase, Irvine, USA) was used for the activation of the agent with 70 Joules energy in continuous wave mode with 3.5 W power for 2.5 min. The operator applied the bleaching agent to the entire labial surface of the tooth and at least 1 mm thick. Then the agent was left on tooth surface without activation for 10 minutes.

### Preparation and Application of Antioxidants

The teeth were randomly divided into seven groups after the bleaching application (Table I).

**Table I -** Study Group.

| Groups | Bleaching agent | Antioxidant |
|---|---|---|
| Group Control (n=12) | %35 hydrogen peroxide | None |
| Group Proanthocyanidin (n=12) | %35 hydrogen peroxide | % 5 proanthocyanidin |
| Group Sodium Ascorbate (n=12) | %35 hydrogen peroxide | % 5 sodium ascorbate |
| Group Lycopene (n=12) | %35 hydrogen peroxide | % 5 lycopene |
| Group Green Tea (n=12) | %35 hydrogen peroxide | % 5 green tea |
| Group White Tea (n=12) | %35 hydrogen peroxide | % 5 white tea |
| Group α-tocopherol (n=12) | %35 hydrogen peroxide | % 5 α-tocopherol |

Five grams of each antioxidants in the form of powder was dissolved in 100 ml of distilled water and stirred for 1 min, and then, the solution was filtered [26]. The solutions were applied for 10 minutes using a syringe to place 0,02 ml of each antioxidant: 5% proanthocyanidin (GNC Proanthocyanidin, GNC, Inc. Nutra, Greenville, S.C., USA), 5% sodium ascorbate (Alfasol Kimbiotek Corporation, Istanbul, Turkey), and 5% lycopene (GNC Lycopene, GNC, Inc. Nutra,Greenville, S.C., USA), %5 green tea (Emerald Gunpowder, Lipton, Istanbul,Turkey), %5 white tea (Organic White Tea, Caykur, Istanbul, Turkey) and %5 α-tocopherol (Alfasol Kimbiotek Kimbiotek

Corporation, Istanbul, Turkey). Antioxidant applications were performed by another operator. Control group was left without any antioxidant applications. A soft toothbrush was used for making five back-and- forth movements over the tooth and washing with distilled and deionized water. They were then immersed in artificial saliva solution in containers and kept in incubator at 37°C for three days. And color measurements were repeated for the third time after the antioxidant applications.

### Spectrophotometric Color Analysis

L*, a* and b* values of the teeth were measured by a spectrophotometer (Spectroshade™ Micro, MHT, Verona, Italy). The teeth were placed on a neutral gray background, and the labial surfaces of the specimen were positioned for measurement. Color measurements were repeated before bleaching, after bleaching and antioxidant applications. Spectrophotometeric measurements were done by a single operator who was blinded to bleaching procedures and antioxidant applications The CIE L*, a* and b* values, obtained with a spectrophotometer, were computerized. The differences in shade according to the bleaching and antioxidant applications were calculated by quantifying ΔE (mean color difference), with the use of ΔE DE2000 ($\Delta E_{00}$) color difference formulas representing the distance from the measured values (L), (C), (h), (a), and (b) to the 3D space of two colors. The color change ($\Delta E_{00}$) was calculated using the following formula:

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

The following criteria were used to determine whether the obtained $\Delta E_{00}$ values affect clinical usage and for the discernibility of color difference:

(a) $\Delta E_{00}$ >1.77 mean the magnitude that constitutes an unacceptable alteration to

dental aesthetics

(b) $\Delta E_{00}$ =0.81 mean the color difference can be visually detected [27].

Color changes due to bleaching application was recorded as $\Delta E_{001}$ $\Delta L_1$, $\Delta a_1$ and $\Delta b_1$ while color change due to antioxidant application was noted as $\Delta E00_2$ $\Delta L_2$, $\Delta a_2$ and $\Delta b_2$.

### Statistical Analysis

The data were analyzed using IBM SPSS V23. The suitability of normal distribution was evaluated with the Shapiro-Wilk test. And the data was normally distributed. One-way ANOVA was used to determine the differences among the groups. Multiple comparisons were examined with Tukey HSD since $\Delta E_{001}$ values were homogeneous and other values were examined with Tamhane's T2.

## RESULTS

There was a difference between the $\Delta E_{001}$ mean values according to the groups (p= 0.003). The reason of these statistical difference in both formula was based on that the mean value in the lycopene group was obtained lower than the proanthocyanidin and sodium ascorbate groups. After the bleaching procedure, color change of lycopene group was calculated as 0.846 for $\Delta E_{001}$. There is no difference between other groups in terms of $\Delta E_{001}$ (p> 0.05). $\Delta E_{001}$ mean values differ according to the groups (p <0.001). The highest mean value was obtained in the lycopene group for both of the calculations (Figure 1). After lycopene applications, color change was calculated 11.712 for $\Delta E_{002}$. That means the color change was visually distinguished by all observers. The average value in the α-tocopherol group was obtained as 3.681 for $\Delta E_{002}$ and the value obtained in this group differs from the others (p<0, 05). There is no difference between proanthocyanidin, sodium ascorbate, green tea and white tea and control groups (p>0.05). (Table II).

$\Delta L_2$ mean values differ according to the groups (p <0.001). The lowest mean value was obtained in the lycopene group with -13.66. There is no difference between white tea and control, sodium ascorbate and green tea (p> 0.05). There is also a difference between the groups in the mean values of $\Delta a_2$ (p <0.001). The highest mean value was obtained in the lycopene group with 13.1. The difference between the other groups is presented in Table II. $\Delta b_2$ mean values also differ according to the groups (p<0.001). In $\Delta b_2$ average values, the highest value is in lycopene group with 8.46. The mean value of α-tocopherol also differs from other groups.

**Table II -** Fracture modes of four one-step adhesive materials (%).

| | $\Delta L_1$ | $\Delta a_1$ | $\Delta b_1$ | $\Delta E_{001}$ | $\Delta L_2$ | $\Delta a_2$ | $\Delta b_2$ | $\Delta E_{002}$ |
|---|---|---|---|---|---|---|---|---|
| **α-tocopherol** | 4.883 ± 3.794ab | -0.575 ± 0.563 | 0.033 ± 1.058 | 2.071 ± 1.308ab | -5.03 ± 1.01d | 2.67 ± 0.67d | 4.79 ± 1.39c | 3.681 ± 0.595a |
| **White Tea** | 4.883 ± 3.794ab | -0.575 ± 0.563 | 0.033 ± 1.058 | 2.071 ± 1.308ab | 0.23 ± 0.22a | -0.22 ± 0.11a | -0.03 ± 0.08a | 0.254 ± 0.106b |
| **Control** | 2.683 ± 2.384ab | -0.483 ± 0.484 | -0.267 ± 0.695 | 1.297 ± 0.794ab | 1.32 ± 1.59ab | -0.58 ± 0.21c | 0.16 ± 1.15a | 1.04 ± 0.503d |
| **Lycopene** | 1.508 ± 1.016a | -0.525 ± 0.347 | -0.342 ± 0.593 | 0.846 ± 0.471a | -13.66 ± 2.98c | 13.1 ± 2.96b | 8.46 ± 3.08b | 11.712 ± 1.992c |
| **Proanthocyanidin** | 6.6 ± 3.49b | -0.517 ± 0.537 | 0.058 ± 0.837 | 2.525 ± 1.347b | -0.46 ± 0.26b | -0.01 ± 0.28a | -0.65 ± 1.8a | 0.675 ± 1.02bd |
| **Sodium Ascorbate** | 6.525 ± 3.534b | -0.908 ± 0.557 | -0.108 ± 1.069 | 2.636 ± 1.344b | -0.48 ± 0.97ab | -0.12 ± 0.32a | 0.42 ± 0.86a | 0.586 ± 0.385bd |
| **Green Tea** | 4.883 ± 3.794ab | -0.575 ± 0.563 | 0.033 ± 1.058 | 2.071 ± 1.308ab | 0.16 ± 0.12a | -0.2 ± 0.1a | 0.13 ± 0.15a | 0.247 ± 0.096b |
| **p*** | 0.002 | 0.497 | 0.893 | 0.003 | <0.001 | <0.001 | <0.001 | <0.001 |

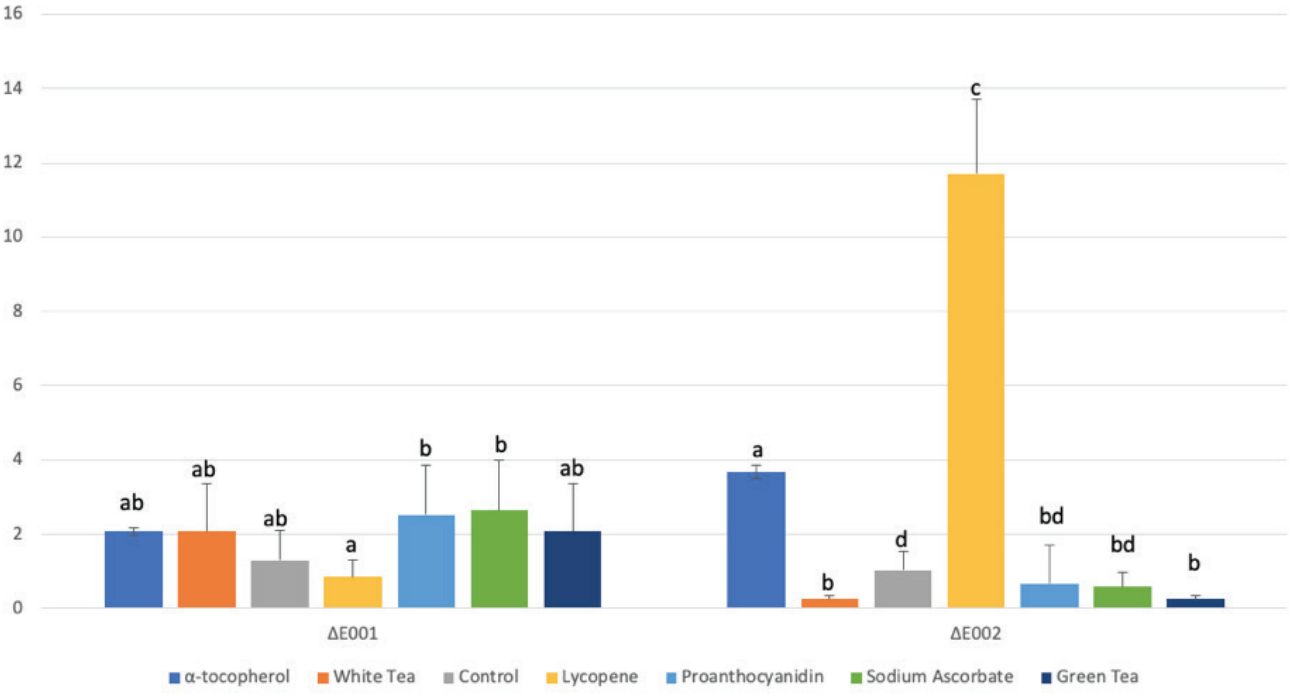



**Figure 1 -** Average and deviation graphic of $\Delta E_{001}$ and $\Delta E_{002}$ according to the groups.

## DISCUSSION

Dental aesthetics is an important issue. Tooth bleaching is one of the most preferred dental treatments. However, the most significant disadvantages of the procedure are the returning of the discoloration, repetition requirement of the bleaching treatment, hypersensivity and an undesired color result after the bleaching [28]. Diode laser of 940 nm is an effective adjunctive tool for reducing hypersensivity originated from high concentration H2O2 bleaching gel [29] but adhesive restorations following tooth bleaching might be required in such cases. A minimum of 2 weeks waiting period is recommended in the literature for the success of adhesive restorations [30]. The reason for this recommendation is that the hydrogen peroxide degradation continues after bleaching, and thus, the produced free radicals inhibit resin polymerization and weaken adhesion [31]. Therefore post-bleaching antioxidant application has been preferred since 1993 [32].

Several studies examined the effect of post-bleaching antioxidant applications on shear bond strength of composite restorations and as a result, they proved the positive effects of antioxidant applications [3, 10, 33]. However, there are limited studies in the literature that investigate the effects of antioxidants on color stability [6].

Sodium ascorbate is the most preferred and studied antioxidant in dental practice [34, 35]. Most of the studies investigated its post-bleaching effects on bonding, and its positive effects were confirmed on shear bond strength [36]. However, there is only one study examining the effects of sodium ascorbate on post-bleaching tooth color. This study has evaluated tooth color change in 169 patients who were applied sodium ascorbate following the endo-bleaching procedure and reported the color change on the surface of the tooth. As a result, no difference was reported in terms of color stability between sodium ascorbate group and the control group, and the use of it was reported to be safe [37]. In this study, sodium ascorbate group showed similar color stability to the control group which was not applied any post-bleaching antioxidant. There was no statistically significant difference between the control and sodium ascorbate groups ($p>0.05$). In this respect, the results of the present study are consistent with the literature.

The success of sodium ascorbate has led scholars to explore other antioxidants, and α-tocopherol has become one of the primary dental research foci [13]. α-tocopherol is most profusely found in nature and is responsible for the reversal of Vitamin E deficiency symptoms in humans [38]. Kavitha et al. evaluated the ten percentage α-tocopherol, and it has shown high efficacy in shear bond strength reversal of enamel and dentin submitted to a home-use bleaching treatment [39]. In 2009, Sasaki et al. compared the efficacy of two different antioxidizing agents in increasing the shear bond strength of bleached enamel and dentin, reporting that 10% α-tocopherol was successful, whereas 10% sodium ascorbate was not [12]. However, there is no study evaluating the effects of α-tocopherol solution on color stability of bleached teeth. In this study, it was found that the application of α-tocopherol solution provokes the discoloration which is clinically distinguished ($\Delta E_{002}=3.681$). And $\Delta L_2$ values had decreased, $\Delta a_2$ value was 2.67 and $\Delta b_2$ value was 4.79. These results can be explicated that after the application, tooth color was darkened, gone yellow and red. It can be correlated the naturel orange color of α-tocopherolin. Later, the researchers turned their direction to natural antioxidants, and proanthocyanidine was one of the first to come to mind [8]. The main reason for this is that proanthocyanidin is easy-to-obtain from grape seed extract and has bonding properties even better than the antioxidants, which were proven to improve the bonding such as sodium ascorbate [40]. However, bond improvement is not the sole criterion for the use of post-bleaching antioxidants; they should also not have any negative effect on color stability. In their study, Taneja et al. found that grape seed extract reduces post-bleaching discoloration [6]. However, proanthocyanidin (PAC) was also included in this study, an active ingredient of grape seed extract and it was observed a significant color change in the related group in our results. In this respect, the results are not consistent with this study. This difference might be explained by the differences in the procedure, such as concentration and duration, which are known to affect bond formation results in antioxidant procedures [41, 42]. No significant change was observed in $\Delta L_2$, $\Delta a_2$ and $\Delta b_2$ values. That indicates no yellowness or redness occured in tooth color and no study was found evaluating color stability following proanthocyanidin application in the literature.

Nowadays, researchers have a focus on white and green tea which have strong antioxidant capacity. But the main aim of this interest is evaluating the effects on bond strength of composite and bleached enamel or

dentine [14, 35]. In this study, the main goal is evaluating the effects on color stability and we found no difference between green tea, white tea and control groups based on $\Delta E_{002}$ values ($p>0.05$). $\Delta E_{002}$ value was calculated as 2.071 after application of green tea and white tea. These results can be related with their colorless nature and no distinguishable color difference occurred. Due to the new focus, there are only few research that consider the effects of green tea on color stability of dental materials like lithium disilicate,zirconia based ceramics and composite resin etc [43, 44]. But there is no study about the post-bleaching applications. When comparing the green tea, white tea is a subject of broad and current interest and studies are limited and focused it as a dentin bonding treatment agent [45]. Lycopene is a widely used antioxidant in medicine, and in the field of dentistry, its effects were examined only on periodontal healing [46, 47]. In this study, the most substantial color change was reported in Lycopene group. Moreover, $\Delta L_2$ values decreased while $\Delta a_2$ and $\Delta b_2$ values significantly increased. This result shows that the teeth in group 4 have darkened, became more yellow and redder. The redness may be associated with the red pigments in lycopene, as it is obtained from tomato extract. Its applicability to bleached teeth may be limited due to its significant effect on color stability; however, it may be useful to evaluate its effects on bond formation.

Limitations of this study could be listed as follows: assessing only one type of bleaching agent, and at one concentration level, not evaluating antioxidant form and application times and that no stratification approach was performed after the bleaching based color change to distribute the specimens equally in the antioxidant groups. Further antioxidant studies with clinical follow-up are required.

## CONCLUSION

Proanthocyanidin, sodium ascorbate, green tea and white tea are reliable post-bleaching antioxidants for maintaining color stability. The application of lycopene and α-tocopherol after bleaching cannot be recommended even if they have an antioxidant effect, due to the significant color change they caused

### Conflict Of Interest Statement

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