

# Effects of Two Different Colorant Solutions on the Color Stability of Bleached Enamel in Association with CPP-ACPF: An In Vitro Study

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## ABSTRACT

**Background:** One of the drawbacks of vital teeth bleaching is color stability. The aim of the present study was to evaluate the effects of tea and tomato sauce on the color stability of bleached enamel in association with the application of MI Paste Plus (CPP-ACPF).

**Materials and Methods:** Sixty enamel samples were bleached with 10% carbamide peroxide for two weeks then divided into three groups (A, B and C) of 20 samples each. After bleaching, the samples of each group were subdivided into two subgroups (n=10). While subgroups A1, B1 and C1 were kept in distilled water, A2, B2, and C2 were treated with MI Paste Plus. Then, the samples were immersed in different solutions as follow: A1 and A2 in distilled water (control); B1 and B2 in black tea; and C1 and C2 in tomato sauce for half an hour/day for seven days. Using a colorimeter, Teeth color measurements were recorded at baseline, after bleaching, staining, and polishing. Color changes were recorded according to the Vita shade guide and the CIE Lab system. Student's t-test was used to analyze differences between the subgroups at  $p < 0.05$ .

**Results:** Significant color changes were recorded for the tea group after staining, but not after polishing ( $p < 0.05$ ). No significant differences in color measurements were recorded between the subgroups of each group at all periods ( $p > 0.05$ ).

**Conclusion:** Only tea produced clinically perceivable color change of bleached enamel after staining as well as after polishing. MI Paste Plus did not affect enamel color change for all the groups.

**Key Words:** Carbamide peroxide, staining affinity, MI Paste Plus, colorimeter. . (J Bagh Coll Dentistry 2017; 29(2):1-6)

## INTRODUCTION

At-home vital tooth bleaching technique has become widely under focus as it has proven to be effective in improving the color of discolored teeth. Using 10% carbamide peroxide (CP) bleaching agent in a mouth guard for two weeks has been addressed to be more effective in whitening vital teeth compared with other bleaching techniques (1). However, teeth bleaching has shown some drawbacks and one of which is the regression of teeth whitening after bleaching (1,2).

This color regression has not been fully understood. Patients have been advised to follow some precaution measures in order to minimize this phenomenon. Clinicians have suggested that during and after the bleaching regimen, patients need not to consume darkly colored foods and drinks. Several researchers have evaluated the effect of different colored food materials such as: coffee, tea, cola, red wine, and dark colored fruits on the staining susceptibility of bleached teeth during and after the bleaching procedure (3-5).

Cortes et al. in 2013 (3) and Matis et al. in 2015 (4) showed that during the bleaching treatment, consuming dark drinks such as coffee, tea, cola and red wine could not minimize the effect of teeth bleaching.

However, Karadas and Seven in 2014 addressed that after bleaching, tooth color re-staining is significantly increased with red wine, cola, and tea solutions (5). Cortes et al. concluded that after bleaching red wine can produce more color changes than coffee (3).

Several researchers have reported the association of bleaching agents with changes in the properties of enamel such as; surface porosity and irregularities, decreasing microhardness, and demineralization (6-9). These changes have been suspected to cause increasing in dye accumulation into bleached tooth surface.

Clinicians have advised their patients to resume normal habits of consuming colored foods and drinks after the first 24-48 hours of bleaching. They have assumed that after bleaching the absorption and precipitation of calcium and phosphate from saliva can compensate some of those micro structure defects, and hence reducing the staining absorption of bleached teeth (10). On the other hand, several in vitro studies have supported the positive effects of fluoride and/or casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) on eroded and demineralized teeth surfaces (11,12). Treatment of bleached tooth surface with such agents can be also promising in decreasing bleaching agents' related adverse changes (7-9,13).

Until recently, few researchers have studied the effect of CPP-ACP (tooth mousse) or CPP-ACPF (MI paste plus, which contains 10% CPP-

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ACP and 0.2% NaF) on enamel staining susceptibility after bleaching<sup>(10,14)</sup>. While Singh et al. evaluated the effects of CPP-ACP or fluoride on tea stain absorption after 24 hours of at-home bleaching<sup>(10)</sup>, Imamura et al. used CPP-ACPF after in-office bleaching<sup>(14)</sup>. However, evidence based facts on the staining susceptibility of bleached enamel after bleaching are still insufficient. Therefore, the purpose of the present study was to investigate the effects of two different colorant dietary solutions (black tea and tomato sauce) on the enamel staining susceptibility after bleaching with 10% CP at-home bleaching agent.

In addition, evaluate the effect of MI paste plus (CPP-ACPF) on enamel staining susceptibility after bleaching. Thus, the null hypotheses tested in the present study were: 1) no color change can be seen when bleached enamel is immersed in black tea or tomato sauce and 2) surface treatment of bleached enamel with CPP-ACPF can produce no effect on its staining affinity to black tea or tomato sauce.

## MATERIALS AND METHODS

This study was approved by the Ethics Committee of the related University. Upon approval, 30 human premolars, extracted for orthodontic reasons, were collected. The teeth were cleaned of gross debris and polished with pumice. The teeth were divided into three groups (A, B and C) of ten teeth each. The roots of all the selected teeth were cut about 1mm below the cemento-enamel junction. Then, the crowns of the teeth were sectioned mesiodistally into 2 halves to produce 20 buccal and lingual enamel samples for each group.

The samples were mounted in plastic moulds with chemically cured acrylic resin, with the enamel surface facing upward. All the samples were bleached with a 10 % CP at-home bleaching agent (Opalescence PF, Ultradent Products, USA). The bleaching gel was applied on each sample for 8 hours/day. After daily bleaching, each sample was rinsed under running water for 10 seconds and stored in distilled water at room temperature for the rest of the day. This procedure was repeated for 14 consecutive days.

After 24 hours of the bleaching treatment, the samples of all the three groups were subdivided into two subgroups (n=10). Subgroups A1, B1 and C1 were stored in distilled water at room temperature. Subgroups A2, B2 and C2 were treated with casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF; GC MI Paste Plus, Ultradent Products Inc.). About 1 mm thick of the paste was applied with a microbrush

on the enamel surface and left for half an hour. Then, the paste was wiped off with dampened gauze and the samples were stored in distilled water for the rest of the day. The application of the paste was repeated for three consecutive days.

### Staining procedure

During the staining period, the samples of group A (A1 and A2) were stored in distilled water as control. The staining procedure was performed for group B and C after 24 hours from the last surface treatment with CPP-ACPF. Two types of colorant solutions were used to stain the samples of group B and C; black tea and tomato sauce as follow: B1 and B2 were immersed in black tea and C1 and C2 were soaked in tomato sauce. Tea solution was prepared by boiling 2 g of black tea (Ceylon tea, Akbar Brothers, Colombo 10, Sri Lanka) in 100 mL of distilled water for five minutes. The solution was then filtered to remove the tea from the infusion.

Tomato sauce solution was prepared by boiling one full table spoon of tomato paste (altunsa, Altunkaya ins, Beylerbeyi-Gaziantep, Turkey) in 100 mL of distilled water for five minutes. The pH of the tea and tomato sauce solutions was 5.95 and 4.98, respectively. The samples in each group were immersed in their corresponding solutions for half an hour/day. After the staining period, the samples were washed under running water for 10 seconds and kept in distilled water at room temperature for the rest of the day. This procedure was repeated with fresh staining solutions for seven consecutive days. After 24 hours, all samples were polished for 10 seconds each with a rubber cup in a low-speed hand piece and pumice to remove any extrinsic stains.

### Teeth color measurements

The color measurements were adopted objectively using a colorimeter (Vita Easy shade, Zahnfabrik; H. Rauter GmbH and Co, KG, Bad Sackingen, Germany). The measurements were performed for all the subgroups at four periods: at the baseline (T0), by the end of the 2 weeks bleaching period (T1), after 24 hours of the staining procedure (T2) and after polishing (T3). To ensure a standard sample spot measurement by the colorimeter, a custom-fabricated positioning template was fabricated for all the samples with a polyvinyl silicone putty material (Zetaplus, Zhermack, Rovigo, Italy). Each template had a 6 mm diameter spot facing the center of each sample for positioning the colorimeter tip.

The color measurements were evaluated according to the Vita classical shade guide

provided by the colorimeter and in reference to the CIE L\*a\*b\* parameters established by the Commission International de l'Eclairage in 1976 (15).

According to Vita shade guide tabs, the tabs are arranged from the lightest with their corresponding values starting from (B1=1) to the darkest (C4=16) as represented in Table 1.

**Table 1: Numerical values of Vita classic shade guide tabs (SGT)**

SGT	Value	SGT	Value
B1	1	A3	9
A1	2	D3	10
B2	3	B3	11
D1	4	A3.5	12
A2	5	B4	13
C1	6	C3	14
C2	7	A4	15
D2	8	C4	16

Based on the CIE L\*a\*b\* color system, the color of an object is located in a three-dimensional color space. The coordinate L\*, representing the lightness; a\*, represents shade and saturation in the green-red axis; and b\*, represents saturation in the blue-yellow axis. Color differences ( $\Delta L^*$ ,  $\Delta a^*$ , and  $\Delta b^*$ ) were calculated between the records after polishing (T3) and after bleaching (T1).

Total color difference ( $\Delta E^*$ ) was calculated at two different periods using the following equation (15):

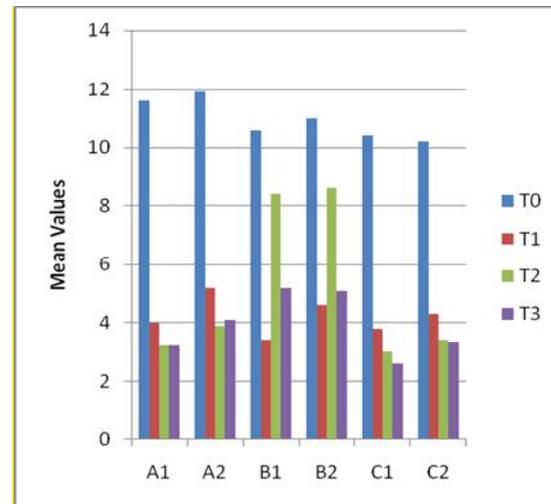
$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

$\Delta E^*1$  was calculated between the records after bleaching (T1) and after staining (T2), while  $\Delta E^*2$  between after bleaching measurements (T1) and after polishing (T3).

The results were analyzed using SPSS program (SPSS 19.0 for windows). One-way ANOVA and post hoc tests were used to evaluate differences among the groups. Student's t-test was used to analyze differences between the subgroups at  $p < 0.05$ .

## RESULTS

Mean values of Vita shade guide at the four measuring periods were represented in figure 1. No significant differences were recorded among the mean values of the shade guide at T0, T1 or T3 ( $p > 0.05$ ). However, at T2 significant differences were seen between both subgroups of group B compared with the other four subgroups ( $p < 0.05$ ). No significant differences were recorded between the subgroups of each of the three groups ( $p > 0.05$ ).



**Figure 1: Mean values of Vita shade guide for all subgroups at baseline (T0), after bleaching (T1) after staining (T2) and after polishing (T3).**

Table 2 presents the means (SD) of  $\Delta L^*$ ,  $\Delta a^*$ , and  $\Delta b^*$  for all groups. Significant differences in  $\Delta a^*$  were seen between the subgroups of the control and those of group C.

**Table 2: Means (SD) of  $\Delta L^*$ ,  $\Delta a^*$ , and  $\Delta b^*$  for all groups**

Groups	Mean(SD) $\Delta L^*$	Mean(SD) $\Delta a^*$	Mean(SD) $\Delta b^*$
A1	-1.74 (1.95)	1.09 (0.95)	-0.52 (2.50)
A2	-1.31 (2.36)	0.23 ab (0.91)	-1.53 (2.02)
B1	-2.07 (2.33)	0.22 cd (0.91)	-2.90 ab (3.28)
B2	-1.15 (2.40)	0.62 (1.28)	-1.47 (3.66)
C1	-0.76 (1.78)	1.01 ac (0.63)	-0.20 a (2.17)
C2	-0.57 (1.41)	1.13 bd (0.66)	-0.45 b (1.64)

Values with the same letters are significantly different at  $p < 0.05$

Table 3 presents the means (SD) of  $\Delta E^*1$  and  $\Delta E^*2$  for all subgroups. Mean values of  $\Delta E^*1$  showed significant differences between the subgroups of group B with the subgroups of group A and C ( $p < 0.05$ ). However, no significant differences were recorded between the subgroups of group C and those of group A ( $p > 0.05$ ). Mean values of  $\Delta E^*2$  for both group B and C showed no significant differences compared with the control group ( $p > 0.05$ ). Neither  $\Delta E^*1$  nor  $\Delta E^*2$  showed any significant differences between the subgroups of all of the three groups ( $p > 0.05$ ).

**Table 3: Means (SD) of  $\Delta E^*1$  and  $\Delta E^*2$  for all subgroups**

Groups	Mean (SD) $\Delta E^*1$	Mean(SD) $\Delta E^*2$
A1	3.30 (1.49)ab	3.36(1.84)
A2	3.40(1.90) cd	3.26(1.81)
B1	7.71(2.47)acef	4.92(2.08) ab
B2	7.78(2.97) bdgh	4.21(2.34)c
C1	2.12(2.21) eg	2.70(1.41)a
C2	2.60(2.60) fh	2.35(.99)bc

Values with the same letters are significantly different at  $p < 0.05$

## DISCUSSION

At-home bleaching technique with 10% CP has been accepted by the American Dental Association (ADA) assuring its safety and effectiveness<sup>(16)</sup>. However, teeth whitening stability after bleaching treatment is still a concern. Researchers have evaluated the effect of different colorant drinks such as; tea, coffee, cola, etc., on bleached enamel during and after bleaching. It has been reported that after bleaching, different colored diets may cause significant color changes<sup>(3,5,9)</sup>.

The two staining solutions used in the present study were selected as they are most commonly consumed in the Middle East countries. While black tea is a very popular beverage consumed on a daily basis, tomato sauce is regularly used as an indispensable food ingredient in each cuisine. And the staining time used (half an hour a day) was to simulate the time and frequency of consumption of these materials during the day.

In the present study, the recorded color differences in  $\Delta L^*$ ,  $\Delta a^*$ , and  $\Delta b^*$  after polishing, showed significant difference in  $\Delta a^*$  between the subgroups of the control and only for those of tomato sauce. However, no significant changes for  $\Delta L^*$  nor  $\Delta b^*$  were recorded between all groups. It has been reported that color change produced by bleaching procedure is more related to  $\Delta L^*$  and  $\Delta b^*$  parameters than  $\Delta a^*$ . Besides,  $\Delta a^*$  is very small compared with  $\Delta L^*$  and  $\Delta b^*$  which could be the reason for the different results of the studies performed under different protocols<sup>(17)</sup>. Interpretation of color change between different groups using  $\Delta E^*$  gives a more meaningful value than the other three color parameters<sup>(18)</sup>.

According to the results of the present study, after staining with tea both  $\Delta E^*1$  and Vita shade guide values for both subgroups were significantly higher than those of the control. However, after polishing both  $\Delta E^*2$  and Vita shade guide values for the tea group were not significantly different from the control group. Thus, the first hypothesis provided in this study

should be rejected for the tea group before polishing and accepted after polishing.

Tea produced significant external tooth surface staining which was not significant after mechanical staining removal. On the other hand, tomato sauce group showed no significant differences neither in  $\Delta E^*$  nor in Vita shade guide values before and after polishing compared with the control. Therefore, for the tomato sauce group, the first null hypothesis should be accepted at both measuring periods. It is well accepted that the value of  $\Delta E^*$  is important to determine teeth color changes clinically. It has been concluded that when the value of  $\Delta E^*$  is  $< 1$ , the difference is considered to be not perceivable clinically, while when  $\Delta E^*$  is  $> 3.7$ , color change is considered as easily visible, but when  $\Delta E^*$  is between 3.7 and 1, color difference is considered clinically acceptable<sup>(19)</sup>.

In the present study, the values for  $\Delta E^*$  for the tea group were the highest before and after polishing ( $\Delta E^* > 3.7$ ). However, both the control and tomato sauce groups recorded  $\Delta E^* < 3.7$ . In the present study, although tea did not produce significant difference in  $\Delta E^*$  compared with the control after polishing, its  $\Delta E^*$  value is considered clinically perceivable. Such a result is in accordance with other studies reported significant color changes of bleached enamel produced by tea<sup>(5,14)</sup>.

In the later studies and after bleaching, increasing color change was obvious by increasing the staining periods. Continued and frequent consumption of colored drinks can increase the staining susceptibility of bleached enamel<sup>(20)</sup>. Several studies have reported that tea has shown to have a high capacity to stain not only teeth<sup>(21)</sup> but also tooth-colored restorative materials<sup>(22)</sup> and denture base acrylic resins<sup>(23)</sup>.

Staining has a multi-factorial etiology with chromogens derived from dietary sources. Teeth color imparted is determined by the natural color of the chromogen<sup>(24)</sup>. It has been reported that teeth discoloration is influenced by low pH and food color rather than the dietary pigment alone<sup>(25)</sup>. In the current study, although tomato sauce was more acidic than tea (4.98 vs. 5.95), its staining affinity was less. Thus, staining affinity of a staining solution is correlated to its essence chromogens rather than to its acidity.

Several studies have reported that after bleaching, both fluoride and CPP-ACP can remineralize eroded enamel<sup>(6)</sup>, and enhance tooth roughness and microhardness<sup>(8,9)</sup>. On the other hand, using CPP-ACPF (MI paste plus) could produce smoother enamel surface and higher level of remineralization<sup>(11,12)</sup>. According to the

manufacturer, MI paste plus has not only the same benefits of regular MI paste, but also enhanced with 0.2% sodium fluoride (900 ppm) to further diminish demineralization.

In the present study, surface application of CPP-ACPF was intended to accommodate the assumed bleaching agents' related changes immediately after bleaching. The results of the present study showed that, no significant differences were seen between the subgroups of all of the three groups. Such a result requires the acceptance of the second null hypothesis that CPP-ACPF could not affect the staining absorption susceptibility for the stained groups. This result is in consistence with Ley et al.<sup>(26)</sup> who recorded no significant change in  $\Delta E^*$  after severe red wine staining of bleached enamel with or without fluoride application. The results are not in accordance with those reported by Singh et al.<sup>(10)</sup>. This could be related to two reasons; shorter immersion period into the tea solution and the storage media used during the procedure. In the later study, they have used tea solution for only ten minutes in only two different timings (after one hour and 24 hours of bleaching).

In the current study, the staining procedure was carried out for half an hour/day for seven days. Imamura et al. reported that increasing the immersion time in tea, bleached enamel treated with CPP-ACP can gradually re-stained<sup>(14)</sup>. Matis et al.<sup>(4)</sup> reported that even during bleaching, a positive but weak association between tooth whitening and diet may be produced when consuming large amounts of coffee/tea.

For the storage media, researchers have used artificial saliva in *in vitro* studies to simulate the natural salivary function, thus less evident detrimental effects of bleaching agents may be induced during and after bleaching regimen<sup>(3,27)</sup>. In the present study, the use of normal saline as a storage media was intended in order to restrict any potential effect other than that proposed to the use of CPP-ACPF. Cortes et al.<sup>(3)</sup> reported that during and after bleaching, enamel staining is effectively prevented by remineralization of the enamel with artificial saliva.

In the present study, no significant color change difference was recorded between the subgroups of the control group. After bleaching, enamel color regression for the control group was not affected by the application of CPP-ACPF. This color regression after at-home bleaching with 10% CP was also recorded by clinical studies as well as in *in vitro* studies<sup>(2,28)</sup>. Li et al.<sup>(29)</sup> reported that color regression of bleached enamel

can be recorded even in mineral containing environment.

Further investigations are still needed to identify the influence of prolong use of other colorant food materials on teeth whitening stability.

In conclusion and within the limitations of the present study, it can be concluded that:

1. Black tea can produce clinically perceivable color change of bleached enamel after staining as well as after polishing.
2. Tomato sauce did not affect the teeth whitening neither after staining nor after polishing.
3. MI Paste Plus did not influence neither teeth whitening regression nor staining susceptibility of the used colorant solutions.

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### المستخلص

اهداف البحث: عدم استقرار تبييض مينا الأسنان بعد استخدام التبييض البيئي للأسنان الحية هي واحدة من موقات هذا النوع من العلاج. الهدف من الدراسة الحالية هو تقييم تأثير الشاي وصلصة الطماطم على ثبات التبييض لمينا الأسنان بالترافق مع استخدام مادة MI Paste Plus. طريقة العمل: ستون عينة من مينا الأسنان تم تبييضها لمدة اسبوعين باستخدام 10% كاربوميد البيروكساييد ثم تم تقسيم العينات الى 3 مجموعات من عشرين عينة لكل مجموعة (A, B and C). بعد التبييض، كل مجموعة قسمت الى جزئين حيث تم تخزين (A1, B1 and C1) في الماء المقطرا والجزء الآخر (A2, B2, and C2) تم معالجتها بمادة MI Paste Plus. ثم وضعت عينات المجموعة الاولى (A) في الماء المقطر والمجموعة الثانية (B) في الشاي والمجموعة الثالثة (C) في محلول صلصة الطماطم لنصف ساعة يوميا ولمدة سبعة ايام متتالية. تم قياس التغير باللون للعينات باستخدام مقياس الالوان وذلك قبل التبييض، بعد التبييض، بعد التلوين بالسوائل الملونة، واخيرا بعد التلميع. التغير باللون تم قياسه اعتمادا على Vita shade guide وايضا CIE Lab system. تم تحليل الاختلافات احصائيا بين المجموعات الثلاث بقسميها باعتماد قيمة  $p < 0.05$ . النتائج: تم تسجيل اختلاف مؤثر بين العينات التي عوملت بالشاي ولكن ليس بعد عملية تلميعها ( $p < 0.05$ ). لم يسجل اي فرق بين قسيمي اي مجموعة من المجموعات الثلاث في جميع القراءات.

الاستنتاجات: محلول الشاي سبب تأثيرا ملحوظا على لون مينا الاسنان المبيضة بعد التلون بالسائل. مادة MI Paste Plus لم تؤثر على عملية التلون لجميع المجموعات. الكلمات المفتاحية: كاربوميد البيروكساييد، الميل للتلون، MI Paste Plus، جهاز قياس الالوان.