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Effect of Different Food Coloring Additives on the Color Stability of Microabraded, at-Home Bleached and Resin Infiltrated Tooth

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Objective: Color stability of at-home bleaching and resin infiltration is one of the main goals of these procedures. The primary aim of present study was to evaluate color stability of at-home bleaching and resin infiltration to four common colorants present in Indian foods (turmeric, beetroot, coffee and artificial food colorants).
Materials and Methods: 128 human maxillary central incisors were used in this study. Teeth were randomly divided into groups as (i) subjected and (ii) not-subjected to microabrasion. Further, the teeth in each group (abraded/non-abraded) were randomly distributed to four subgroups (16 teeth/subgroup). Subgroups were control, bleached, combined treatment with at-home bleaching followed by resin infiltration and infiltrated. After treatment procedures the teeth were immersed in

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the four different food colorants. Objective photographic color change evaluation from pre-/ post staining was done using the CIEDE2000 formula.
Statistical Analyses: Data were analyzed using parametric and nonparametric tests (α =0.05).
Results: Turmeric solution caused significantly (P < 0.05) highest post-staining values b*and ΔE values in Combine-Rx group and Infiltration groups.
Conclusions: Subsequent discoloration of esthetically treated teeth does not necessarily depend on the type of treatment but on the coloring additives present in the diet, of which turmeric has the highest discoloration potential.
Clinical Significance: Turmeric is one of the essential ingredients in Indian foods therefore, both patients and operator must be aware that resin infiltrated teeth can discolored from regular diets that contains turmeric as coloring additives.

Keywords: At-home bleaching; dental fluorosis; food colorants; microabrasion; resin infiltration; turmeric.

1. INTRODUCTION

Retaining the color achieved after bleaching and resin infiltration procedures is one of the main objectives of these treatment procedures. Several in-vitro and clinical studies investigated the stability of the resultant color gained with at-home bleaching treatment [1–5]. Ritter et al [6]. showed that the color achieved with at-home bleaching with 10% carbamide peroxide remained stable for approximately 10 years. Resin infiltration in combination with at-home or in-office bleaching procedure has been recommended to mask white spot lesions of the anterior teeth and the management of fluorotic teeth [7–9]. Very little information is available regarding the color stability achieved by this treatment combination when Indian food additives are used. An in-vitro 4-wk study concluded that bleached and resin infiltrated teeth prevented the diffusion of the food additive pigments, thereby hindering the discoloration effect [7]. Reports in literatures on color stability following resin infiltration procedure contrast that of earlier study that showed deterioration of the tooth color [10,11]. However, these in-vitro studies utilized only coffee solutions for evaluating the susceptibility of resin infiltrated teeth to staining and not other food coloring additives.

The primary objective of the present study was to evaluate the color stability of maxillary central incisors exposed to four common colorants present in Indian foods (turmeric, beetroot, coffee and artificial food colorants) after treatment by combined at-home bleaching and resin infiltration. The secondary objective was to compare the color stability with different treatment protocols.

2. MATERIALS AND METHODS

The sample size calculation that was based on a power analysis, were performed using a statistical software (G Power 3.1.9.2 software, Universität Kiel, Germany). Based on the effect size of 0.38 determined from the results of previous studies on color stability of resin infiltrated teeth [10,11], an effective size of 124 teeth will have power greater than 0.95 with a 0.05 one-sided significance level to detect statistical significant difference in outcome (number of groups = 4, ANOVA: Fixed effects, omnibus, one-way). However, 128 extracted caries free human maxillary central incisors were utilized in the study to make provision for 3% loss. The approval of the institutional ethics committee was obtained (CSICDSR/IEC/0124/2019) prior to teeth collection. A tooth was excluded if it had any non-carious lesions, restorations, root resorption, discoloration or fractures. Using a computer-generated numbers, the teeth were randomly assigned to the following two main groups; teeth subjected (n=64) and not-subjected (n=64) to 3 times microabrasion (Opalustre, Ultradent Inc., UT, US).

2.1 Microabrasion

Approximately 1 mm thick layer of slurry was applied over the labial surface of each tooth, and using rubber prophy cup attached to a micromotor handpiece, a moderate pressure was applied for 60 seconds at a speed of 500 RPM. Paste from the teeth was suctioned and then rinsed. This procedure was repeated 3 times on each tooth.

Following microabrasion, the teeth in each main group (abraded/non-abraded) was further

randomly distributed to the following four subgroups (16 teeth/subgroup). Control subgroup was immersed in coloring solution without subjecting to any further treatment procedure (Control subgroup). Bleached subgroup was treated with at-home bleaching gel containing 16% carbamide peroxide gel (VOCO Perfect Bleach, VOCO GmbH, Germany) for 8hrs overnight for 2 weeks (Bleached group). Combined-treatment subgroup was treated by at-home bleaching as per earlier protocol followed by resin infiltration (Icon™, DMG Chemisch-PharmazeutischeFabrik GmbH, Hamburg, Germany) two weeks after bleaching procedure according to manufacturer's instructions (combined-Rx group). Infiltrated subgroup received only resin infiltration procedure as per previously mentioned protocol (Infiltrated group).

2.2 Home Bleaching

Using a custom-made bleaching tray, syringe gel containing 16% carbamide peroxide (VOCO Perfect bleach; VOCO GmbH, Cuxhaven, Germany) was applied over the teeth for 8 hours overnight. Then teeth were rinsed and stored in artificial saliva (Wet Mouth, ICPA Health Products Ltd, Mumbai, India). This procedure was repeated for two weeks (14 nights).

2.3 Resin Infiltration

Resin infiltration was performed with the ICON™resin infiltration kit (DMG Chemisch Pharmazeutische Fabrik GmbH). Etch gel was applied over tooth surface for 2 minutes, and excess material was removed followed by rinsing and air drying for 30 seconds. Etching procedure was repeated once. Following rinsing and air drying of the tooth surface, ethanol solution was applied and allowed to penetrate for 30 seconds followed by air drying. This is followed by application of two coats of resin infiltrant for 3 minutes for each coating. Infiltrant was light-cured for 40 seconds after removal of excess infiltrant. Final polishing was done using Super snap polishing kit (Shofu Inc., Kyoto, Japan).

After completion of each treatment procedure, the teeth were preserved in artificial saliva at room temperature for 48 hrs prior to baseline photographic recording. This was done to avoid photographing dehydrated teeth since the teeth is constantly bath in saliva in vivo, and to mimic clinical scenario since the patients were being instructed to avoid stainable food substances for 48-hrs after treatment. All teeth were photographed using a Nikon D5300 (Nikon Corp, Tokyo, Japan) camera under controlled lighting and at the same distance from the maxillary incisors using a tripod. The same light source, camera, and exposure settings were used for post treatment photos. A focal length of 55mm with flash and auto white balance was used as the camera settings for all photographs.

Four different food colorant solutions were freshly prepared daily as follows. Turmeric solution was prepared by dissolving 5 g of turmeric (Aachi Masala Foods Pvt Ltd, Chennai, India) in 500 ml of water. Coffee solution was prepared by dissolving 50 g of coffee powder (Nescafe classic, Nestlé India Ltd, Gurgaon, India) in 500ml of water. Beet-root solution was prepared by dissolving 50g of beet-root grated into small particles in 500 ml of water. The artificial food colorant (Gold-Camel synthetic food color preparation, Chennai, India) solution was prepared in similar way to turmeric solution. All the prepared solutions were boiled for 10 minutes and allowed to cool prior to tooth immersion.

The 16 teeth in each of the 4 subgroups of the two main groups (abraded/non-abraded) were distributed to the four coloring solutions (4 teeth/colorant). To facilitate treatment with the colorants, 8 teeth (4 abraded and 4 non-abraded) drawn from similar subgroup of the two main groups (abraded/non-abraded) and assigned to the same colorant (Turmeric, coffee, beet root or artificial-colorant) were mounted in one edentulous maxillary typodont (Fig. 1). Flowchart of the experimental procedure is shown in Fig. 2. The 8 teeth in each typodont were immersed into their respective food colorant for 5 minutes 3 times daily (Morning, afternoon and evening) for 10 days. After each immersion the teeth were cleaned under running water for 3 minutes. Following each immersion episode, the teeth were returned back to the artificial saliva storage in room temperature. After 48-hrs of completion of the 10 days coloring treatment, post-treatment photographs were taken with the same settings as the pre-treatment photographs.

2.4 Objective Color Evaluation

Objective analysis of the photographs was performed by a blinded operator as described in our previous publication [12]. Briefly, the pre- and post-colorant treatment photographs were opened in Adobe Photoshop CS5 ("Ctrl + O"; Adobe Inc, San Jose, CA, USA). "View > show >

grid" was used to superimpose a grid on the photographs. "Edit > preferences > guides > grids," and "slices > grid line every" were chosen, and values of 4000 mm and 5 were input into the "gridline every" and "subdivision," respectively, to change the size of the grid to 70 × 70 mm. This grid size was chosen to enable the maxillary central incisor to be incorporated into 3 × 3. Each of these grids was numbered from right to left, as shown in Fig. 3. The layer panel was made visible by selecting "windows > layers," and the layers were unlocked by double-clicking the lock symbol to right of the "background." The image was "zoomed in" using the "zoom tool (Z)" to have only one maxillary incisor in the viewing window. All photographs were analyzed using similar settings. To minimize the errors due to different ambient lighting conditions, the photographs were taken with a gray card. From each of the grids on the incisor, one random point was selected. From the "windows" menu, the "info" tab was selected, and the pointer was moved to the selected points to obtain the "x" and "y" coordinates and CIE L*a*b values. CIE L*a*b values were calculated using the "color sampler (I)" tool by right-clicking on selected points and choosing the "lab color" option with a dimension of 1 × 1 pixels. All the CIE L*a*b values from the 9 grids were averaged for each tooth. To estimate the ΔE values (color difference between pre – and post coloring agent exposure) the points selected in the pre-coloring agent exposure photographs were precisely relocated in the post-coloring agent exposure images using the reference "x" and "y" coordinates and were also averaged as mentioned earlier. The ΔE values were obtained using the CIEDE2000 formula with an online ΔE calculator (http://www. colormine.org/delta-e-calculator/Cie2000).

$$\Delta E_{00} = \sqrt{\left(\frac{\Delta L'}{K_L S_L}\right)^2 + \left(\frac{\Delta C'}{K_C S_C}\right)^2 + \left(\frac{\Delta H'}{K_H S_H}\right)^2 + R_T \left(\frac{\Delta C'}{K_C S_C}\right)^2 \left(\frac{\Delta H'}{K_H S_H}\right)^2}$$

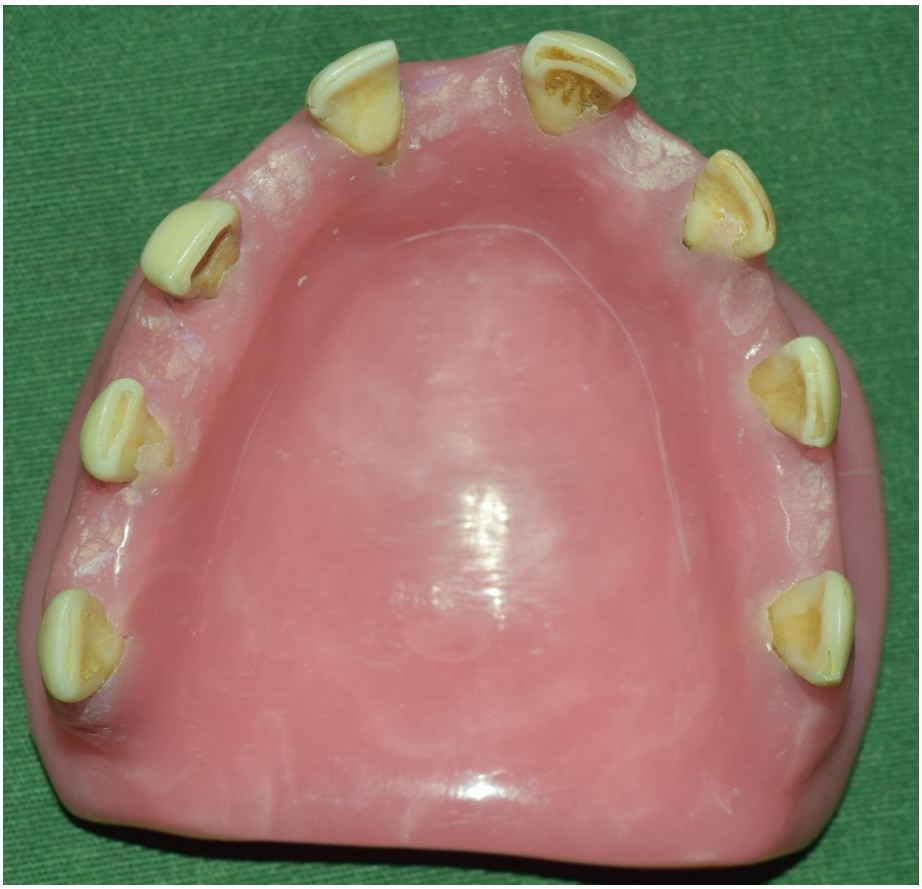

Fig. 1. Maxillary incisors mounted in acrylic edentulous arch

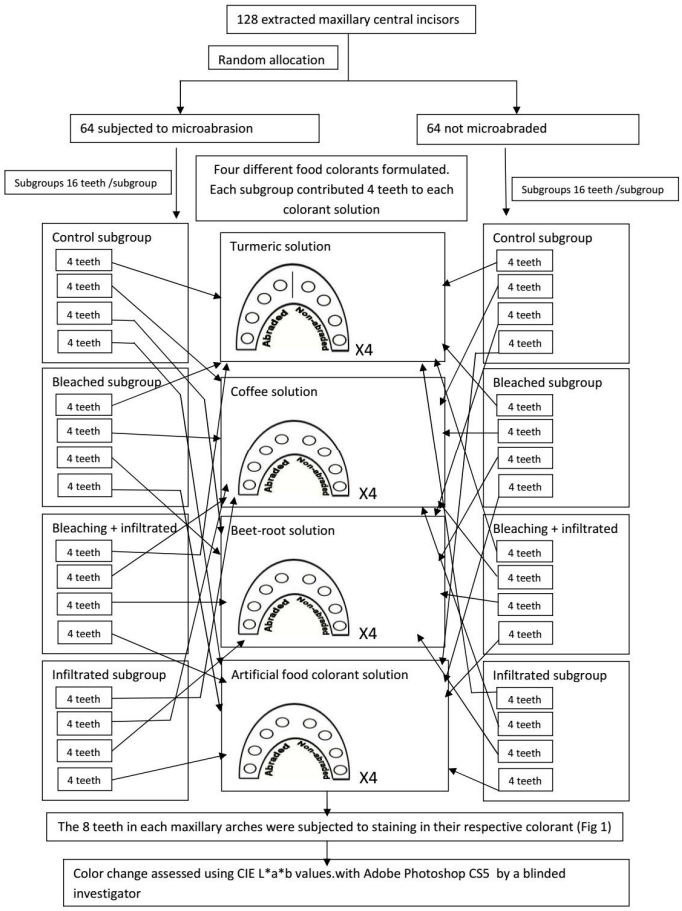

Fig. 2. Experiment flowchart

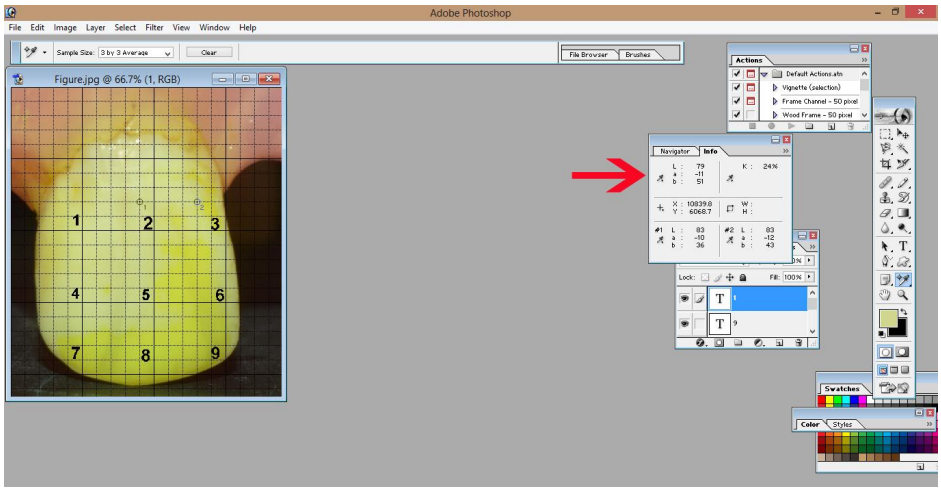

Fig. 3. Screenshot of Adobe Photoshop software showing different stained points marked on the maxillary central incisor. The arrow mark shows the measured L*and b* values in subdivisions 2 and 3 of the grid

2.6 Statistical Analysis

Statistical analyses were performed using SPSS 23.0 (IBM Corp, USA). Normality of the data was assessed using the Shapiro-Wilk test, as data could not be assumed to be distributed normally. L color values followed normal distribution and mean values of pre- and post-treatment L values were analyzed with paired t-test. ΔE (color differences), b* between pre- and post-treatment images were compared using the Kruskal-Wallis test. For all tests, the probability level for statistical significance was at α = 0.05.

3. RESULTS

Fig. 4 shows the mean post staining L, b* and ΔE values with no significant difference for microabraded and non-microabraded teeth. Table 1 and 2 depicts the average pre-/post-staining L, b* and ΔE values for each tooth in the four different treatment regimen and in coloring media respectively. The control group (subjected to only staining solution) has significantly lower post-staining L value. Turmeric solution caused significantly higher post-staining b* and ΔE values. Difference in post-staining L, b* and ΔE values for the four treatment groups with staining solutions is shown in Table 3. Significantly (P< 0.05) lowest L was recorded for artificial colorant in bleached group. Turmeric solution caused significantly highest post-staining values b*and ΔE values in Combine-Rx group and Infiltration groups.

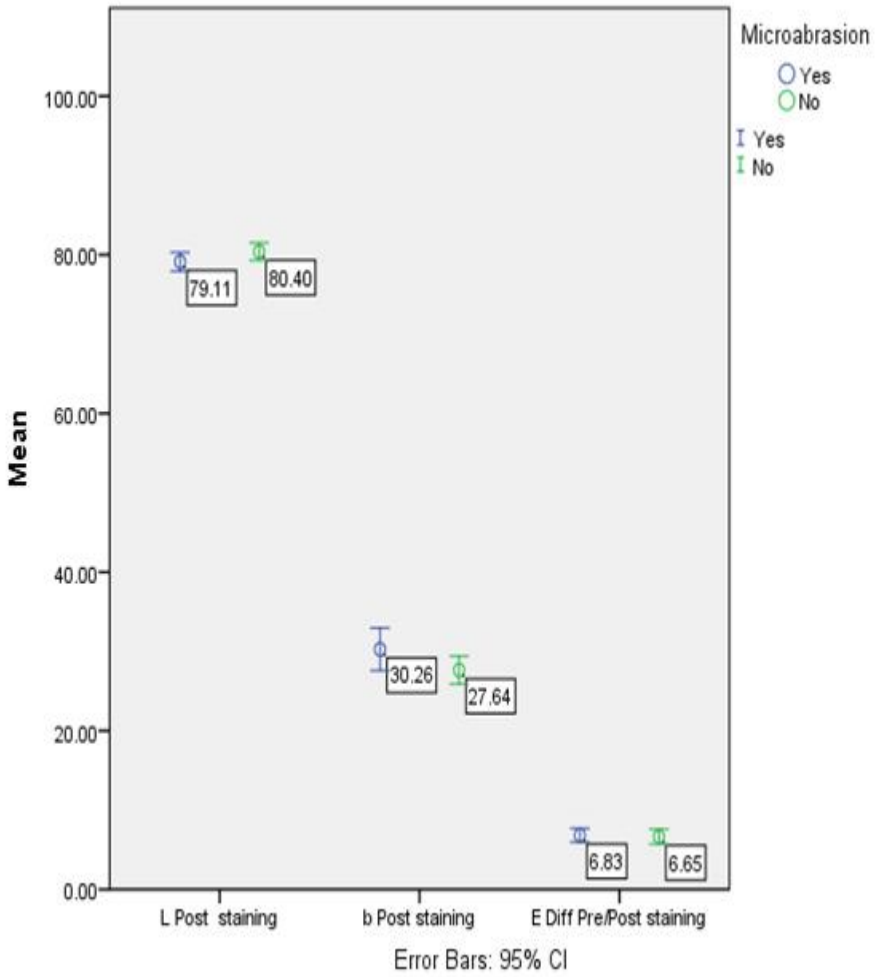

Fig. 4. Mean (with error plots) of L, b* and ΔE post staining values for microabraded and non-micrabraded teeth

Table 1. Mean L, b* and ΔE values for the four treatment groups

	Mean L value		Mean b* value		Mean ΔE
	Pre-staining ± SD	**Post- staining value ± SD**	**Pre- staining ± SD**	**Post- staining value ± SD**	**ΔE between pre-/post-staining ± SD**
Coloring solution only (n^ = 32)	81.30 ± 2.37*	76.53 ± 3.24 $^{A\,\Delta}$	20.72 ± 4.94 *	27.40 ± 10.64 $^{a\,\Delta}$	6.36 ± 2.90 ^{b}
At-home bleaching only (n^ = 32)	80.43 ± 4.09*	79.09 ± 3.88 $^{B\,\Delta}$	21.17 ± 5.02 *	27.18 ± 7.16 $^{a\,\Delta}$	5.97 ± 3.25 ^{b}
At-home bleaching + resin infiltration (n^ = 32)	83.62 ± 2.79*	78.29 ± 3.50 $^{C\,\Delta}$	20.26 ± 4.89 *	30.63 ± 10.87 ^{a} *	8.38 ± 4.66 ^{b}
Resin infiltration only (n^ = 32)	85.91 ± 3.52*	85.09 ± 2.76 $^{D\,\Delta}$	20.14 ± 5.09*	30.58 ± 6.94 $^{a\,\Delta}$	6.22 ± 2.79 ^{b}

^n – Number of samples
SD – Standard deviation
$^{a, A}$- A difference in letters across vertical direction indicates a statistically significant difference; difference in case (lowercase vs uppercase) indicates two different statistical tests (upper case – One-way ANOVA test; lower case – Kruskal-Wallis test).
$^{*\Delta}$ – Different symbols across horizontal direction indicates a statistically significant difference in paired t- test

Table 2. Mean L, b* and ΔE values for the different coloring media

	Mean L value		Mean b* value		Mean ΔE
	Pre- staining value ± SD	**Post- staining value ± SD**	**Pre- staining value ± SD**	**Post- staining value ± SD**	**ΔE between pre-/post-staining ± SD**
Turmeric (n^ = 32)	82.72 ± 2.97*	80.76 ± 3.01$^{A\,\Delta}$	18.59 ± 5.03*	35.05 ± 11 $^{a\,\Delta}$	9.37 ± 4.42 ^{e}
Beetroot (n^ = 32)	82.36 ± 3.87*	78.83 ± 4.50 ^{A}*	22.27 ± 4.52*	29.67 ± 10.08 ^{b}*	6.21 ± 2.15 ^{f}
Coffee (n^ = 32)	84.27 ± 3.79*	80.42 ± 4.89 $^{A\,\Delta}$	19.77 ± 4.27*	23.78 ± 4.74 $^{c\,\Delta}$	4.98 ± 1.86 ^{g}
Artificial food colorant (n^ = 32)	81.84 ± 4.45*	78.94 ± 5.63 $^{A\,\Delta}$	21.74 ± 5.17*	27.47 ± 5.17 $^{d\,\Delta}$	6.41 ± 3.73 ^{h}

^n – Number of samples
SD – Standard deviation
$^{a, A}$- A difference in alphabets letter across vertical direction indicates a statistically significant difference; difference in case (lowercase vs uppercase) indicates two different statistical tests (upper case – One-way ANOVA test; lower case – Kruskal-Wallis test).
$^{*\Delta}$ – Different symbols across horizontal direction indicate a statistically significant difference in paired t- test

Table 3. Mean L, b* and ΔE values for the different coloring media in the four treatment regimens

Treatments	Staining solutions	Mean ± SD post staining L value	Mean ± SD post staining b* value	Mean ± SD ΔE between pre-/post-staining
Coloring solution only	Turmeric (n^ = 8)	79.59 ±1.05^{A}	20.67 ±3.79^{b}	5.04 ±1.59 ^{a}
	Beetroot (n^ = 8)	74.99 ±2.62^{B}	37.51 ±16.73^{c}	6.48 ±1.61 ^{a}
	Coffee (n^ = 8)	74.96 ±3.02^{C}	26.97 ±2.98^{d}	6.20 ±0.81 ^{a}
	Artificial food colorant (n^ = 8)	76.62 ±3.65^{D}	24.47 ±4.44^{e}	7.72 ±5.24^{a}
At-home bleaching only	Turmeric (n^ = 8)	79.58 ±1.79^{E}	32.54 ±4.98^{f}	8.02 ±2.49^{j}
	Beetroot (n^ = 8)	80.76 ±2.68^{F}	22.65 ±3.42^{g}	3.36 ±1.12^{k}
	Coffee (n^ = 8)	81.28 ±2.29^{G}	20.74 ±4.25^{h}	4.31 ±1.56^{l}
	Artificial food colorant (n^ = 8)	74.69 ±4.47^{H}	33.04 ±5.11^{i}	8.10 ±4.05^{m}
At-home bleaching + resin infiltration	Turmeric (n^ = 8)	79.86 ±2.67^{I}	47.46 ±2.50^{n}	15.43 ±1.14^{r}
	Beetroot (n^ = 8)	75.68 ±2.15^{I}	29.00 ±2.26^{o}	8.00 ±1.52^{s}
	Coffee (n^ = 8)	79.22 ±4.36^{I}	22.67 ±6.48^{p}	5.54 ±2.59^{t}
	Artificial food colorant (n^ = 8)	78.43 ±3.49^{I}	23.40 ±3.46^{q}	4.57 ±1.82^{u}
Resin infiltration only	Turmeric (n^ = 8)	84.04 ±3.51^{J}	39.56 ±6.92^{v}	9.02 ±3.31^{z}
	Beetroot (n^ = 8)	84.15 ±2.20^{J}	28.67 ±4.47^{w}	6.67 ±1.37^{aa}
	Coffee (n^ = 8)	86.14 ±1.20^{J}	25.14 ±2.04^{x}	3.96 ±1.40^{ab}
	Artificial food colorant (n^ = 8)	86.03 ±3.26^{J}	28.99 ±2.95^{y}	5.25 ±1.95^{ac}

^n – Number of samples
SD – Standard deviation
$^{a, A}$- A difference in alphabets letter across vertical direction indicates a statistically significant difference; difference in case (lowercase vs uppercase) indicates two different statistical tests (upper case – One-way ANOVA test; lower case – Kruskal-Wallis test)

4. DISCUSSION

The stability of the resultant color following esthetic treatment of discolored teeth with either at-home bleaching and/or resin infiltration has been a matter of concern. Previous studies have reported contrasting results on this issue [6-11]. However, none of these studies investigated the effects of Indian food coloring additives on the color stability of these recommended esthetic treatments of discolored teeth. Thus the primary objective of the present study was to evaluate the color stability of at-home bleaching and resin infiltration for Indian food colorants. These treatment options are the current esthetic conservative treatments for stained fluorotic teeth [13,14]. In this study the control group was non-microabraded group with no esthetic treatment procedure to permit examination of the influence of the microabrasion on discoloration potential of food colorants. In the present study microabrasion did not significantly influence the potential of the food colorants to discolor teeth.

Results of the present study on staining of resin infiltrated teeth are in agreement with the report of previous investigations. Previous in-vitro studies on color stability of resin infiltration reported a significant discoloration from food staining and this was reversible [15-18]. However, the staining potential of Indian foods on teeth subjected to microabrasion, at-home bleaching and resin infiltration has not been investigated previously, so there are no previous literatures to compare with the present observation. Nevertheless, the Adobe Photoshop CD5 software that was used for CIE L*a*b* assessment in the present study has been used in other studies evaluating color change following esthetic treatments [12,19], thus authenticating the present observation.

It was observed in the present study that teeth treated with combined at-home bleaching and resin infiltration and with resin infiltration only had significantly higher b* and ΔE values than those treated by at-home bleaching only. This may be attributed to the fact that the resin infiltrant used in the present study is a TEGDMA based resin matrix with increased water sorption and decreased color stability, and as such the possibility of absorbing colorants may be high [16]. This was evident in the results of the present investigation that showed more staining of the treatments involving resin infiltration by turmeric solution than by the other colorants. However, the present study also showed turmeric solution to possess significantly high discoloration potential compared to the other colorants. Thus one may attribute the staining of the resin infiltrated treatments more to the high discoloration potential of the turmeric solution than to the quality of the resin infiltrant (increased water sorption). This argument is supported by the observation in Table 1 where there was no significant difference in the level of discoloration (ΔE) among the four treatment modalities, while Table 2 showed significantly higher staining potential by the turmeric among the four colorants. Furthermore, in this study b*value was the most affected parameter with turmeric indicating more yellowness of the tooth in agreement with previous review work [20]. Earlier experiments has shown food staining of resin infiltrated teeth to be reversible by repolishing or bleaching [15,16], thus we believed that turmeric staining could also be reversible. This may need to be confirmed by a research study. Turmeric is one of the essential ingredients in Indian foods therefore, both patients and operator must be aware that resin infiltrated teeth can discolored from regular diets that contains turmeric as coloring additives.

5. CONCLUSION

Based on the result of the present study, it can be concluded that the subsequent discoloration or the color stability of an esthetically treated teeth does not necessarily depend on the type of treatment but on the coloring additives present in the diet, of which turmeric has the highest discoloration potential.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The approval of the institutional ethics committee was obtained (CSICDSR/IEC/0124/2019) prior to teeth collection.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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