



## Evaluating the Efficiency of Humic Acid to Remove Stain from Artificial Teeth



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### Abstract

**Objective:** to assess the color stability of artificial teeth after immersion in different staining solutions and to determine the effect humic acid on removing stain from teeth. **Background:** Old denture wearer needs effective, easy-use and safe denture-cleaning material.

**Material and Methods:** Sixty maxillary incisors were divided into the three groups according to staining solution as tea (T), cherry juice (CJ) and cola (C). Baseline color measurements were performed with a spectrophotometer. Specimens were immersed in a staining solutions for 14 h (2 h \_ 7 days) and then second color measurements were performed. Each group was further divided into four sub-groups according to denture cleanser as Corsodyl (Cr), Corega tablet (Ct), humic acid (H) and distilled water (W) (n=5). Specimens were immersed in denture cleansers for 8h and third color measurements were made. Thus, the weekly simulation period was completed. This cycle was repeated 12 times to simulate a 3-month time period and measurements were performed. Color differences were calculated and data were analyzed with 2-way repeated measures ANOVA and Tukey tests.

**Results:** The highest color difference after staining was noted in T. After the 12-times repeated immersion beverage and cleanser cycle, discoloration of teeth stained in beverages and cleaned with different solutions was demonstrated. H and W were more effective than others for the teeth stained in T and Cj. However there was no difference among the cleaning solutions for teeth stained in C.

**Conclusions:** Humic acid could be used as an alternative “natural” solution for denture-cleaning agent.

**Keywords:** Humic acid; Denture cleanser; Complete denture

### Introduction

Despite major advances in dentistry and dental prosthesis, removable dentures are still commonly used for oral rehabilitation of older edentulous people. Artificial teeth used as an essential part of dentures can be made of porcelain or acrylic resin. Acrylic resin is the most widely used material for manufacturing artificial teeth due to its superior properties<sup>[1]</sup>.

Discoloration of artificial teeth may be caused by composition, wear, hygiene of patient and exposure to staining solutions<sup>[2]</sup>. The extent of discoloration in the oral cavity may be associated with dietary habits<sup>[3]</sup>. Moreover, denture-cleaning agents that are indicated for denture overnight immersions cause color change of acrylic resin

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teeth<sup>[4]</sup>. Color change can be assessed by colorimetry, which is based on the digital expression of the color perceived from the object<sup>[5]</sup>. The American Dental Association (ADA) recommends the use of CIE L\*a\*b system for assessing chromatic differences<sup>[6]</sup>.

Efficient and easy cleaning procedures for dentures are important for maintaining good oral and general health of the patients<sup>[7-9]</sup>. Mechanical cleaning requires a degree of manual dexterity that is often lacking, especially among older patients<sup>[10-12]</sup>. The chemical denture cleaning is preferred due to ease in use<sup>[13]</sup>.

Chemical denture cleansers are classified into five groups as alkaline peroxides, alkaline hypochlorites, acids, disinfecting agents and enzymes.



Humic acid has recently been introduced as an alternative denture-cleaning agent. The effect of humic acid to remove microorganisms from denture base material was reported<sup>[14]</sup>. Humus is that organic portion of soil that remains after millions of years of microbial decomposition of plants and vegetation. Humic acid consumption and treatment has been linked to several health benefits such as its antiviral, antimicrobial and detoxifying properties.

The purpose of this study was to assess the color stability of acrylic resin denture teeth after immersion in different staining solutions and to determine the effect of humic acid experimental solution on removing stain from acrylic resin denture teeth.

### Materials and Methods

Artificial teeth, three staining solutions and three denture-cleansing agents were used in the present study. Sixty maxillary central incisors of shade D2 (Samed, Eciyes Dental, Turkey) were used. Teeth were randomly divided into three equal groups according to staining solution as tea with sugar, cherry juice and cola. Each group was further divided into four subgroups according to denture-cleansing protocol as Corsodyl, Corega tabs, Experimental solution and distilled water (control group) (n = 5).

Before staining, after staining and after denture cleansing protocols, color measurements were performed and ΔE values were calculated.

### Color Measurements

Before immersing in staining solutions, CIE L\*a\*b\* color coordinates of each specimen were recorded with the use of a clinical spectrophotometer (VITA Easyshade Compact, VITA Zahnfabrik, Bad Säckingen, Germany). The contact probe tip of VITA Easyshade Compact is ~5 mm in diameter and, during the measurement process, the tooth is illuminated by the periphery of the tip, directing the light from white LEDs into the tooth surface. Each specimen was stored in distilled water at 37 ± 1 °C for 24 h before color measurements. A silicon mold and a positioning transparent jig were prepared for each brand of teeth to standardize the repetitive color measurements. Specimens were placed into the silicone mold and measurements were repeated 3-times for each specimen, CIE L\*a\*b\* values were recorded.

### Staining and Denture Cleansing Procedures

Tea (Lipton Yellow Label Tea, Unilever, Turkey), cherry juice (Pinar Dairy Products INC. Turkey), cola (The Coca-Cola Company, USA) and were used as staining solutions in this study. Tea solution was prepared by mixing 4 g of tea in 300 ml boiling water for 10 min and 10 g of white sugar was added. Twenty specimens were used for each staining solutions and each group was randomly subdivided into four groups for evaluating the denture cleansing protocols. Each specimen was stored in a separate plastic container and the containers were coded according to the staining solution and denture cleanser.

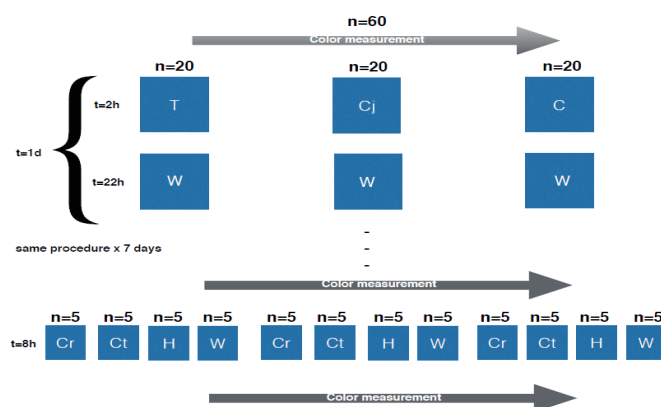
The specimens were immersed in staining solutions for 14 h to simulate the weekly exposure time (2 h X 7 days) with the beverages<sup>[15]</sup>. After the storage, the specimens were taken out of the solutions and they were rinsed with distilled water and air-dried. Then, the color measurements were repeated as described

above. After that, specimens were immersed in 200cc denture cleansers that were prepared according to the manufacturers' instructions or distilled water (control group) for 8 h. The details of denture-cleaning agents were shown in Table 1.

**Table 1:** Details of denture-cleaning agents

Denture-cleaning agent	Manufacturer	Active ingredient
Corsodyl	GlaxoSmithKline Consumer, Health Group, Canada	0.2% chlorhexidine gluconate
Corega Tabs	GlaxoSmithKline, Brentford, United Kingdom	Sodium carbonate, sodium carbonate peroxide.
Experimental humic acid solution	NA	0.3% humic acid, distilled water

Then, they were rinsed with distilled water and air-dried and the third color measurements were performed. In this manner, the weekly procedure was completed (Figure1). Subsequently, the specimens were immersed in staining solutions preceding the immersion in denture cleansers as described previously. This cycle was repeated 12-times to simulate the 3-month usage of denture. The color measurements were repeated at the end of the 4<sup>th</sup>, 8<sup>th</sup> and 12<sup>th</sup> cycles<sup>[15]</sup>.



**Figure 1:** Color measurement, staining and denture cleansing procedure cycle for a week. T: tea with sugar, Cj: cherry juice, C: cola, W: distilled water, Cr: Corsodyl, Ct: Corega tablet solution, H: Humic acid solution.

The CIE L\*a\*b\* color differences between the baseline–1st week and–3rd month were calculated for each specimen with the following formula:

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

where DL\*, Da\* and Db\* indicate the differences between the baseline and staining solution immersion and baseline and denture cleanser immersion. DE value >5.5 was considered clinically unacceptable and DE value >2.6 was considered as perceptible to the human eye<sup>[16]</sup>.

### Statistical Analysis

Normality of the data distribution was checked by the Shapiro–Wilks test and parametric tests were chosen since the data were distributed normally. Two-way repeated measures ANOVA was used for analysis and post-hoc comparisons were performed by using the Tukey test when significance was detected. Values of p < 0.05 were accepted as statistically significant.

## Results

The mean values and standard deviations of  $\Delta E$  after staining, 1<sup>st</sup> week and 3<sup>rd</sup> month period are presented in Table 2. There are statistically significant differences between  $\Delta E$  after staining and 3<sup>rd</sup> month period for all the groups except teeth immersed in cherry juice solution and cleaned with distilled water. When the staining ability of solutions was evaluated, tea showed significantly higher baseline-staining  $\Delta E$  values compared to cherry juice and cola. At the end of 1<sup>st</sup> week, it was showed that there was no significant difference among the stain removal efficiency of denture cleansers for all of the beverage groups. After 3<sup>rd</sup> month period the  $\Delta E$  of teeth exposed to tea and cherry juice and immersed in either corsodyl or corega were higher than humic acid or distilled water; however, there was no statistically significant difference of  $\Delta E$  exposed in cola in different denture cleansers.

**Table 2:** Means and standard deviations of  $\Delta E$  values

Beverage	$\Delta E$ 1 (Staining)	Denture cleanser	$\Delta E$ 2 (1st week)	$\Delta E$ 3 (3rd month)
	Mean (SD)		Mean (SD)	Mean (SD)
TEA	4.05 (0.97) <sup>*.a</sup>	Corsodyl	3.16 (0.90) <sup>A.b</sup>	3.86 (1.82) <sup>D.b</sup>
	4.13 (1.32) <sup>*.c</sup>	Corega	4.24 (0.94) <sup>A.d</sup>	5.70 (1.26) <sup>D.d</sup>
	4.91 (1.87) <sup>*.e</sup>	Humic acid	4.13 (1.90) <sup>A.f</sup>	1.48 (0.76) <sup>E.g</sup>
	4.59 (1.53) <sup>*.h</sup>	Distilled water	2.84 (1.70) <sup>A.i</sup>	1.77 (1.75) <sup>E.i</sup>
CHERRY	1.79 (0.49) <sup>#.a</sup>	Corsodyl	3.22 (1.12) <sup>B.b</sup>	5.07 (1.66) <sup>F.c</sup>
	2.22 (1.13) <sup>#.d</sup>	Corega	4.10 (0.56) <sup>B.e</sup>	4.12 (1.72) <sup>F.e</sup>
	1.65 (0.51) <sup>#.f</sup>	Humic acid	3.50 (1.29) <sup>B.g</sup>	2.62 (1.66) <sup>G.g</sup>
	1.63 (0.39) <sup>#.h</sup>	Distilled water	4.24 (1.14) <sup>B.i</sup>	1.39 (0.41) <sup>G.i</sup>
COLA	3.08 (4.06) <sup>#.a</sup>	Corsodyl	6.84 (1.52) <sup>C.b</sup>	6.55 (4.30) <sup>H.b</sup>
	2.54 (1.41) <sup>#.c</sup>	Corega	5.31 (2.01) <sup>C.d</sup>	6.95 (1.51) <sup>H.d</sup>
	2.16 (0.66) <sup>#.e</sup>	Humic acid	4.19 (0.97) <sup>C.f</sup>	5.12 (1.59) <sup>H.f</sup>
	2.28 (2.70) <sup>#.g</sup>	Distilled water	4.91 (1.92) <sup>C.h</sup>	3.49 (1.05) <sup>H.h</sup>

Same symbols (\*#) shows no statistically significant color difference after staining among the beverages

Same capital letters shows no statistically significant difference within each beverage group in the same column. Same lower letters shows no statistically significant difference in the same row.

## Discussion

In the present study the effects of denture cleansers and the beverages on the color stability were investigated and the values obtained after staining, 1-week and 3-month storage times were compared.

It is clear that three of the investigated beverages in the study cause staining on the artificial teeth of dentures. Ruyter et al.<sup>[17]</sup> described discoloration of  $\Delta E > 3.3$  as no longer clinically acceptable levels, therefore the results in the present study may be clinically noticeable.

It was evaluated that total beverage consumption drops steadily with age, besides this daily beverage consumption for people aged 71 or older was around 1,5 grams<sup>[18]</sup>. Three different beverages which are the regular soft drinks preferred by elders were tested in the study. The maximum color difference ( $\Delta E$ ) after staining was noted in tea solution. This result is in agreement with many authors<sup>[19-21]</sup> due to higher polarity components of tea (yellow colorants). The effect of sugar on color difference

of restorative materials due to the sticky effect of sugar on the staining was reported previously<sup>[21]</sup>.

According to the findings from the results of this study, the color of samples changed with time except the samples that immersed in tea and cleaned with either humic acid or distilled water in 3rd month period. It is well known that immersing denture into the distilled water alone is not enough to remove microorganisms from dentures. Therefore it may be concluded that humic acid is an efficient material to remove tea stain from artificial teeth.

After the 12-times repeated immersion beverage and cleanser cycle, it was demonstrated that the color changes of teeth in cola and cherry juice was changed. This result may be due to the low pH of the beverages that is a contributing factor to change in the color of the materials in longer-term period. It has been demonstrated that denture cleansers increase the surface roughness of acrylic resins<sup>[22]</sup>. A rougher surface may cause the acrylic resin to be more prone to staining. Furthermore, Paranhos et al.<sup>[4]</sup> reported that immersion of acrylic resin specimens in denture cleansers caused a noticeable color change even if they were not exposed to staining solutions. Therefore, in the present study, increased  $\Delta E$  values of artificial teeth after a 3-month simulation period can be explained by the effect of denture cleansers on the surface roughness and color change of acrylic resins.

3<sup>rd</sup> month period  $\Delta E$  of teeth immersed in cherry juice and cleaned with either humic acid and distilled water lower than that cleaned with either corega tablet and corsodyl solutions. Thus it can be concluded that there is an effect of humic acid on removing cherry juice stain from artificial teeth.

The previous study<sup>[14]</sup> showed that humic acid solution significantly reduced the microorganisms on dentures. However, beside the effectiveness of the introduced experimental solution, the side effects of the solution, such as discoloration or surface deterioration of dentures, were not evaluated in the study. In the present study the effect of humic acid on removing stain from denture teeth was evaluated. These results lead our ongoing studies focusing on the development of the experimental denture-cleaning agent.

## Conclusion

Humic acid solution is an effective experimental cleaning solution for removing stain from non-acidic beverages. Therefore it can be used as an alternative “natural” solution for denture-cleaning agent.

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