

## COLOR STABILITY OF CONVENTIONAL, 3D PRINTED, AND MILLED DENTURE TEETH AFTER IMMERSION IN COLORING AGENTS: AN *IN-VITRO* STUDY

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**Objectives:** This study investigates the color stability of denture teeth made from different materials when subjected to various staining solutions.

**Methods:** Forty-five A1 shade upper central incisors were divided into three groups: prefabricated, 3D printed, and milled denture teeth (N=45). Each group was further divided into three subgroups, with teeth immersed in coffee, wine, and artificial saliva for up to 120 days (n=5).  $\Delta E$  values for color change were measured using an Optishade colorimeter.

**Results:** The type of teeth, type of solution, and immersion time had a significant effect on color stability ( $p < 0.001$ ). Interactions between the three variables were all significant ( $p < 0.001$ ). Conventional denture teeth showed slight color changes with saliva ( $\Delta E = 1.256$  by day 120), moderate changes with wine ( $\Delta E = 2.893$ ), and more significant changes with coffee ( $\Delta E = 5.208$ ). Milled denture teeth exhibited no color changes with saliva, noticeable changes with wine ( $\Delta E = 5.359$ ), and moderate changes with coffee ( $\Delta E = 3.137$ ). 3D printed denture teeth showed no color changes with saliva, but increased changes with wine ( $\Delta E = 2.533$ ) and highly significant changes with coffee ( $\Delta E = 10.546$ ). Coffee consistently caused the highest  $\Delta E$  values, especially in 3D printed teeth.

**Conclusions:** The study found that material choice in denture teeth fabrication significantly affects color stability, with milled teeth showing the highest color stability and 3D-printed denture teeth exhibiting the least color stability. In addition, coffee has been found to be the most chromogenic staining agent.

**Keywords:** 3D printed, Color change, Computer-aided design, Denture, Polymethyl methacrylate, Staining.

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**Conflicts of interest:**

The authors declare no conflicts of interest.

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## **STABILITÉ DE COULEUR DES DENTS PROTHÉTIQUES CONVENTIONNELLES, IMPRIMÉES EN 3D ET FRAISÉES APRÈS IMMERSION DANS DES SOLUTIONS COLORANTES: UNE ÉTUDE IN-VITRO**

**Objectifs:** Cette étude étudie la stabilité de la couleur des dents prothétiques fabriquées à partir de différents matériaux lorsqu'elles sont soumises à diverses solutions de coloration.

**Méthodes:** Quarante-cinq incisives centrales supérieures de teinte A1 ont été divisées en trois groupes : dents préfabriquées, imprimées en 3D et fraisées (N = 45). Chaque groupe a ensuite été divisé en trois sous-groupes, les dents étant immergées dans du café, du vin et de la salive artificielle pendant une période allant jusqu'à 120 jours (n = 5). Les valeurs  $\Delta E$  pour le changement de couleur ont été mesurées à l'aide d'un colorimètre Optishade.

**Résultats:** Le type de dent, le type de solution et le temps d'immersion ont eu un effet significatif sur la stabilité de la couleur ( $p < 0,001$ ). Les interactions entre les trois variables étaient toutes significatives ( $p < 0,001$ ). Les dents prothétiques conventionnelles ont montré de légers changements de couleur avec la salive ( $\Delta E = 1,256$  au jour 120), des changements modérés avec le vin ( $\Delta E = 2,893$ ) et des changements plus significatifs avec le café ( $\Delta E = 5,208$ ). Les dents prothétiques fraisées n'ont montré aucun changement de couleur avec la salive, des changements notables avec le vin ( $\Delta E = 5,359$ ) et des changements modérés avec le café ( $\Delta E = 3,137$ ). Les dents prothétiques imprimées en 3D n'ont montré aucun changement de couleur avec la salive, mais des changements accrus avec le vin ( $\Delta E = 2,533$ ) et des changements très significatifs avec le café ( $\Delta E = 10,546$ ). Le café est constamment à l'origine des valeurs  $\Delta E$  les plus élevées, en particulier dans les dents imprimées en 3D.

**Conclusions:** L'étude a révélé que le choix du matériau dans la fabrication des dents de prothèse affecte considérablement la stabilité des couleurs, les dents fraisées présentant la stabilité de couleur la plus élevée et les dents de prothèse imprimées en 3D présentant la stabilité de couleur la moins grande. De plus, le café s'est avéré être le colorant le plus chromogène.

**Mots clés:** Impression en 3D, Changement de couleur, Conception assistée par ordinateur, Prothèse, Polyméthacrylate de méthyle, Coloration.

## Introduction

The incidence of total edentulism has exhibited a discernible reduction over the years. Nevertheless, there is an escalation in the demographic of individuals experiencing complete tooth loss, attributable to heightened life expectancy consequent to advancements in healthcare [1]. Edentulism poses complications such as challenges in chewing food, resulting in compromised health, diminished aesthetic appeal, and speech difficulties. These factors collectively contribute to physical impairment impacting the overall health of the affected individual [2]. Polymethylmethacrylate (PMMA) resin, following its clinical validation in the late 1930s, has been widely used as an optimal material for denture base, as well as denture teeth. [3]. While PMMA has its limitations, its widespread use is supported by a combination of strengths rather than depending on one perfect characteristic. Its enduring popularity is particularly evident in fulfilling aesthetic criteria, where the judicious application of clinical expertise, coupled with meticulous selection and arrangement of artificial acrylic teeth, contributes to its efficacy [3]. Digital technology has revolutionized the field of dental practice leading to the creation of dentures using computer-aided design and manufacturing (CAD/CAM) workflow [4]. Additionally, recent years have seen a significant increase in the production of dental materials using these advanced techniques [5]. There are two main techniques for digital manufacturing denture teeth: additive, which involves 3D printing, and subtractive, where milling processes are employed [6]. Denture teeth can be fabricated through subtractive manufacturing by milling a pre-polymerized resin blank. Subsequently, either 3D-printed or milled denture teeth are bonded to the denture base using a suitable adhesive or attaching them through cold or heat polymerization methods. The pre-polymer-

ized acrylic resin blocks, generated under elevated heat and pressure conditions, yield a densely compacted resin with presumed diminished microporosities and reduced release of residual monomers [7]. Color stability becomes evident optical property when contemplating the substantial number of patients requiring prosthodontic treatment due to the growing lifespan of the people [8], maintaining color stability is crucial for achieving optimal esthetics in denture teeth which can affect patient contentment with his denture and long-term quality of life. Staining of teeth can occur due to both internal and external factors. External staining is typically caused by the accumulation of plaque or coloration from consuming substances such as coffee, tea, and tobacco smoke on the tooth surface [9]. On the other hand, internal staining happens due to the physical and chemical changes within a substance, leading to an increased uptake of colorants, enhanced reactivity to these substances, and higher water absorption. Factors like the level of polymerization, the amount of monomer and filler used, the size of the filler particles can affect the color stability [9]. This study aims to evaluate color change of conventional, 3D printed and milled denture teeth while being immersed in three types of coloring solutions. The null hypothesis is that there is no significant color change among conventional, milled, and 3D printed denture teeth.

## Material and methods

The Ethics Committee of Saint Joseph University of Beirut-Health Science Centre, approved the protocol (Tfemd/2024/9).

To determine the sample size, a power analysis was performed using G\*Power software for Repeated Measures ANOVA analysis, taking into consideration power=80%, alpha=5% error, and effect size=0.40. The minimum sample size required was 4 teeth per group (36 in total). It

was decided to increase the sample size beyond the minimum.

A total of forty-five upper central incisors (N=45), all shade A1, were sourced from various materials: Conventional (Ivoclar Vivodent, Bendererstr. 2, Schaan, Liechtenstein 9494, Liechtenstein), 3D printed (Saremco Print CROWNTEC, Gewerbestrasse 4-CH-9445 Rebstein, Switzerland) by a Dentrattec 3D printer (Figure 1), and milled denture teeth (Yilink medical, No. 57, Innovation Park, No. 1 Ruike Road, Shuanggang Economic and Technological Development Zone, Jinnan District, Tianjin, China). Subsequently, the specimens underwent meticulous cleaning and polishing to eliminate any debris or surface contaminants.

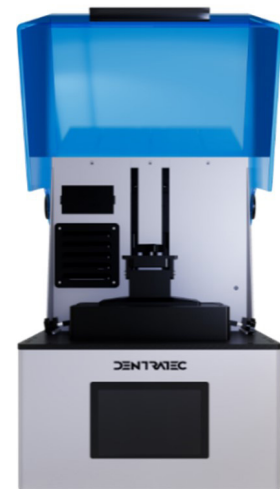


Figure 1. Dentrattec 3D printer used to manufacture 3D printed denture teeth

In adherence to specific inclusion criteria, the upper central incisors were obtained from a dental supplier, ensuring each type (conventional, milled, 3D printed) met stringent standards: absence of visible defects or damage, consistent shade and translucency, uniform size and shape within each type, application of identical staining solutions (coffee, red wine, and artificial saliva) across all groups, use of a designated colorimeter for color measurement, and implementation of standardized cleaning processes.

Exclusion criteria were established to eliminate teeth with visible

defects, different shades or translucencies within types, varied sizes or shapes within types, use of different resin materials, staining solutions, or color measurement techniques for distinct groups, exposure to staining agents, and any history of repair or restoration.

The maxillary central incisors were organized into three groups of fifteen teeth each consisting of on their manufacturing type, and within each group, further categorized into three subgroups of five teeth (n=5) each based on the colorants used: coffee, wine, and artificial saliva, maintaining consistency across the testing parameters.

The artificial teeth will be immersed in the selected coloring solution and placed in three separate containers filled with artificial saliva (pH=6.8), coffee (Nescafe Matinal, Nestlé S.A., avenue Nestlé 55, 1800 Vevey, Switzerland). and wine (Reserve du Couvent, Chateau Ksara, Nakhle Hanna Bldg, Charles Malek Avenue, Tabaris, Beirut, Lebanon). The coffee solution was prepared by mixing two teaspoons of coffee with 150 ml of room temperature mineral water. The color of each tooth will be measured using a Optishade spectrophotometer (Smile Line, St-Imier, Switzerland) as in figure 2 before immersion and for five time periods: 7, 14, 21, 30, 40 and 120 days. A silicone mold will be prepared for stabilization of the artificial tooth while measuring the shade, so that the determination of the shade will always be from the same area of the tooth as figure 3 demonstrates.



Figure 2. Smile Line Optishade colorimeter.

Table 1. National Bureau of Standards (NBS) ratings.

NBC Unit	Critical remarks of color difference	
0-0.5	Trace	Extremely slight change
0.5-1.5	Slight	Slight change
1.5-3	Noticeable	Perceivable change
3-6	Appreciable	Marked change
6-12	Much	Extremely marked change
12.0 Or more	Very much	Change to another color

The color stability of each tooth will be calculated using the CIE Lab\* color space. " It includes the three-color dimensions: L\* (lightness), a\* (red-green), and b\* (yellow-blue), which allows for a more accurate and uniform measurement of color. The L\* dimension ranges from 0 (black) to 100 (white), while the a\* and b\* dimensions can range from -128 to +128, representing different hues of colors [10].

Before taking measurements, Optishade colorimeter was calibrated following the manufacturer's guidelines, and teeth were cleaned with distilled water to eliminate superficial stains. In addition, solutions were changed every week.

The color change of each tooth will be calculated by subtracting the baseline color measurement (T0) from the color measurement at the end of each time already set. This formula can be used to calculate the total color change ( $\Delta E_{ab}$ ).

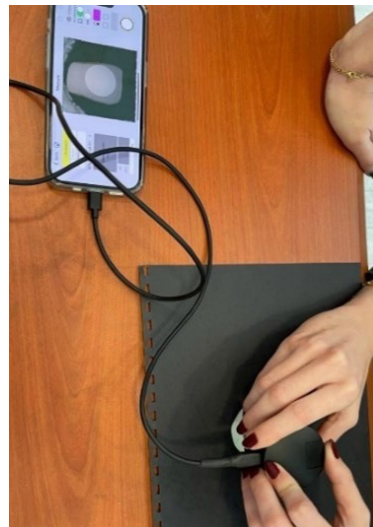


Figure 3. Color change measurement

$$\text{Equation (1): } \Delta E_{ab} = (1)$$

To derive the National Bureau of Standards unit (NBS), the color difference degree was multiplied by 0.92, as indicated by Equation (2): NBC unit =  $\Delta E \times 0.92$ . This system serves as a metric for assessing color stability, providing insight into the perceptibility of color changes by the human eye [10]. NBS ratings are showed in table 1.

The statistical analysis of the data was conducted using Rstudio Version 2023.12.1. A significance threshold of  $p < 0.05$  was employed. Three-way ANOVA was used.

A mixed linear model was used to evaluate the effects of factors such as the type of fabrication, the staining solutions in and the number of days on the color stability of the teeth.

## Results

The mean color changes ( $\Delta E$ ) in NBC units, for the three types of teeth (Conventional, milled and 3D printed), after immersion in three staining solutions (Wine, Saliva and Coffee) for 7, 14, 21, 30, 40, and 120 days, are summarized in Table 2.

In evaluating the color stability of diverse types of denture teeth, we observe distinct patterns of change over time. For conventional denture teeth, there are slight color changes with saliva, with  $\Delta E$  reaching 1.256 by day 120. Similarly, color differences with wine increase steadily, reaching  $\Delta E$  of 2.893 by day 120. The most significant change occurs with coffee, with  $\Delta E$  reaching

Table 2. Color changes ( $\Delta E$ ) over time according to the type of teeth and staining solution (NBC unit)

Type of teeth	Type of solution	Day 7	Day 14	Day 21	Day 30	Day 40	Day 120
Conventional	Wine	0.471	0.471	0.672	1.339	1.339	2.893
	Saliva	0.109	0.522	1.050	1.050	1.050	1.256
	Coffee	1.185	1.228	1.607	1.607	1.607	5.208
Milled	Wine	0.206	4.042	4.869	5.359	5.359	5.359
	Saliva	0.000	0.000	0.000	0.000	0.000	0.000
	Coffee	0.000	0.000	0.000	0.000	0.823	3.137
3D printed	Wine	0.643	1.836	2.176	2.176	2.176	2.533
	Saliva	0.000	0.000	0.000	0.000	0.000	0.000
	Coffee	3.511	5.013	5.005	5.147	7.296	10.546

5.208 by day 120. Milled denture teeth show no color changes with saliva, maintaining  $\Delta E$  of 0.000 from day 7 to day 120. However, with wine, there is a noticeable increase in color differences, reaching  $\Delta E$  of 5.359 by day 120, and an increase of 3.137 is observed with coffee. For 3D printed denture teeth, there are no color changes with saliva, with  $\Delta E$  remaining at 0.000 from day 7 to day 120. There is an increase in color differences with wine, reaching  $\Delta E$  of 2.533 by day 120. The most significant change is observed with coffee, where  $\Delta E$  increases from 3.511 to 10.546 by day 120.

The marginal means in figure 4 summarize these color changes according to the type of teeth, revealing that 3D printed denture teeth show a consistent and pronounced increase in  $\Delta E$  values over time, with the most significant change on day 120.

The marginal means in figure 5 summarize the color changes according to the type of solution immersed in over different days. Examining each solution individually reveals distinct trends. Coffee exhibits a consistent and steady increase in  $\Delta E$  values over time, with the highest values observed on day 120. In contrast, saliva demonstrates consistently low  $\Delta E$  values across all days, indicating minimal color changes.

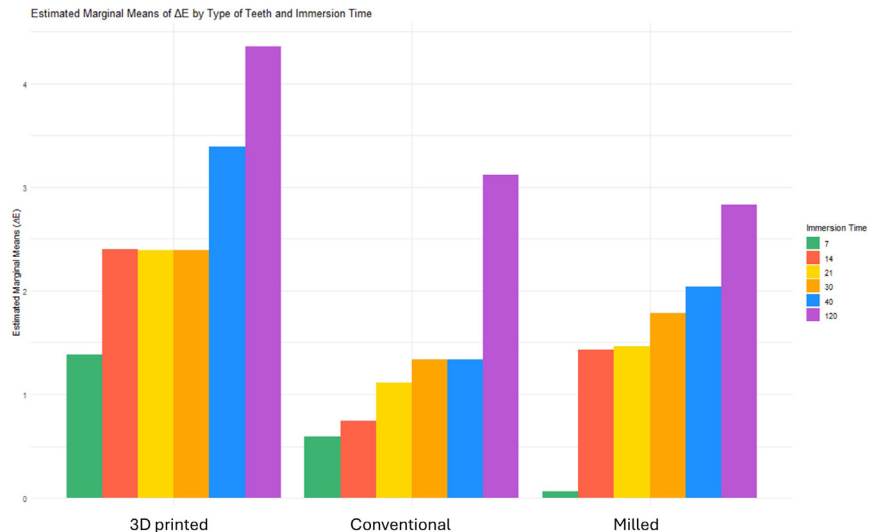
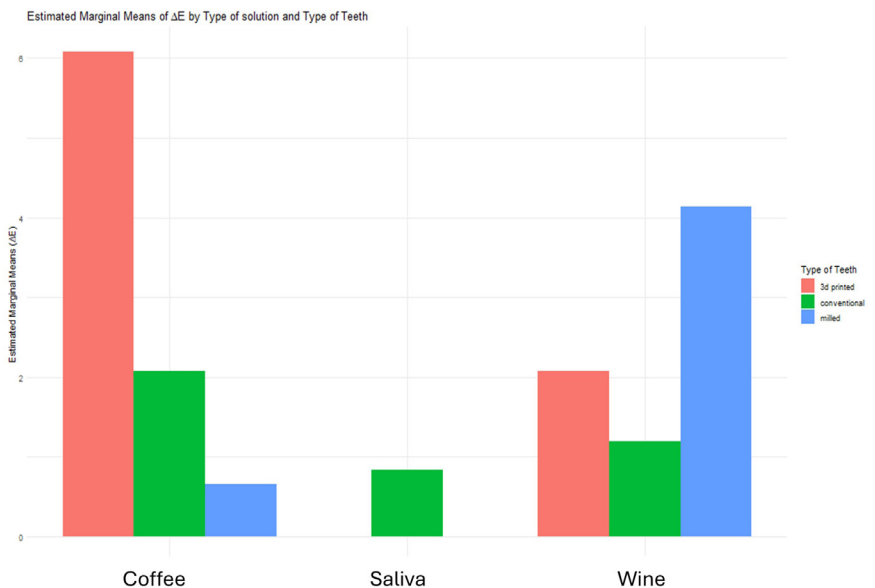
Figure 4. Estimated Marginal Means of  $\Delta E$  by Type of Teeth and Immersion TimeFigure 5. Estimated Marginal means of  $\Delta E$  by Type of Solution and Immersion Time

Figure 6 illustrates the marginal means of the color change  $\Delta E$  induced by different staining solutions (coffee, saliva, and wine) across various fabrication methods. Each solution's impact varies across teeth type, with notable observations discernible. When exposed to coffee, 3D printed samples exhibit the highest  $\Delta E$  values, indicating a significant color change, followed by moderate changes in conventional samples, and the least changes in milled samples.

A linear model was performed (to determine whether there is any statistical significance between the value of  $\Delta E$  and the type of teeth; the immersion time; and the type of solution as independent variables, as well as between their interaction terms. The value of the confidence interval used for the test was  $\alpha = 0.05$ . The results are presented in Table 3. The results reveal significant effects of teeth type, solution type, immersion time, and their interactions on the response variable. Type of teeth demonstrates a highly significant influence ( $p < 0.001$ ), indicating notable differences in the mean response among tooth types. Similarly, type of solution exhibits a highly significant effect ( $p < 0.001$ ), reflecting significant differences in the mean response among distinct types of solutions. Immersion time also shows an extremely significant effect ( $p < 0.001$ ), suggesting substantial variations in the mean response across different time intervals. Furthermore, interactions between type of teeth and type of solution, type of solution and immersion time, and type of teeth, type of solution, and immersion time are all highly significant ( $p < 0.001$ ), underscoring the combined influence of these factors on the response variable. Overall, this analysis reveals that the change in length ( $\Delta E$ ) is significantly influenced by the type of fabrication, the liquid type, and the day of measurement, with notable interactions between these factors.

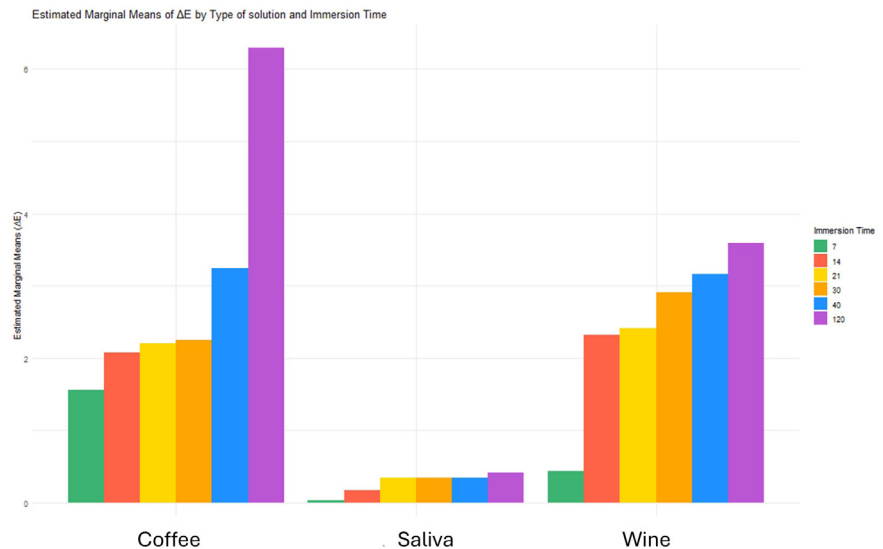


Figure 6. Estimated Marginal Means of  $\Delta E$  by Type of Solution and Type of Teeth

Table 3. Three-Way ANOVA for  $\Delta E$

Source of Variation	Sum Sq	Mean Sq	NumDF	DenDF	F value	p value
Type of teeth	33.516	16.758	2	36	23.1285	3.470e-07 ***
Type of solution	129.913	64.957	2	36	89.6504	1.044e-14 ***
Immersion time	188.031	47.738	5	180	51.9025	< 2.2e-16 ***
Type of teeth x type of solution	190.953	47.738	4	36	65.8864	4.607e-16 ***
Type of teeth x Immersion time	14.572	1.457	10	180	2.0112	0.03454 *
Type of solution x Immersion time	129.849	12.985	10	180	17.9212	< 2.2e-16 ***
Type of teeth x type of solution x Immersion time	62.608	3.130	20	180	4.3205	4.068e-08 ***

\* Significant if  $p, 0.05$

## Discussion

The aim of the study was to assess the color change ( $\Delta E$ ) of conventional, milled and 3d-printed denture teeth after immersion in coloring agents: red wine, coffee, and artificial saliva across six-time intervals: 7, 14, 21, 30, 40, and 120 days. Color changes can be evaluated either visually or through instrumental techniques. Colorimeters like Optishade are preferred over visual evaluation as they eliminate subjective interpretations in color comparison [11]. Many studies have evaluated color change through colorimeters and spectrophotometers [12-14]. In addition, the middle third area was chosen for color changes investigation because studies have proven that it was the most convenient one [15].

Three-way ANOVA was used to evaluate the differences in color change ( $\Delta E$ ) across time, between the types of denture teeth, and among the different staining agents (coffee, wine, and artificial saliva). ANOVA results showed significant main effects and interactions, indicating that the color change varied significantly not only over time but also between the diverse types of teeth and staining solutions.

Estimated marginal means in figure 4 shows that teeth immersed in coffee had the most color change, especially at day 120 with  $\Delta E$  reaching a value of 6. Studies performed by Tieh et al., Koksai et al., Al-Qarni et al., and Hipolito et al found that coffee exhibits the most powerful capacity for staining [7, 12, 16, 17]. In contrast, Dimitrova et al., Arana-Correa et al., and Kurtulmus-Yilmaz et al. have found that red wine had the strongest staining capability. Top of Form among other coloring agents [13, 18, 19]. Artificial saliva was used in this study as a control. Few studies have used it as a control knowing that it can simulate oral conditions [16, 20-22]. However, other studies preferred to use distilled water as a control group when comparing coloring agents [17, 23-25].

The null hypothesis in this study was that there is no significant difference in color change among conventional, milled, and 3D printed denture teeth, it was rejected. 3D printed denture teeth experienced the highest color change  $\Delta E$  reaching 10.546 at day 120 when exposed to coffee causing the most substantial impact. Comparable results were found by Tieh et al. [7]. The extent of color change increased over time, highlighting the importance of considering the long-term aesthetic performance of these materials when selecting denture teeth for clinical use. Milled denture teeth showed higher  $\Delta E$  values in red wine over time reaching 5.359 at day 120, with particularly minimal  $\Delta E$  values in coffee and no color changes in artificial saliva. Conventional teeth when compared to milled teeth showed higher  $\Delta E$  values while being immersed in coffee and saliva but lower than those of 3D printed teeth. This result is consistent with Alouch et al. finding which suggested that the color stability of milled denture teeth was higher than conventional one [14]. In contrast, studies made by Tieh et al. showed that color stability of conventional teeth was similar to milled denture teeth [7]. A systematic review conducted by Tieh et al. collected data from several articles about type of teeth, type of control group used in the studies [26]. A few authors included 3d-printed and milled denture teeth together in their studies [13, 23].

Immersion time also shows an extremely significant effect ( $p < 0.001$ ) meaning that the color change over time was not random but rather a consistent trend that could be attributed to the staining effect of the solutions. In addition, immersion time of denture teeth was 120 days in this study. In fact, studies have shown that 30 days of consistent immersion in coloring agent simulates 2.5 years of regular consumption [27], which means that 120 days of immersion would simulate 10 years of daily consumption. However, in Koksai et al. and Dimitrova et

al. studies, immersion time was 30 and 21 days respectively [12, 13].

This study has limitations. The use of specific coloring agents and continuous immersion does not accurately replicate the oral environment. In reality, coloration is influenced by various chemical and mechanical changes in the mouth, including the composition of saliva, differences in foods and drinks, chewing efforts, and temperature fluctuations [17]. Furthermore, denture teeth have not undergone a thermocycling process which helps to simulate oral conditions. Additionally, factors such as abrasion and corrosion can exacerbate discoloration, which are also influenced by oral health, dietary habits, and denture care practices [17].

## Conclusion

The findings in this study indicated that milled denture teeth have the highest color stability with 3d-printed teeth being the least resistant to color change. Furthermore, coffee has been found as the most potential staining solution leading to the highest  $\Delta E$  values.

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