In vitro color change of three dental veneering resins in tea, coffee and tamarind extracts

J.K. Subramanya¹, S. Muttagi²

¹Professor, Department of Prosthodontics and Crown and Bridge, Al-Badar Rural Dental College and Hospital, Gulbarga, Rajiv Gandhi University of Health Sciences, Bangalore, India.
²Associate Professor, Department of Biochemistry, Gulbarga University, Gulbarga, India.

Abstract:
Objective: To study the in vitro color changes of three dental resin veneering materials when immersed in tea, coffee and tamarind extracts.

Materials and Methods: The color changes of heat polymerized tooth colored acrylic resin (Stellondetrey, B, F14, DPI Dental products of India Ltd, Mumbai), auto polymerized tooth colored acrylic resin (DPI, B, QV5, DPI Dental products of India Ltd, Mumbai) and light polymerized resin composite (Herculite XRV, Enamel A2, part no. 22860, lot no. 910437, Kerr Corporation, West Collins Avenue, Orange, CA, USA) when immersed in water extracts of tea (Tata Tea Ltd. Bangalore, India), coffee (Tata Coffee Ltd. Coorg, India) and tamarind were evaluated using computer vision systems. The color images were recorded in R (red), G (green) and B (blue) form and converted into H (hue), S (saturation) and V (value).

Results: Significant color change occurred for auto polymerized tooth colored acrylic resin in tamarind extract, for heat polymerized tooth colored acrylic resin in tea extract and for light polymerized resin composite in coffee extract. Auto polymerized tooth colored acrylic resin samples showed an overall higher color change. However, for all the material samples coffee extract produced more color change.

Conclusion: These results suggest that the color stability of the resins is influenced by the presence of secondary metabolites such as tartaric acid, tannins, caffeine, saponins and phenols in tamarind, tea and coffee extracts.

Key Words: Color, Dental Resins, Secondary Metabolites, Tannins

INTRODUCTION
The use of esthetic restorative materials such as porcelain and resins have enabled us to contribute to the character, charm and dignity of a person, thereby restoring that elusive esthetic appeal. Tooth colored materials should have intrinsic color stability and resistance to surface staining in order to have a high degree of esthetics [1]. Various teeth colored veneering materials have been used e.g. porcelain, light and dual cured composites, heat and auto polymerized tooth colored acrylic resins [2]. Tooth colored resins have been improvised over the years to meet the challenges of esthetic dentistry [3]. Compositions of materials have been altered, thereby improving the properties of these resins. However, the lack of color stability has always remained a problem for resin veneering materials [3]. Color stability is the property of a material that allows col-
There are many factors responsible for the color changes of tooth colored dental resins [3,5]. Factors involving changes within the resin matrix and those occurring at the interface of matrix and fillers are called intrinsic factors. Color changes can also occur due to changes on the surface of the dental resin by adhesion of ions or molecules of certain liquids to the surface [3,5]. This process of adsorption contributes to extrinsic color change on the surface of the resin [5]. Oxidation of amine accelerators or entry of colored mixtures leads to discoloration of denture base polymers [6,7]. Change in the color of denture base materials has been demonstrated due to exposure to oral fluids and denture cleansers [6]. The structure and physical characteristics of inorganic fillers present in the resin may have an effect on the ability of the resin to maintain a smooth surface for extended periods of time [3]. Along with the size of the filler particles, the chemical properties of the resins also influence the resistance to color change[8].

In this study, three types of dental veneering resins i.e. heat polymerized tooth colored acrylic resin, auto polymerized tooth colored acrylic resin and light polymerized resin composite were tested for color change in water extracts of tea, coffee, and tamarind, using drinking water as control. The purpose of this study was to investigate and discuss the factors responsible for change in the color of samples when exposed to various foods.

**MATERIALS AND METHODS**

Three commercial resins were selected according to the type of polymerization; namely, heat polymerized tooth colored acrylic resin (Stel-lon, B, F14, DPI Dental products of India Ltd, Mumbai), auto polymerized tooth colored acrylic resin (DPI, B, QV5, DPI Dental products of India Ltd, Mumbai) and light polymerized resin composite (Herculite XRV, Enamel A2, part no. 22860, lot no. 910437, Kerr Corporation, West Collins Avenue, Orange, CA, USA). Commercially available tea powder (Tata Tea Ltd. Bangalore, India), coffee powder (Tata Coffee Ltd. Coorg, India) and tamarind which are naturally available were used and drinking water was used as control.

| Constituents     | Tea   | Coffee | Tamarind |
|------------------|-------|--------|----------|
| Alkaloids        | +     | +      | –        |
| Carbohydrates    | +     | +      | –        |
| Glycosides       | –     | –      | –        |
| Steroids         | –     | –      | –        |
| Flavonoids       | +     | +      | +        |
| Phenols          | +     | ++     | +        |
| Saponins         | +     | +      | ++       |
| Tannins          | ++    | ++     | ++       |
| Proteins and Amino acids | ++ | +     | +       |

++ = considerable, + = detected, – = not detected
Preparation of samples
Samples were prepared in three material groups.
- Heat polymerized tooth colored acrylic resin - Group 1
- Auto polymerized tooth colored acrylic resin - Group 2
- Light polymerized resin composite - Group 3

Twenty samples were prepared in each group, with five samples for each solution in every group. Twenty samples of each material were prepared using a rectangular acrylic mold, fabricated with four 20 mm wide and 2 mm deep central perforations, according to ISO 4049 [9]. The mold was placed on a glass slab enrolled with cling film. For group 1, the central perforations were filled with wax (Modeling Wax, DPI Dental products of India Ltd, Mumbai). The mold was then tightly covered by another glass slab wrapped with cling film to provide a smooth surface of the wax pattern. These wax patterns were then invested in a dental flask using dental stone. After wax elimination, the gypsum mold space was packed with heat polymerized tooth colored acrylic resin which was mixed according to manufacturer’s instructions.

The samples were retrieved from the mold after curing. The samples were then polished using an abrasive wheel followed by the use of sandpaper. This process removed any surface irregularities such as nodules and produced a smoother surface. The mixture of water and pumice was then used over the surface of the sample with a cloth wheel. Finally, RxCreate diamond polishing paste (Dental Life Sciences, Ince, Wigan, UK) was applied for 1 minute to both sides of the samples.

For group 2, the central perforations were filled directly with autopolymerized tooth colored acrylic resin, which was mixed according to manufacturer’s instructions and allowed to polymerize in the mold space between the two glass slabs wrapped with cling film. Resin samples having smooth surfaces were then retrieved from the mold. Finishing and polishing was done following the same steps as for group 1.

For group 3, using an agate spatula a single increment of composite resin was inserted in each of the mold’s upper surface perforations after placing on a glass slab enrolled with cling film. The mold was then tightly covered by another glass slab wrapped with cling film to provide a smoother surface.

Table 2. Mean, standard deviation (SD) and $P$-value of hue-H, saturation-S and value-V for heat polymerized tooth colored acrylic resin samples before and after immersion in drinking water, tamarind extract, tea extract and coffee extract

| Heat polymerized tooth colored acrylic resin samples | Immersed in drinking water | Immersed in tamarind extract | Immersed in tea extract | Immersed in coffee extract |
|-----------------------------------------------------|----------------------------|-----------------------------|-------------------------|---------------------------|
| Before H                                            | Mean±SD 0.58±0.01          | Mean±SD 0.57±0.01           | Mean±SD 0.58±0.01       | Mean±SD 0.59±0.01         |
| After H                                             | Mean±SD 0.63±0.10          | Mean±SD 0.59±0.10           | Mean±SD 0.22±0.01       | Mean±SD 0.74±0.01         |
| Before S                                            | Mean±SD 0.20±0.02           | Mean±SD 0.18±0.00           | Mean±SD 0.18±0.01       | Mean±SD 0.19±0.01         |
| After S                                             | Mean±SD 0.15±0.01           | Mean±SD 0.13±0.01           | Mean±SD 0.11±0.01       | Mean±SD 0.13±0.01         |
| Before V                                            | Mean±SD 0.75±0.04           | Mean±SD 0.74±0.01           | Mean±SD 0.74±0.01       | Mean±SD 0.75±0.01         |
| After V                                             | Mean±SD 0.79±0.01           | Mean±SD 0.82±0.00           | Mean±SD 0.81±0.01       | Mean±SD 0.82±0.00         |

*P<0.01 is statistically significant
a finished surface. The resin was cured for 40 seconds on each surface of the sample through the cling film and glass slab with a tungsten-halogen lamp (Trilight, 3M, ESPE, Seefeld, Germany). The curing light intensity was measured at 800mW/cm\(^2\) and monitored using a built-in radiometer.

An additional 20 seconds of curing was done on both sides of the specimens after removing the films and glasses.

The surfaces of all specimens were then polished with fine and superfine polishing disks (Sof-lex, 3M/Espe Dental Products, St Paul, MN) with a low-speed handpiece.

After completion of the polymerization, all the samples were coded by numbering from one to twenty on their back in each group. To reduce variability, all sample preparation and finishing/polishing procedures were performed by the same operator. The samples were immersed in distilled water at 37°C for 24 hours after they were photographed for the registration of their initial color.

**Preparation and biochemical screening of tea, coffee and tamarind extracts**

Normal drinking water of about 600 ml was taken in a clean 1000 ml round bottom flask, to which about 60 gm of tea powder was added and it was subjected to soxhlet extraction for about 30 minutes. The content was then cooled and the extract was stored at 4°C in a clean sterilized conical flask for further experiment. This extract is called tea extract. The same procedure was repeated for coffee and tamarind and extracts were used till the experiments were completed. All the three extracts were biochemically screened for the detection of secondary metabolites present in them [10]. The results were tabulated as shown in Table 1.

Five samples of each group were immersed in a clean 100 ml conical flask containing 40 ml of the solution. Samples immersed in tea, coffee and tamarind extracts were kept in a thermostat shaker with 18 rpm at 37°C. Samples immersed in water were kept in another thermostat shaker with 18 rpm at 37°C acting as a control. The solutions were changed every twenty four hours to minimize bacterial growth. The experiment was continued for fifteen days. The samples were then cleaned using an electric toothbrush (Colgate Motion, Colgate-Palmolive, NY, USA) with toothpaste (Colgate Total, Colgate-Palmolive, India Ltd.) for 10 seconds on each side of them.

| Auto polymerized tooth colored acrylic resin samples | Immersed in drinking water | Immersed in tamarind extract | Immersed in tea extract | Immersed in coffee extract |
|------------------------------------------------------|-----------------------------|-----------------------------|-------------------------|---------------------------|
| Before H                                             | Mean±SD                     | P-value                     | Mean±SD                 | P-value                   | Mean±SD                 | P-value               |
|                                                      | 0.58±0.00                   | 0.88                        | 0.58±0.01               | 0.00*                     | 0.58±0.01               | 0.00*                 |
| After H                                              | 0.58±0.04                   |                             | 0.61±0.01               | 0.00*                     | 0.58±0.01               | 0.36±0.07            |
| Before S                                             | 0.18±0.01                   | 0.01                        | 0.19±0.01               | 0.00*                     | 0.18±0.01               | 0.00*                 |
| After S                                              | 0.16±0.02                   |                             | 0.16±0.01               | 0.00*                     | 0.15±0.01               | 0.13±0.01            |
| Before V                                             | 0.73±0.00                   | 0.00*                       | 0.74±0.01               | 0.00*                     | 0.74±0.00               | 0.00*                 |
| After V                                              | 0.81±0.01                   |                             | 0.79±0.01               | 0.00*                     | 0.78±0.01               | 0.79±0.01            |

*P<0.01 is statistically significant
This was followed with gentle rinsing in distilled water and drying with a paper towel.

**Color measurement**

Color may be rapidly analyzed by computerized image analysis techniques, also known as computer vision systems [11].

After review of literature regarding the techniques used for color measurements, it was thought that a simpler, quicker technique was required where the procedure could be repeated a number of times, data recorded and interpreted easily. In addition, the equipment used here is easily available, operated and maintained. Color measurements of the surface of the samples were obtained using image analysis. This method has been used to explain the color measurements of agricultural foods [11].

All the samples of the three materials used were measured for a baseline color before immersion in the extracts. Samples were illuminated using two 40 watt fluorescent lamps. A Color Digital Camera, model PowerShot A70 (Canon, USA) was located vertically over the background at a distance of 30 cm.

A digital color image represented in R (red), G (green) and B (blue) form with three components per pixel in the range 0-255 was obtained and stored using eight bits per color component.

These three intensity images (R, G and B) were electronically combined to produce a digital color picture.

Care was taken to avoid external light and reflections, the images were captured on a matte black background and stored in a JPEG format.

Image processing and color analysis were written in MATLAB 6.5. RGB form was converted into H (hue), S (saturation) and V (value) using the function rgb2hsv available in MATLAB®.

**Statistics**

All the experiments were done in triplicate. Mean and standard deviation (SD) were calculated for each variable. Paired Student’s t-test was applied to compare before-after values. Two-way ANOVA was done and a $P$-value of $<0.01$ was considered as statistically significant.

| Light polymerized resin composite samples | Immersed in drinking water | Immersed in tamarind extract | Immersed in tea extract | Immersed in coffee extract |
|------------------------------------------|-----------------------------|-------------------------------|-------------------------|---------------------------|
| Mean±SD | $P$-value | Mean±SD | $P$-value | Mean±SD | $P$-value | Mean±SD | $P$-value |
| Before H | 0.57±0.01 | 0.03 | 0.57±0.01 | 0.04 | 0.64±0.01 | 0.00* | 0.57±0.01 | 0.00* |
| After H | 0.61±0.02 | 0.49±0.07 | 0.76±0.01 | 0.00* | 0.61±0.00 | 0.00* |
| Before S | 0.18±0.01 | 0.00* | 0.19±0.01 | 0.00* | 0.19±0.01 | 0.00* | 0.19±0.01 | 0.00* |
| After S | 0.21±0.01 | 0.12±0.02 | 0.14±0.02 | 0.21±0.00 | 0.00* |
| Before V | 0.57±0.01 | 0.23 | 0.65±0.01 | 0.04 | 0.64±0.01 | 0.14 | 0.65±0.01 | 0.00* |
| After V | 0.54±0.06 | 0.62±0.02 | 0.63±0.01 | 0.75±0.01 | 0.00* |

$P<0.01$ is statistically significant

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*Table 4. Mean, standard deviation (SD) and $P$-value of hue-H, saturation-S and value-V for light polymerized resin composite samples before and after immersion in drinking water, tamarind extract, tea extract and coffee extract.*
RESULTS

In the present study, color changes observed in the samples of heat polymerized tooth colored acrylic resin, auto polymerized tooth colored acrylic resin and light polymerized resin composite were noted down as RGB and converted to HSV and statistically analyzed as shown in tables 2-4. The color change observed in heat polymerized tooth colored acrylic resin samples is more significant in tea extract, followed by coffee extract; whereas, there is no significant color change in samples immersed in tamarind extract when compared with samples immersed in water as shown in table 2.

Auto polymerized tooth colored acrylic resin samples showed a significant color change in tamarind extract followed by coffee extract and tea extract, when compared with the control as shown in table 3. Light polymerized resin composite samples showed the most significant color change in coffee extract. Samples immersed in tea extract have shown some change in hue and saturation but no significant deviation in the overall color. There was no significant color change in samples immersed in tamarind extract when compared with samples immersed in water as shown in table 4.

DISCUSSION

Color perception is an art as well as a science which deals with the sensation induced from light of different wavelengths reflecting from a surface and reaching the eye. Color is usually determined by measuring its three variables which are hue, saturation and value [2]. Hue is the dominant color of an object indicated by the dominant wavelengths present. This may be red, green or blue. Saturation or chroma is the degree of saturation of a particular hue. It refers to the intensity of a color. Value refers to the relative lightness or darkness of a color. A lighter shade means a higher value and a darker shade means a lower value [2]. Although all the three variables may be measured independently, they are not considered separately in dentistry to describe a particular tooth or restoration. This is because changing any one of these parameters can result in an overall change in the perception of a particular color.

Heat polymerized tooth colored acrylic resin and auto polymerized tooth colored acrylic resin have the same basic chemical structure; difference is in the activation of the polymerization reaction. In the auto polymerized resin, chemical activation is accomplished through tertiary amines such as N,N-dimethyl-paratoluidine or N,N-dihydroxyethyl-paratoluidine [2].

When color changes of acrylic resins were studied, tea, coffee and tamarind extracts were found to produce a significant color change. Previous studies have shown that polymethyl methacrylate resins are hydrophilic and attract more water soluble dyes on the surface and staining, which occur as a result of electrostatic charges [6].

The extracts of tea, coffee and tamarind may have more ionizable groups or highly reactive secondary metabolites like flavonoids, phenols, saponins and tannins which are water soluble and much of them are stable at high temperature. The sorption of these watersoluble secondary metabolites on the surface may have caused more color change. Table 2 shows maximum staining of heat polymerized tooth colored acrylic resin samples in tea extract, because tannins are higher in tea extract than coffee and tamarind extract.

Acrylic resins exhibit the property of water sorption that is directly related to the polar properties of resin molecules, the physical process of water diffusion through intermolecular space and the amount of residual monomer in the polymerized mass. Acrylic resins are made up of several interpolymeric chains which have gaps between them. The absorbed water enters these gaps and remains there. The size and number of these interpolymeric gaps determine the amount of water absorption...
Therefore, the color change observed here is due to the diffusion of water in the interpolymeric gaps along with the diffusion of water soluble secondary metabolites like tannins, phenols and saponins present in the tea extract. These metabolites may cause the discoloration of resin material. Better polymerization of acrylic resin may increase the cross linking and reduce water sorption values [12]. However, in table 3 auto polymerized tooth colored acrylic resin shows significant color change in tamarind extract followed by coffee and tea extracts. Chemical discoloration has been attributed to a change or oxidation in the amine accelerator, oxidation in the structure of the polymer matrix and oxidation of the unreacted pendant methacrylate groups [13]. It is also possible that discoloration in auto polymerized acrylic resin is due to the change of the tertiary amine by UV rays, the change of dimethyl metha toluidine contained as an impurity in the amine and the change of hydroquinone [14]. As amines are basic, they neutralize acids to form the corresponding ammonium salts. The un-reacted tertiary amine in the auto polymerized acrylic resin may have reacted with high percentage of tartaric acid (8-23.5mg) present in the tamarind extract [15] which forms respective salts that dissolve in the surrounding solution and create porosity at the surface, which then leads to more sorption of tannic acid and other secondary metabolites causing color change. It has been shown that resin materials using urethane dimethacrylate as matrix have more color stability than resin materials using bis-GMA as matrix [3,16].

Water enters the resin matrix mainly by direct absorption. Glass filler particles present in the resin matrix do not absorb, but can adsorb water on the surface. Thus, the total amount of water sorption depends on the resin matrix, the glass fillers and the quality of bond between them. Extra water sorption may decrease the life of resin composites by expanding and plasticizing the resin matrix.

The excess water absorbed by the resin matrix hydrolyzes the silane and forms micro-cracks. These micro-cracks or the interfacial gaps at the interface between filler and matrix allow penetration of stain and influence the amount of color change [1].

The resin polymerization degree may also affect the color change. An inadequate composite resin polymerization is directly associated with color instability, which is due to easy and pigment diffusion through the resin matrix [3,7,17]. Therefore, significant change in color is observed in the light polymerized resin composite samples immersed in coffee extract and to some extent in tea extract as shown in table 4. Coffee extract contains more amounts of phenols, saponins, tannins and caffeine alkaloid. Polymerization inhibition by oxygen at the sample surface and at the periphery of porosities may also induce composite discoloration [18].

CONCLUSION

Within the limitations of the present study, there is a definite role of secondary metabolites influencing color change. Tannins, caffeine, tartaric acid and phenols are predominantly responsible. Computer image analysis seems to be a good technique to quantify easily, quickly and precisely the color changes observed in the resin samples.

Results of the present study suggest that a similar process may occur clinically and indicate the importance of the patient’s habits in the longevity of restorative materials.

REFERENCES

1-Bagheri R, Burrow MF, Tyas M. Influence of food-simulating solutions and surface finish on susceptibility to staining of aesthetic restorative materials. J Dent 2005 May;33(5):389-98.
2-Anusavice KJ. Phillips’ Science of Dental Materials. 11th ed. Philadelphia: W.B. Saunders Co; 2003. p. 164-763.
3-Mundim FM, Garcia Lda F, Pires-de-Souza Fde
C. Effect of staining solutions and repolishing on color stability of direct composites. J Appl Oral Sci 2010 May-Jun;18(3):249-54.
4-Assuncao WG, Barao VA, Pita MS, Goiato MC. Effect of polymerization methods and thermal cycling on color stability of acrylic resin denture teeth. J Prosthet Dent 2009 Dec;102(6):385-92.
5-Villalta P, Lu H, Okte Z, Garcia-Godoy F, Powers JM. Effects of staining and bleaching on color change of dental composite resins. J Prosthet Dent 2006 Feb;95(2):137-42.
6-Hersek N, Canay S, Uzun G, Yildiz F. Color stability of denture base acrylic resins in three food colorants. J Prosthet Dent 1999 Apr;81(4):375-79.
7-Galvao AP, Jacques LB, Dantas L, Mathias P, Mallmann A. Effect of lipstick on composite resin color at different application times. J Appl Oral Sci 2010 Dec;18(6):566-71.
8-Lu H, Roeder LB, Lei L, Powers JM. Effect of surface roughness on stain resistance of dental resin composites. J Esthet Restor Dent 2005;17(2):102-9.
9-International Standard Organization. ISO 4049: Dentistry-polymer-based filling, restorative and luting materials. Switzerland:ISO;2000.
10-Trease EG, Evans WC. Textbook of Pharmacognosy. London:BalliereTindall; 1983. p. 383. (vol 12)
11-Mendoza F, Dejmek P, Aguilera JM. Calibrated color measurements of agricultural foods using image analysis. Postharvest BioTechnol 2006 Sep;41(3):285-95.
12-Meloto CB, Silva-Concilio LR, Machado C, Ribeiro MC, Joia FA, Rizzatti-Barbosa CM. Water sorption of heat-polymerized acrylic resins processed in mono and bimaxillary flasks. Braz Dent J 2006;17(2):122-5.
13-Topcu FT, Sahinkesen G, Yamanel K, Erdemir U, Oktay EA, Ersahan S. Influence of different drinks on the colour stability of dental resin composites. Eur J Dent 2009 Jan;3(1):50-6.
14-Fusayama T, Hirano T, Kono A. Discoloration test of acrylic resin fillings by an organic dye. J Prosthet Dent 1971 May;25(5):532-9.
15-Singh D, Wangchu L, Moond SK. Processed products of Tamarind. Nat Prod Rad 2007 July-Aug;6(4):315-21.
16-Khokar ZA, Razzoog ME, Yaman P. Color stability of restorative resins. Quintessence Int 1991 Sept;22(9):733-7.
17-Costa E Silva D, Tiradentes SBSP, Parente RCP, Bandeira MFCL. Color change using HSB color system of dental resin composites immersed in different common Amazon region beverages. ActaAmaz 2009 Oct;39(4):961-8.
18-Dietschi D, Campanile G, Holz J, Meyer JM. Comparison of the color stability of ten new generation composites: an in vitro study. Dent Mater 1994 Nov;10(6):353-62.