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Original Research

Color Stability of Reline Resin after Microwave Disinfection and Immersion in Drinks for Different Periods

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Abstract:

Background: This study investigated the color stability of reline resin after microwave disinfection and immersion in drinks for different periods.

Materials and Methods: Disks of lucitone were made, relined with Tokuyama (15 mm × 10 mm) and divided (n = 15) according to immersion solution: W - water, W+MD - water and microwave disinfection for 3 min at 650 W, once a week (MD), CE - coffee, CE+MD - coffee and disinfection, T - tea, T+MD - tea and disinfection, CA - cola, CA+MD - cola and disinfection, SJ - strawberry juice, SJ+MD - strawberry juice and disinfection, GJ - grape juice, GJ+MD - grape juice and disinfection. Color parameters in L*a*b* were recorded by a spectrophotometer, before immersion and after 7, 15, 30, 90, 180, 270 and 365 days and the color difference was calculated. Data were analyzed by three-way analysis of variance and Tukey tests (α = 0.05).

Results: Disinfection produced color change after 7, 15 and 30 days irrespective of the drink. After 15 days, W, W+MD, CE, CE+MD, GJ and GJ+MD resulted in significantly higher color change when compared with 7 days. For the other groups, the color change was observed after 30 days. After 180 days, no significant change was found for all solutions. When the staining agents compared, there were no significant differences in color change for each storage period.

Conclusion: Color change of microwaved resin was observed after 7, 15 and 30 days. Staining agents caused color change in different periods when compared with 7 days. There were no significant differences in color change for all solutions after 270 days and for each storage period when staining agents were compared.

Key Words: Color, coloring agents, denture relining, disinfection, water storage

Introduction

It has been demonstrated that esthetics is a significant contributor to rating of general satisfaction of patients with complete dentures.[1] Esthetic value of a denture is influenced by several factors, such as contour, texture and color. Maintenance of color affects the durability of treatment because perceptible color change may compromise the acceptability of the dentures. To minimize those failures, the material must have optimal color stability.

Color is the way the human visual system measures a part of the electromagnetic spectrum, approximately between 300 and 830 nm.[2] It is possible to quantify color by using instrumental determination that confers advantages such as repeatability, sensitivity, and objectivity.[3,4] The evaluation of color change can be measured using the spectrophotometers which quantify the fraction of light that passes through a given sample. In assessing chromatic differences, Commission Internationale de l'Eclairage (CIE) lab provides a three-dimensional representation for the perception of color stimuli, describing all the colors visible to the human eye.[5] The L*-axis is known as the lightness and extends from 0 (black) to 100 (white). The other two coordinates a* and b* represent redness-greenness and yellowness-blueness, respectively. The difference or distance between two colors is represented by ΔE^* which can be calculated using the relationship:[6,7]

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

Color change on dental materials can be significantly increased upon exposure to different drinks, like tea, cola, coffee, juices, and wine.[8-11] Bagheri *et al.*,[12] compared the staining properties of five foods (red wine, coffee, tea, soy sauce, cola). They

tested six tooth-coloured restorative materials and observed that the materials were susceptible to staining by all stains especially coffee, red wine, and tea. Guler *et al.*[13] reported that resin composite provisional restorative materials may showed staining after immersion in red wine, tea, and coffee. Haselton *et al.*,[5] show that provisional crown and fixed partial denture resins may be stained over a range of time periods when immersed in artificial saliva and artificial saliva-coffee solutions. Patel *et al.*,[14] demonstrated that storage solutions significantly affect the surface staining of resin-based dental materials.

It has been demonstrated that the lack color stability is one of the most common failures of several autopolymerizing reline resins.[15] However, use of reline resins is often indicated to maintain adaptation of the denture bases to the mucosa covering the residual ridges. According to the glossary of prosthodontic terms,[16] reline is "the procedures used to resurface the tissue side of a denture with the new base material, thus producing an accurate adaptation to the denture foundation area." Such procedure maintains supporting tissues in good health and satisfy patients by improving oral function and self-esteem.

A growing number of autopolymerizing reline resins have been introduced for relining denture bases.[17,18] However, the physical and mechanical properties of these materials can varied considerably.[15,17-19,20] Although improvements have been made, it is common the colonization of *Candida* spp. on and within the reline material, which predisposes to the development of denture stomatitis.[21]

Denture stomatitis may be treated through microwave disinfection of complete upper dentures.[22] Microwave irradiation for 3 min in water at 650 W, performed on contaminated hard chairside reline specimens proved to eliminate pathogenic microorganisms.[23] However, this disinfection protocol can decreased the hardness of acrylic resins and the tooth/acrylic resin impact strength.[24,25]

No information could be identified by the authors describing the influence of microwave disinfection on the color stability of reline resin after drinks immersion. Therefore, the aim of this study was to evaluate the color stability of reline resin after microwave disinfection and drinks immersion in different periods.

Materials and Methods

An autopolymerizing denture base relines resin and a conventional heat-polymerized denture base acrylic resin were selected for this study (Table 1). To fabricate denture base disk-shaped specimens intended to be relined, silicone impression material (Zeta Plus, Zhermack, Badia Polesine, Rovigo, Italy) was adapted in a stainless steel mold with a breakaway compartment (15 mm × 5 mm). The silicone patterns were individually invested by sandwiching them between two glass slides in Type IV stone (Vel-Mix, Kerr, Romulus, Mich, USA), using a conventional denture processing flask. The L material was mixed, packed under pressure, and processed according to the manufacturer's recommendations (Table 1). After polymerization, the flasks were bench cooled at room temperature for 30 and 15 min under running water before the L disk-shaped specimens were removed from the flasks. The edges of the specimens were finished with 400-grit silicon carbide paper (3M ESPE, St. Paul, MN, USA)to remove irregularities. The finished specimens were stored in distilled water at 37 ± 1°C for 50 ± 2 h before relining (ISO/FDI 1567).[26] After water storage, the L disk-shaped surfaces to be bonded were abraded with 240 silicon carbide paper (3M ESPE, St. Paul, MN, USA)in an automatic grinding and polishing unit (Metaserv 2000, Buehler, Lake Bluff, IL, EUA) at 350 rpm for 40 s, brushed with liquid detergent (Limpol, Bombril-Cirio, Sao Paulo, SP, Brazil) for 20 s, washed in distilled water, and blot-dried. The relining surface of the L disk-shaped specimens was treated with adhesive according to the reline resin manufacturer' recommendation and air-dried. The L disk-shaped specimens were then placed into a stainless steel mold (15 mm × 10 mm) placed on the center of a glass plate covered with an acetate sheet. The reline material was proportioned and manipulated following the manufacturer' instruction and poured into the mold. A second glass plate was placed over the reline material, and the pressure was applied until polymerization was complete. The edges of the specimens were finished.

After relining, the baseline color of all specimens was determined in the spectrophotometer Color Guide 45/0 (BYK-Gardner, Santo André, SP, Brazil) according to the CIE $L^*a^*b^*$ system. All measurements were repeated twice and mean for the L^*, a^* and b^* values were calculated. After baseline color measurements were made, 180 reline disk-shaped specimens were divided into 12 groups to provide a sample size of 15. The color changes were evaluated after the experimental conditions described in Table 2.

Each of the specimens was immersed separately in vials containing 25 mL of control or test solution 30 min each day at 37°C. To prepare the coffee solution (Nescafé Original, Nestlé Brasil S.A., Araras, SP, Brazil), 16 g of coffee were dissolved in 1000 mL of distilled water at 100°C. Strawberry and grape

Table 1: Materials used.

Product	Code	Manufacturer	Powder liquid ratio	Curing cycle
Lucitone 550	L	Dentsply International, Rio de Janeiro, RJ, Brazil	4.2 g/2 mL	90 min at 73°C and then 100°C boiling water for 30 min
Tokuyama rebase II	T	Tokuyama Dental Corporation, Tokyo, Japan	2.4 g/1 mL	5.5 min at room temperature

juices (Tang, Kraft Foods Brasil S.A., Curitiba, Paraná, Brazil) were prepared from 1000 mL of distilled water at 37°C to which one juice bag (35 g) was added. Tea (Lipton Ice Tea Limon, AmBev, Rio de Janeiro, RJ, Brazil) and cola (Coca-cola, Coca-cola Brasil, Rio de Janeiro, RJ, Brazil) were also used as staining solutions.

After exposure to the staining agent described above, the specimens were stored in water at 37°C. The color changes were again evaluated after 7, 15, 30, 90, 180, 270 and 365 days. The total color change (ΔE^*) of each test specimen was then calculated using the relationship:

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

The data were evaluated statistically (Statistica 6.0; StatSoft, Tulsa, Okla) using three-way analysis of variance (ANOVA). The 3 factors analyzed were material, disinfection, and immersion time. The Tukey honestly significant difference (HSD) *post-hoc* test was used to determine differences between mean values ($a = 0.05$).

Table 2: Test groups.

Group code	Group description
W (control)	Specimens immersed in distilled water at 37°C
W+MD	Specimens were immersed in distilled water and individually microwave disinfected (650 W for 3 min) once a week being immersed in 200 mL of water
CE	Specimens immersed in a coffee solution
CE+MD	Specimens immersed in a coffee solution and individually microwave disinfected (650 W for 3 min) once a week being immersed in 200 mL of water
T	Specimens immersed in a tea solution
T+MD	Specimens immersed in a tea solution and individually microwave disinfected (650 W for 3 min) once a week being immersed in 200 mL of water
CA	Specimens immersed in cola
CA+MD	Specimens immersed in cola and individually microwave disinfected (650 W for 3 min) once a week being immersed in 200 mL of water
SJ	Specimens immersed in strawberry juice
SJ+MD	Specimens immersed in strawberry juice and individually microwave disinfected (650 W for 3 min) once a week being immersed in 200 mL of water
GJ	
GJ+MD	Specimens immersed in grape juice and individually microwave disinfected (650 W for 3 min) once a week being immersed in 200 mL of water

W: Water, MD: Microwave disinfection, GJ: Grape juice, SJ: Strawberry juice, CE: Coffee, T: Tea, CA: Cola

Results

The three-way ANOVAs and the indication of significance for the different factors and interactions are shown in Table 3. From Table 3, it can be seen that significant differences were found for the 3 main factors, disinfection, staining agent, and storage period. The three-way interactions were not significant. The 2-factor interactions for "storage period X disinfection" and "storage period X staining agent" were detected as a source of variation ($P < 0.001$).

Table 3: Summary of three-way ANOVA for the color change.

Effect	df	Mean square	*F*	*P*
Disinfection	1	1.096	9.75	0.002*
Staining agent	5	0.269	2.39	0.040*
Disinfection X-staining agent	5	0.086	0.76	0.578
Error (a)	168	0.112		
Storage period	6	3.796	355.69	<0.001*
Storage period X disinfection	6	0.262	24.56	<0.001*
Storage period X-staining agent	30	0.025	2.38	<0.001*
Storage period X disinfection X-staining agent	30	0.013	1.21	0.202
Error (b)	1008	0.011		

*Significant difference at $P<0.05$

Table 4 presents the mean values for color change and the results of Tukey HSD *post-hoc* test ($a = 0.05$). Microwave disinfection produced specimens with significantly color change values after 7, 15 and 30 days regardless of the staining agent when compared with specimens that were not irradiated (P values of 0.00002, 0.00015 and 0.005, respectively).

The three-way ANOVA and Tukey HSD *post-hoc* tests showed significant differences among the storage periods ($P < 0.001$) for each staining agent regardless the microwave disinfection. After 15 days, distilled water (W and W+MD groups), coffee (CE and CE+MD groups) and grape juice immersions (GJ and GJ+MD groups) resulted in significant color change than those immersed for 7 days. For the other groups, the color change was observed after 30 days of immersion when compared to 7 days. After 270 days, no statistically significant color change was found for all solutions. When compared the staining agents, there were no significant differences in color change at each storage period.

The color change (ΔE) observed after 180 days of immersion in all staining agents (Graph 1) could be categorized as "much" based upon the National Bureau of Standards system for expressing color difference (Table 5).

Discussion

The color stability of denture materials is determining factor for the aesthetics of removable partial or complete prostheses. The visual assessment of color includes many subjective factors, such as the light source, the object being viewed and the observer viewing the object, and thus is not enough.[7] Paul *et al.*,[3] observed that instrumental color determination is more exact than the naked eye in the shade selection. Therefore, in this study, the measurement of color was performed by spectrophotometer that confers reproducible, objective, and statistically utilizable results.[7]

Table 4: Mean color change and SD for each group at different storage period.

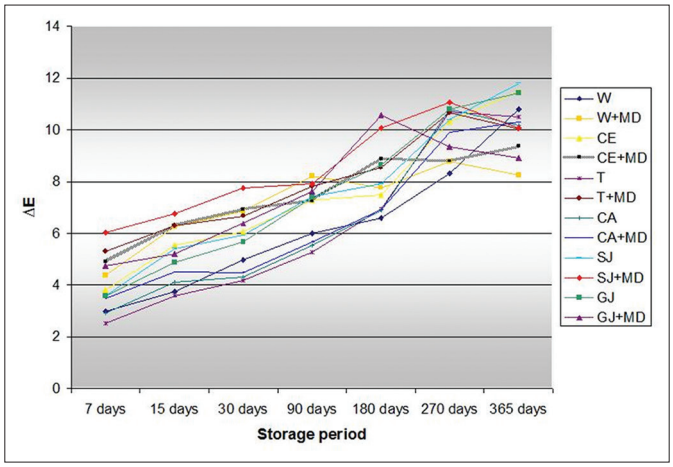
Group	7 days	15 days	30 days	90 days	180 days	270 days	365 days
W	2,99 (2,45)	3,75 (2,53)	4,97 (2,83)	6,00 (3,01)	6,59 (3,88)	8,31 (2,32)	10,80 (2,95)
W+MD	4,38 (2,42)	6,25 (3,20)	6,87 (4,25)	8,21 (4,63)	7,77 (3,96)	8,78 (2,67)	8,25 (2,97)
	A	B	BC	CD	CD	DE	E
CE	3,81 (2,47)	5,53 (3,25)	6,07 (3,48)	7,28 (3,68)	7,49 (3,64)	10,32 (2,90)	11,51 (3,28)
CE+MD	4,91 (2,49)	6,31 (2,68)	6,93 (3,63)	7,27 (3,88)	8,89 (2,44)	8,80 (2,09)	9,37 (2,15)
	A	B	B	BC	CD	DE	E
T	2,52 (2,18)	3,60 (2,37)	4,17 (1,76)	5,28 (2,27)	6,95 (2,66)	10,71 (2,10)	10,51 (2,01)
T+MD	5,31 (2,52)	6,31 (2,55)	6,68 (2,18)	7,82 (2,66)	8,55 (2,96)	10,68 (1,53)	10,03 (1,60)
	A	AB	BC	CD	D	E	E
CA	2,93 (2,02)	4,12 (2,28)	4,30 (1,72)	5,54 (2,03)	6,90 (2,39)	10,79 (1,98)	10,18 (2,47)
CA+MD	3,50 (1,41)	4,50 (2,29)	4,49 (1,68)	5,67 (2,04)	6,91 (2,09)	9,90 (1,91)	10,30 (2,38)
	A	AB	B	BC	CD	D	D
SJ	3,59 (2,13)	5,41 (2,34)	5,92 (2,87)	7,42 (2,53)	7,92 (2,38)	10,40 (2,33)	11,80 (2,14)
SJ+MD	6,04 (2,30)	6,77 (2,27)	7,76 (2,58)	7,91 (3,66)	10,08 (3,22)	11,06 (3,19)	10,08 (2,11)
	A	AB	BC	C	D	D	D
GJ	3,59 (2,40)	4,88 (3,67)	5,66 (3,59)	7,38 (5,14)	8,64 (4,11)	10,80 (2,81)	11,42 (3,22)
GJ+MD	4,74 (1,88)	5,21 (2,82)	6,38 (2,82)	7,63 (2,99)	10,57 (3,26)	9,34 (2,99)	8,91 (2,56)
	A	B	BC	CD	D	E	E

Horizontally, mean values designated with identical capital letters were not statistically different ($P>0.05$). Vertically, there were not significantly differences between staining agent at each storage period ($P<0.05$), SD: Standard deviation, W: Water, MD: Microwave disinfection, GJ: Grape juice, SJ: Strawberry juice, CE: Coffee, T: Tea, CA: Cola

Table 5: NBS system of expressing color difference.

Critical remarks of color difference	ΔE NBS units
Trace	0.0-0.5
Slight	0.5-1.5
Noticeable	1.5-3.0
Appreciable	3.0-6.0
Much	6.0-12.0
Very much	12.0≥

NBS: National Bureau of Standards



Graph 1: Means of color change for each group at different storage periods.

The color change of resin-based materials may be determined by the combined effects of intrinsic and extrinsic factors. Intrinsic factors are associated with physicochemical reactions of the material itself, oxidation of residual monomers, degree of polymerization and water sorption.[27] Extrinsic staining is related with absorption and adsorption of staining foods or mouth rinses, plaque and surface roughness of the denture.[27]

The results showed that microwaved specimens have higher color change than specimens did not microwave after 7, 15 and 30 days regardless of the staining agent. Microwave irradiation increase the temperature of the resin, and structural changes may occur during this process. The heating generated by microwave irradiation for denture could leave the resin near its glass transition temperature (Tg), which provide greater mobility of the molecules of residual monomer and as a result, reaction of additional polymerization and greater degree of conversion of monomer into polymer.[28] According to Wallace *et al.*,[29] the conventional heating by conduction and the dielectric heating generated by microwave irradiation are different. In dielectric heating, external and internal parts of the object are uniformly heated and the temperature increases more rapidly. Microwave energy does not depend on the thermal conductivity and, therefore, is more efficient for heating materials such as acrylic resins. It has been shown that microwave irradiation in an already polymerized denture significantly reduces the content of residual monomer of autopolymerizing acrylic resin, because of higher content of residual monomer of this materials when compared to heat-polymerized acrylic resins.[30,31] In the 1st month, heating generated by microwave irradiation probably resulted in unreacted monomer vaporization. This process can cause porosities in sections of the polymerized reline acrylic resin and consequently, make easy absorption of staining foods. The results also demonstrated that microwave disinfection produced no significant increase in the color change after 1 month. These findings suggest that initial cycles of microwave irradiation may have accelerated the reduction of the content of residual monomer, which took place with time.

Color change was observed after 15 days of water immersion when compared to 7 days. The susceptibility to color change may be attributed to the degree of absorption of water and hydrophilic of reline material. The poly(ethyl methacrylate), the major component of Tokuyama Rebase II powder, is hydrophilic.[32] Therefore, this material may show high degree of water absorption and increase of discoloration compared to hydrophobic materials.[12] Sham *et al.*,[33] observed that color change of the methyl/ethyl methacrylate polymers is due to the high absorption of water. Buyukyilmaz and Ruyter[34] evaluated seven resins-based materials for dentures and observed that materials exhibited absorption of water from 2.5 to 3.5%. They also observed color change for denture base materials. Previous studies have reported that water could induce chemical degradation by hydrolysis and formation of pores on the surface of composite.[12]

Furthermore, porosities inside reline material may have facilitated the absorption of liquids. Acrylic resins porosity has been attributed to a number of factors that include the inclusion of air during the mixture of material, shrinkage and monomer evaporation during the polymerization process associated with the exothermic reaction, insufficient incorporation of the powder into the liquid and the presence of residual monomer.[35,36]

Surface porosity may also be the result of an inadequate mixture of the powder and the liquid, and therefore, one might expect a higher residual monomer content in this sections of the polymerized reline resin. Probably, these regions shrank more than the adjacent that produced localized gaps and make it easy to absorption of aqueous solutions.

With respect to water immersion, color change may also have occurred due to intrinsic discoloration produced by oxidation in the structure of the polymer matrix, oxidation of unreacted pendant methacrylate groups and oxidation of the accelerator.[37] In addition, water might act as a carrier for staining agents in the water sorption process.[38] In this study, a period of 15-day was enough to induce color change on the reline resin after coffee and grape immersion. Color change may be occurred due to extrinsic staining by absorption and adsorption of colorants.[39,40] Different types and percentage of colorants may be found in the beverages used in this study (coffee, tea, cola, strawberry juice and grape juice).

A percentage of 15-28% of brown pigments, resultant of non-enzymatic browning reactions, as Maillard reaction and caramelization, is found in the instant coffee.[41] These reactions occur in the roasting process of the coffee beads. In the Maillard reaction, there were chemical interactions between an aldehyde or ketone group of a reducing sugar and amino group, resulting in brown polymers called melanoidins.[42] In the caramelization reaction, it occurs the decomposition of sucrose to glucose, fructose and finally to caramel at temperatures close to its melting point.[43] Melanoidins and caramels are naturally occurring pigments and that produced brown color in the coffee. Probably, immersion in this beverage enables pigmentation of reline resin by coffee, which confirms its chromogenic effect. These results are in agreement with those reported by several authors.[37,44-49]

Reline resins also were color instable after exposure to grape juice for 15 days. Grape beverage contains an inorganic natural pigment (titanium dioxide) and synthetic colorants. Titanium dioxide is used to provide whiteness and opacity in foods, however its utilization as food colorant in the United States is limited to 1% by weight.[50] Allura red AC (red), Brilliant blue FCF (Greenish blue) and Amarantha (red) colorants are included in composition of grape juice. Probably, color instable of reline resin by grape juice immersion was mainly due to present of Brilliant blue FCF that confer greenish-blue coloration.

It is important to emphasize that tea immersion resulted in color change after 30 days. Lipton ice tea lemon has extract of black tea in its composition. Black tea leaves contains natural pigments, such as chlorophyll and carotenoids. Green chlorophylls pigments, by the action of degradative enzymes and acids, are converted to pheophorbides (brownish in color) and pheophytins (black) during the processing of tea. In addition, enzymatic oxidative reactions, which involved phenolic groups, amino acids, carotenoids and unsatured fatty acids, are of essential importance for the formation of colorants. The pigments formed as a result of this reaction are theaflavins (bright red color), epitheaflavic acids (bright red color) and thearubigins (reddish-yellow color) that impart color to black tea.[41] Furthermore, final color of Lipton Tea lightens by the addition of lemon juice. This occurs because the lemon juice is considered a strong acid and its non-dissociated form becomes apparent that reveal the yellow color.[42] Therefore, tea pigmentation derives from the process of adsorption of reddish-yellow pigments on the resin surface and absorption in the sub-surface layer facilitated by porosities of the material.

With regard to the staining produced by cola after 30 days, it is important note that the caramel pigment is included in the composition of this soft drink. Heating of a sucrose solution with ammonium bisulfite results in the formation of the caramel IV used in cola that imparts dark brown to black color.[41] Probably, the relevant effect of cola immersion on the color change of the reline material may be attributable to exogenous discoloration arising from the absorption of caramels. Furthermore, the polarity of the poly(ethyl methacrylate) molecules and of the cola, coffee, and tea pigments facilitates absorption process.[12] According to Lai *et al.*,[51] different polar properties of the materials may affect both the affinity of a resin to extrinsic stains and the diffusion of water molecules. Thus, hydrophilic materials with high water absorption are stained by hydrophilic colorants in aqueous solution.[51]

Strawberry juice caused color change after 30 days. This drink has synthetic colorant (Allura red AC), inorganic natural pigment (titanium dioxide) and caramel colorants that may be conferred brownish-red coloration for the prosthetic material.[50]

Staining agents caused no statistically significant color change after 270 days. However, reline material after 180 days of immersion in all staining agents showed color change categorized as "much" based upon the National Bureau of Standards system for expressing color difference. One might assume that endogenous irreversible discoloration attributed to changes in the chemical structure of the material or bulk discoloration derived from the incomplete conversion of photoinitiators and the unconverted C=C bonds shall have occurred due to aging of the reline resin.[27] In addition, exogenous discoloration from the superficial penetration of colorants after chemical degradation of the material surface and surface discoloration from absorption of color pigments may be concerned all layers of the material until 9 months.[27]

The results also demonstrated that there were no significant differences in color change at each storage period when compared the staining agents. The degree of staining may be influenced by the characteristics of the reline material and by solutions used. The pH and alcohol concentration of drink may alter the surface properties of this material. Solutions with pH values lower than 7 may cause a greater plasticizer effect on the surface layer of the material and consequently, a softening of surface, which make it predisposed to degradation and staining.[14] However, all solutions used showed acid pH, resulting in similar alterations on the color for same storage period.[12,52-55]

Although this *in vitro* study evaluated the color stability of reline resin after drinks immersion, it did not reproduce the clinical situation ideally. Dietary conditions, tooth brushing, use of mouthrinses or of other chemical plaque control agent, salivary flow rate, denture surface characteristics, physical and chemical properties of the material may affect the color stability. Further investigations are required to clinically evaluate the color stability of relined dentures after a long service period.

Conclusion

Color change of microwaved reline resin was observed after 7, 15 and 30 days irrespective of the staining agent when compared with specimens that were not irradiated. Immersion in distilled water, coffee, and grape juice caused significant color change after 15 days when compared with 7 days. After 30 days, immersion in tea, cola and strawberry juice resulted in significantly greater color change than that which occurred in specimens immersed for 7 days. There were no significant differences in color change for all solutions after 270 days. No statistically significant color change was found for each storage period when the staining agents were compared.

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