ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 11, Issue, 06, pp. 47580-47584, June, 2021
https://doi.org/10.37118/ijdr.22008.06.2021

RESEARCH ARTICLE OPEN ACCESS

PIGMENTATION OF RESIN COMPOSITE IMMERSED IN COLORED DRINKS: AN *IN VITRO* COMPARATIVE STUDY

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ARTICLE INFO

Article History:
Received 17th March, 2021
Received in revised form
11th April, 2021
Accepted 20th May, 2021
Published online 20th June, 2021

Key Words:
Composite Resins; Color; Coloring Agents; Beverages

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ABSTRACT

Aim: To evaluate the potential pigmentation caused by beverages consumed by humans on nanohybrid composite resins. **Methods:** Seventy-five cylindrical samples (5×2 mm) were made with a Teflon matrix and distributed in groups according to the type of composite resin: NT Premium (Coltene), Opallis (FGM), and Beautiful (Shofu) (n = 25). The samples of each group were randomly divided into five subgroups with different colored drinks (n = 05): artificial saliva, red wine, Gatorade, açai juice, and whiskey. The color measurement was performed using a digital spectrophotometer (Vita Easyshade) at the following time intervals: 0 h (before immersion), 1 h, 1 d, and 1 week after the immersion date. The color variation (ΔE) of the composite resins was calculated using CIELab parameters, considering the different immersion times. **Results:** Evaluation after 1 week also revealed a statistically significant difference between the composite resins assessed. When immersed in red wine, Opallis resin showed lesser pigmentation when compared with other resins ($p = 0.013$), and when immersed in Gatorade, Beautiful resin showed lesser pigmentation than the other composite resins ($p = 0.004$). There was a statistically significant difference for red wine, with it being the drink that caused maximum pigmentation in all three composite resins that were tested ($p < 0.001$). **Conclusion:** All composite resins showed an intense degree of pigmentation after immersion in the beverages for 7 days. The time of exposure to these drinks helped determine the pigmentation potential of various beverages on composite resins.

Citation: ***Victoria Eduarda Vasconcelos Liberato Miranda, Luís Felipe Espíndola-Castro, Bruno Lacerda Miranda, Valdenice Aparecida de Menezes, Priscila Dâmaris dos Santos and Claudio Heliomar Vicente Silva.*** "Pigmentation of resin composite immersed in colored drinks: an *in vitro* comparative study*", International Journal of Development Research*, 11, (06), 47580-47584.

INTRODUCTION

Oral esthetics actively influence human relationships;thus, psychological aspects can influence a patient's search for dental care. A harmonic smile can improve an individual's self-esteem, which has a direct consequence on their professional, emotional, and socioeconomic success (Espíndola-Castro *et al*., 2019a).

Thus, esthetic dental restorations have been frequently sought after dental services (Binalrimal, 2019). Among esthetic restorative materials, composite resins stand out (Veloso *et al*., 2019). This class of materials has good physical, mechanical, and optical properties that provide good long-term clinical results (Espíndola-Castro *et al*., 2019b). However, composite resins exhibit sorption and solubility (Alabbadi *et al*., 2020, Espíndola-Castro *et al*., 2020a). In this way, they can absorb liquids from the oral cavity and consequently dietary pigments, which can esthetically compromise the restorative material

(Espíndola-Castro *et al*., 2020a, Espíndola-Castro *et al*., 2020b). The longevity of composite resins in the oral cavity, the photopolymerization technique, the finishing and polishing method, and the accumulation of dental biofilm on composite resins can also influence the degree of pigmentation they undergo (Espíndola-Castro *et al*., 2020a, Espíndola-Castro *et al*., 2020b). Many factors can influence the water diffusion coefficient between the polymeric network of composite resins, including the content of the charged particles and the volume of the resin matrix (Espíndola-Castro *et al*., 2020b, Brito, Oliveira, Monteiro, 2019). In general, composite resins with a higher percentage of filler particles have less sorption and solubility (Alshali *et al*., 2015). The pigmentation of composite resins may have an intrinsic origin that is related to the composition or type of polymerization or may be extrinsically caused by the incorporation of pigments (Espíndola-Castro *et al*., 2020b, Ramos *et al*., 2019).

Several food items contain a large proportion of dyes in their composition (Rodriguez-Amaya, D.M., 2016). These dyes solely add color, making food items or beverages more attractive, thereby increasing the acceptance of these products by consumers (Hamerski, Rezende, Silva,2013). However, they have no nutritional value and in view of manycoloring compounds, it has become necessary to control their use (Hamerski, Rezende, Silva,2013).Among the food items that contain pigmentation potential, coffee, tea, cola-based soft drinks, energy drinks, refreshment syrups, foods with a high concentration of chlorophyll, whiskey, and wine stand out (Espíndola-Castro *et al*., 2020a, Espíndola-Castro *et al*., 2020b, Dinelli *et al*., 2015).

Thus, dentists must know the different types of composite resins available in the market, to select the best material in relation to the mechanical properties and color stability (Espíndola-Castro *et al*., 2019b; Espíndola-Castro *et al*., 2020b). The oral hygiene of the patient is also a major factor in the color stability of composite resins, since the presence of a dental biofilm and its products causes degradation of the resin, facilitating pigmentation (Bourbia *et al*., 2013). This study aimed to evaluate the pigmentation potential of drinks that are part of the human diet on nanohybrid composite resins as a function of time. The null hypotheses tested were as follows: (I) There was no difference in the pigmentation potential of the different substances tested. (II) There was no difference in the color stability of the different restorative materials studied.

MATERIALS AND METHODS

This was an in vitro study carried out at the Universidade Federal de Pernambuco.

Sample making: With a Teflon matrix, 75 cylindrical samples (height = 2 mm and diameter = 5 mm) were fabricated using three different composite resins (n = 25). The 25 samples from each group were randomly divided among five substances (artificial saliva as a control, red wine, Gatorade, açai juice, and whiskey), as shown in Table 1. To prepare the samples, a Teflon matrix was placed on polyester tape (3M-ESPE, Minnesota, USA),which was on a glass plate. The composite resin was inserted into the Teflon matrix in a single increment.Then, a new polyester tape was placed on the composite resin, and a new medium glass plate with an approximate weight of 511 g was placed on the set. Then, the composite resin was polymerized with an EmitterC light-emitting diode device (Schuster, Rio Grande do Sul, Brazil), with a light intensity of 1250mWcm^2, for 40 s.

Immersion of samples: After preparation, the samples were immersed in artificial saliva (Phormula Ativa, Pernambuco, Brazil), red wine (Logowines, Kompass, Porto, Portugal), whiskey (Bacardi-Martini, Hamilton, Bermuda), Gatorade citrus fruits (PepsiCo, New York, USA), and açai juice (homemade). The samples were immersed in 50 ml Falcon tubes containing 20 ml of each pigmenting substance for a maximum of 1 week. The liquids were replaced daily.

Color measurement: Color measurement was performed with an EasyShare digital spectrophotometer (VITA Zahnfabrik, Bad Säckingen, Germany) at the following time intervals: baseline = 0h, T1 = 1h, T2 = 24h, and T3 = 1 week after the immersion date. Three color measurements were performed for each immersion interval, and an average was obtained. The color was determined using the parameters of the CIELab system (L* a* b*), wherein L* indicates the luminosity, with the average varying from 0 (black) to 100 (white) and a* and b* represent the hue, where a* represents saturation on the red-green axis and b* on the blue-yellow axis. The color comparison before and after the treatments was representedas the color variation (ΔE), given by the equation:

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

where,

L*2 - L*1 = (the reading after immersion - the reading before treatment)
a*2 - a*1 = (the reading after immersion - the reading before treatment)
b*2 - b*1 = (the reading after immersion - the reading before treatment)

Statistical methods: The results are expressed as mean ± standard deviation. For the comparison between composite resins in each drink and between drinks for each composite resin, the Kruskal-Wallis test was used, and in the case of significant differences, Conover's multiple comparison tests were used. The Kruskal-Wallis test was chosen due to five samples for each combination of a composite resin and drink, which was insufficient to analyze the normality of the data. In the statistical tests, significant differences were considered if $p < 0.05$. The data were entered into an Excel spreadsheet, and statistical analysis was performed using IMB SPSS version 23.

RESULTS

Tables 2 to 4 show the color variation statistics (ΔE) of composite resins when immersed in the colored solutions after 1 h, 1 d, and 1 week. From the results presented in Table 2, evaluation after 1 h of immersion revealed that for wine, Gatorade, and whiskey, the averages were significantly lower for NT Premium resin than the other two resins. This evaluation revealed that red wine and Gatorade had the greatest pigmentation potential, with statistically significant differences between all the restorative materials tested when compared with the control group (Table 2). Table 3 describes the results obtained after 1 d of immersion in the colored drinks. It is noteworthy that the highest averages of color variation were observed when red wine was used, with the average ranging from 13.01 to 13.29. In this evaluation, there were statistically significant differences between the composite resins only when immersed in Gatorade and whiskey. NT Premium showed greater color stability in this evaluation than other resins. Table 4 highlights that red wine caused maximum pigmentation of all composite resins tested in this study. There were statistically significant differences between the restorative materials only when immersed in red wine and Gatorade.The composite resin that was least pigmented in red wine was Opallis ($p = 0.013$) and the one that was least pigmented in Gatorade was Beautiful ($p = 0.004$).

DISCUSSION

The first null hypothesis was rejected. There were statistically significant differences in the pigmentation potentials of the drinks used in the present study. All drinks tested showed statistically significant differences when compared with the control group after 7 days of evaluation. However, red wine showed the greatest pigmentation potential for the three composite resins assessed ($p < 0.001$).

Table 1. Description of the materials studied and immersion substances

Composite Resins (Manufacturers)	Material Composition	Colorants	n
NT Premium (Coltene, Altstätten, Suíça)	Dimethacrylate (Bis-GMA), triethylene glycol Dimethacrylate (TEGDMA), ethyl 4-dimethylaminobenzoate, bisphenol A ethoxylated dimethacrylate (Bis-EMA), photoinitiator, fillers and pigments/core A2	Saliva (control)	5
		Red /wine	5
		Gatorade	5
		Açai juice	5
		Whiskey	5
Opallis (FGM, Santa Catarina, Brazil)	Bis-GMA, Bis-EMA, TEGDMA, urethane dimethacrylate (UDMA), camphorquinone, Co-initiatorand silane/core A2.	Saliva (control)	5
		Red wine	5
		Gatorade	5
		Açai juice	5
		Whiskey	5
		Saliva (control)	5
Beautiful (Shofu, Kyoto, Japan)	Bis-GMA, TEGDMA, Aluminofluoro-borosilicate glass Al_2O_3, DL-camphorquinone/core A2	Red wine	5
		Gatorade	5
		Açai juice	5
		Whiskey	5

Table 2. Color variation (∆E) after immersing the composite resins for 1 h in the beverages

	Resins			
	NT Premium (Coltene)	Opallis (FGM)	Beautiful (Shofu)	
Drink	Mean ± SD	Mean ± SD	Mean ± SD	p-value
Saliva (control)	6.57 ± 1.15$^{(a)}$	6.43 ± 0.29 $^{(a)}$	6.36 ± 0.90 $^{(a)}$	p $^{(1)}$ = 0.811
Red wine	8.45 ± 0.69 $^{(A, b)}$	11.09 ± 0.35 $^{(B, b)}$	11.44 ± 0.23 $^{(B, b)}$	p $^{(1)}$ = 0.006*
Gatorade	9.17 ± 0.87 $^{(A, b)}$	11.68 ± 0.55 $^{(B, b)}$	10.82 ± 0.87 $^{(B, b)}$	p $^{(1)}$ = 0.012*
Açai juice	8.22 ± 0.69 $^{(A, b)}$	7.24 ± 0.83 $^{(c)}$	7.35 ± 0.85 $^{(c)}$	p $^{(1)}$ = 0.289
Whiskey	6.12 ± 0.37 $^{(A, a)}$	8.04 ± 0.49 $^{(B, c)}$	6.73 ± 0.51 $^{(C, ac)}$	p $^{(1)}$ = 0.006*
p-value	p $^{(2)}$ = 0.002*	p $^{(2)}$<0.001*	p $^{(2)}$ = 0.001*	

SD, standard deviation (*) Significant difference at the 5% level. (1) The Kruskal-Wallis test between resins per drink with multiple comparisons of the Conover test. (2) The Kruskal-Wallis test between resin drinks with multiple comparisons of the Conover test.. If all capital letters in parentheses are distinct, it is possible to verify the significant differences between the corresponding resins. If all lower-case letters in parentheses are distinct, significant differences between the corresponding drinks are verified.

Table 3. Color variation (∆E) after immersing the composite resins for 1 d in the beverages

	Resins			
	NT Premium (Coltene)	Opallis (FMG)	Beautiful (Shofu)	
Drink	Mean ± SD	Mean ± SD	Mean ± SD	p-value
Saliva (control)	6.98 ± 1.28 $^{(AB. a)}$	7.48 ± 0.32 $^{(A, a)}$	5.89 ± 0.59 $^{(B, a)}$	p $^{(1)}$ = 0.032*
Red wine	13.18 ± 1.67 $^{(b)}$	13.21 ± 0.86 $^{(b)}$	13.29 ± 0.62 $^{(b)}$	p $^{(1)}$ = 0.592
Gatorade	9.68 ± 0.66 $^{(A, c)}$	13.20 ± 0.84 $^{(B, b)}$	10.94 ± 0.85 $^{(C, c)}$	p $^{(1)}$ = 0.005*
Açaijuice	8.93 ± 0.94 $^{(c)}$	8.09 ± 1.02 $^{(a)}$	8.99 ± 0.56 $^{(d)}$	p $^{(1)}$ = 0.184
Whiskey	7.21 ± 1.15 $^{(A, a)}$	10.00 ± 0.74 $^{(B, c)}$	7.51 ± 0.53 $^{(A, e)}$	p $^{(1)}$ = 0.009*
p-value	p $^{(2)}$ = 0.001*	p $^{(2)}$<0.001*	p $^{(2)}$<0.001*	

SD, standard deviation (*) Significant difference at the 5% level. (1) The Kruskal-Wallis test between resins per drink with multiple comparisons of the Conover test. (2) The Kruskal-Wallis test between resin drinks with multiple comparisons of the Conover test. If all capital letters in parentheses are distinct, it is possible to verify significant differences between the corresponding resins. Ps. If all lower-case letters in parentheses are distinct, significant differences between the corresponding drinks are verified.

Table 4. Color variation (∆E) after immersing the composite resins for 1 week in the beverages

	Resins			
	NT Premium (Coltene)	Opallis (FGM)	Beautiful (Shofu)	
Drink	Mean ± SD	Mean ± SD	Mean ± SD	p-value
Saliva (control)	3.33 ± 1.22 $^{(a)}$	4.93 ± 0.57 $^{(a)}$	3.94 ± 1.19 $^{(a)}$	p $^{(1)}$ = 0.137
Red wine	20.43 ± 2.65 $^{(A, b)}$	16.02 ± 1.27 $^{(B, b)}$	20.34 ± 1.85 $^{(A, b)}$	p $^{(1)}$ = 0.013*
Gatorade	15.31 ± 0.61 $^{(A, c)}$	15.71 ± 0.40 $^{(B, b)}$	12.67 ± 1.01 $^{(C, c)}$	p $^{(1)}$ = 0.004*
Açai juice	9.64 ± 1.16 $^{(d)}$	9.07 ± 1.07 $^{(c)}$	9.73 ± 0.41 $^{(d)}$	p $^{(1)}$ = 0.619
Whiskey	11.11 ± 1.22 $^{(e)}$	10.97 ± 1.14 (10.39) $^{(d)}$	11.15 ± 0.24 (2.15) $^{(e)}$	p $^{(1)}$ = 0.997
p-value	p $^{(2)}$<0.001*	p $^{(2)}$<0.001*	p $^{(2)}$<0.001*	

SD, standard deviation

(*) Significant difference at the 5% level.

(1) The Kruskal-Wallis test between resins per drink with multiple comparisons of the Conover test.

(2) The Kruskal-Wallis test between resin drinks with multiple comparisons of the Conover test.

If all capital letters in parentheses are distinct, it is possible to verify significant differences between the corresponding resins.

Ps. If all lower-case letters in parentheses are distinct, significant differences between the corresponding drinks are verified.

The second null hypothesis was also rejected. There were statistically significant differences between the restorative materials studied when immersed in red wine and Gatorade after 7 days of immersion ($p = 0.013$ and $p = 0.004$, respectively). When immersed in red wine, the least pigmented material was Opallis resin (FGM); however, when immersed in Gatorade, Beautiful (Shofu) resin was the least pigmented. To analyze the pigmentation of composite resins when immersed in different colored drinks, a digital spectrophotometer was used. This equipment allows an objective evaluation of the color and interpretation of these results in a numerical form. The digital spectrophotometer measures the color from the reflection of the object's light within the visible spectrum, encodes the data, and determines the color coordinates L* a* b* (Johnsen, S., 2016). In clinical practice, color evaluations are performed more frequently using visual color scales; however, this analysis is operator-dependent (Nogueira, Della Bona, 2013).

In visual assessments, several factors such as patient age, clinical experience, visual fatigue, and ambient lightcan influence the result (Espíndola-Castro *et al*., 2020c). In a clinical study of 13 patients who required a single ceramic crown on the upper central incisor, two methods of digital color analysis were compared with visual analysis. Three crowns were fabricated for each tooth, using the Vita Easyshade spectrophotometer, Shadepilot spectrophotometer, and the VitapanClassic color guide for shade selection. Both spectrophotometric systems showed significantly better results than the visual method ($p < 0.05$)(Kalantari, Ghoraishian, Mohaghegh, 2017). Therefore, the use of digital color methods should be encouraged for clinical and laboratory research assessments.

The results obtained in the present study prove that composite resins are subjected to staining according to the substances that are ingested by the patient and the frequency at which they are consumed. Among the tested beverages, red wine was found to have the greatest pigmentation potential, followed by Gatorade, whiskey, and Açai juice after a week of immersion. This result can be justified by the fact that red wine has alcohol in addition to pigments, which is responsible for weakening the resin structure and facilitating the absorption and fixation of dyes (Arocha *et al*., 2013). In addition, red wine and Gatorade have acidic pH that induce discoloration of composite resin as well as a reduction in surface microhardness (Erdemir *et al*., 2013).The sorption, which is the capacity of the resinous material to absorb liquids from the buccal medium, can be increased after exposure to an acidic solution, which implies a possibility of stimulating the degradation of the polymeric resin matrix.

Gatorade is an isotonic sports drink developed scientifically to quickly replace the fluids and minerals lost with sweat, either through physical activity or in situations of intense heat. The composition of Gatorade is similar to that of ordinary saline containing water, sugar, and salt. However, the drink also contains flavoring agents, dyes, and a high concentration of carbohydrates (Proctor *et al*., 2005). The dyes present in this drink were able to pigment the restorative materials from the first hour of immersion in the substance when compared with the control group (artificial saliva). The potential pigmentation caused by whiskey, despite having a moderate color, can also be justified by it having a high alcohol content. Alcohol is a solvent that facilitates pigmentation because it weakens the structure of the resin (Arocha *et al*., 2013), a process similar to wine pigmentation.

An in vitro study evaluated the influence of acidity, presence of alcohol, and beverage pigments on the optical properties, microhardness, and surface roughness of a nanohybrid dental composite resin. The samples were distributed in drinks with pH (2.0, 3.0, 4.0, and 5.5), alcohol (0%, 20%, and 40%), and anthocyanin pigment (0%, 2.5%, and 12 5%). The authors observed that the concentration of 12.5% pigment or 40% alcohol significantly increased the color variation of the samples (Hwang *et al*., 2018). The results of this study also show that, despite intense coloring, açai juice had the lowest pigmentation potential among the tested drinks. Açaí is rich in a natural dye named anthocyanin (Peris *et al*., 2018). This dye binds to hydroxyapatite, even in the absence of salivary proteins.An study evaluated the pigmentation of a nanoparticulate composite resin immersed in açaí juice and mirtillo juice after 7 days. The authors also observed a lower pigmentation potential for açaí juice with $\Delta E = 10.21$. These results are similar to the present study, which observed a color change in groups immersed in açaí juice with values of ΔE that varied from 9.07 to 9.73 after a week of immersion (Dias, Carrera, Rastelli, 2016). The lower pigmentation potential observed in açaí in the present study can be explained by the fact that the product was not used in its pure form. Açaí was diluted in water in this study, which consequently decreased the chances of pigmentation caused by this substance.

Opallis resin (FGM) showed great color stability after 7 days of immersion in the colored beverages. Water sorption is one of the main factors responsible for the degradation of resinous materials, andit occurs mainly by the absorption of water in the resin matrix (Espíndola-Castro *et al*., 2020a, Espíndola-Castro *et al*., 2020b). It is the precursor to a variety of chemical and physical processes, which produce many deleterious effects on the structure and function of these materials, such as plasticization, hydrolysis, solubility, and leaching of the material (Espíndola-Castro *et al*., 2020a, Espíndola-Castro *et al*., 2020b, You *et al*., 2004). The type of monomer present in the resin composition is also related to the degree of staining of the restoration. The resins that have UDMA as the monomer have greater resistance to staining than those with Bis-GMA (Reis, Giannini, Pereira, 2007, Lee, Powers, 2007). All resins tested in the present study have Bis-GMA monomers; however, Opallis is the only resin withUDMA in its composition. This may be related to the lower pigmentation seen with respect to Opallis after a week of immersionin red wine.

CONCLUSION

According to the results obtained, red wine had greater pigmentation potential at different evaluation intervals. All composite resins showed an intense degree of pigmentation after immersion in the beverages for 7 days. The NT premium composite resin (Coltene) revealed a lower degree of pigmentation at the 1-h- and 1-d evaluations. However, after 7 days, Opallis resin (FGM) underwent lesser pigmentation in red wine and Beautiful resin (Shofu) underwent lesser pigmentation in Gatorade. The time of exposure to the beverages in this studyhelped determine the pigmentation potential of various beverages oncomposite resins.

Conflict of interest and sources of funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors declare no conflict of interest

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