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by Wiena Widyastuti

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The colour stability of nanofilled composite resin after immersion in pomegranate juice: a laboratory experimental study

Wiena Widyastuti[1]
Maria Gabriela[1]
Dinie Qurratuaini Zulkifly[2]

[1]Department of Conservative Dentistry, Faculty of Dentistry, Universitas Trisakti, Indonesia
[2]Department of Restorative Dentistry, Kulliyyah of Dentistry, International Islamic University Malaysia

*Correspondence:
wiena@trisakti.ac.id

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ABSTRACT

Introduction: Composite resin is highly valued for its aesthetic appeal, as its colour closely resembles that of natural teeth. Nanofilled composite resins, in particular, offer impressive colour stability. However, restorative materials can undergo colour changes due to intrinsic and extrinsic factors. One such extrinsic factor is fruit pigments, like those found in pomegranates. This study aims to analyze the colour stability of nanofilled composite resin after immersion in pomegranate juice. **Methods**: This laboratory experiment utilized a pre-test and post-test control group design. A total sample of forty nanofilled composite resins (Filtek Z350 XT), each with a diameter of 10 mm and a thickness of 2 mm, was divided into four groups. Each sample in the two treatment groups was immersed in 10 mL of commercial pomegranate juice for one day and seven days, respectively. Meanwhile, each sample in the two control groups was immersed in 10 mL of distilled water for the same duration. Colour measurements were carried out before and after immersion using a spectrophotometer. The data were statistically analyzed using the Shapiro-Wilk normality test to assess distribution. For normally distributed data, a one-way ANOVA was conducted, followed by the Tukey HSD post-hoc test for significant differences. For non-normally distributed data, the Kruskal-Wallis test was applied, followed by the Mann-Whitney post-hoc test if significant differences were observed. **Results**: The one-way ANOVA test for the L parameter yielded a p-value of 0.067 ($p>0.05$), indicating no significant changes after immersion in both pomegranate juice and distilled water. The Kruskal-Wallis test yielded p-values of 0.000 for E, 0.001 for C, and 0.000 for H ($p<0.05$), demonstrating substantial differences after immersion in both pomegranate juice and distilled water. **Conclusion**: The nanofilled composite resin exhibited minimal discolouration after immersion in pomegranate juice, as evidenced by changes in E, C, and H. No significant change was observed in L.

KEYWORDS

Colour stability, restoration material, nanofilled composite resin, pomegranate juice

INTRODUCTION

An attractive appearance increases one's self-confidence physically, socially, and psychologically.[1] When patients undergo dental treatment involving anterior teeth, restorations that match the colour and shape of their natural teeth are highly desired.[2] Both patients and dentists have widely accepted composite resin as an aesthetic restorative material.[3] Nanofilled composite resins, particularly, are suitable for anterior and posterior teeth due to their beneficial properties.[4] The amount of filler in nanofilled composite resin affects its mechanical properties, including its microhardness, flexural strength, compressive strength, diametral strength, and yield.[5] It also minimizes occurrence of polymerization shrinkage. In addition, the zirconia or silica content in nanofilled composites gives good abrasion resistance and surface smoothness, which helps reduce plaque accumulation and maintain resin colour stability over time.[6]

Colour stability is an important consideration for dental restorative materials, as long-term use can cause discolouration due to extrinsic staining and intrinsic degradation.[7] The Munsell colour system recognizes three colour variables: value (lightness), hue, and chroma. Lightness refers to the brightness of a colour, hue represents the dominant colour, and chroma indicates the intensity of the colour.[8] A spectrophotometer, which is considered the gold standard for enhancing accuracy and reliability by minimizing individual differences in colour perception, was used for colour measurement.

Pomegranate (*Punica granatum* L.), a plant native to Iran but also grown in tropical areas like Indonesia, is used to make juice, syrup, and vinegar or consumed directly.[9] They are also widely used in the cosmetic and pharmaceutical industries. The pomegranate market was valued at 8.2 billion USD in 2018 and is predicted to reach 23.14 billion USD by 2026.[10] Pomegranates are rich in polyphenolic compounds, including flavonoids, tannins, and phenolic acids, with anthocyanins acting as natural colour pigments and primary antioxidants that are beneficial for health.[11,12,13] Pomegranate offers various health benefits, such as managing diabetes, obesity, hypertension, wound healing, cancer, and osteoarthritis.[14]

In dentistry, pomegranate bioactive components have been shown to reduce plaque formation as well as the risks of gingivitis and periodontitis, indicating that they could be employed as a natural ingredient in toothpaste, mouthwash, and gum treatments.[15] Additionally, pomegranates can also aid in curing recurrent aphthous stomatitis, promote wound healing, and act as a medium for avulsed teeth. In some parts of Indonesia, pomegranate fruit, flowers, seeds, peel, and roots have long been utilized in traditional medicine. However, while their medicinal properties are well-known within certain communities, broader awareness remains limited. Due to their potential medical benefits, various pomegranate plantations have been developed throughout Indonesia.

A study in 2022 showed that commercially produced pomegranate juice and orange juice caused colour changes in nanohybrid and microhybrid composites.[16] However, further studies need to be conducted on the colour stability of nanofilled composite resin. This study hypothesizes that immersing nanofilled composite resin in pomegranate juice will significantly impact its colour stability, leading to observable changes in Delta E, lightness, chroma, and hue (E, L, C, and H). This study aims to evaluate the colour stability of nanofilled composite resin after immersion in pomegranate juice.

METHODS

This laboratory study involved a pre-test and post-test control group design and was conducted at the Dental Material and Testing Center of Research (DMT Core) Laboratory, Faculty of Dentistry, Universitas Trisakti. Samples were prepared using Filtek Z350XT A2 shade (3M, Saint Paul, Minnesota, USA), molded into disc shapes with a diameter of 10 mm and a thickness of 2 mm (Table 1). The sample size was determined in accordance with ISO 4049 guidelines.[17]

Table 1. Composition of the composite evaluated in this study

Material	Organic phase	Inorganic matrix	Colour	Manufacturer
Filtek™ Z350 XT	UDMA, Bis-GMA, Bis-EMA, TEGDMA	Silica filler (20 nm non-agglomerated / non-aggregated), zirconia filler (4-11 nm non-agglomerated / non-aggregated), aggregated zirconia/silica cluster filler (20 nm silica particles combined with 4-11 nm zirconia particles)	A2E, enamel	3M ESPE, St. Paul, Minnesota, USA

The sample size for each group was determined using the Lemeshow formula based on the standard deviation reported in previous research.[16] This formula takes into account confidence level ($Z\alpha$), power of the test ($Z\beta$), standard deviation (S), and the difference in means (x_1-x_2). In this study, the confidence level and power were set at 1.96 and 0.84. The standard deviation from the previous research was 1.51, and the mean difference between the two groups was 1.56.[16] Applying these values to the Lemeshow formula yielded a calculated sample size of approximately 7.345. This value was rounded up to a sample size of 8. To account for potential errors, an additional 20% was added to the sample size, resulting in a final sample size of 10 samples per group.

The samples were measured on an analytical balance to ensure mass consistency. Using a plastic filling instrument, they were then inserted into a stainless-steel moulds measuring 10 mm in diameter and 2 mm in thickness. A celluloid strip and glass slide were placed over the surface to create a smooth sample with minimal porosity and to remove excess composite material.[18] Polymerization was performed using a light-curing unit (LY-B200, Guangdong, China) with a power output of 5 W, a light intensity exceeding 1500 mW/cm², and a wavelength range of 420-480 nm, applying a curing cycle of two 20-second exposures. The samples were then randomly divided into four groups of ten samples each.

Before immersion, the samples were incubated at 37°C for 24 hours to ensure complete polymerization and the release of any residual monomers. Colour measurements were taken using the Vita *Easyshade* V spectrophotometer (VITA Zahnfabrik, Bad Säckingen, Baden-Wurttemberg, Germany). Each sample was measured three times, with the spectrophotometer tip positioned perpendicular to the sample surface. The pH levels of distilled water (Onemed, Sidoarjo, East Java, Indonesia) and commercial pomegranate juice (Rabenhorst, Unkel, Rhineland-Palatinate, Germany) were monitored daily.

The pomegranate juice used in this study was a commercially available product. It was prepared following the manufacturer's instructions and stored under recommended conditions until use. The groups were organized as follows: Group 1 (control), immersed in 10 mL of distilled water for one day; Group 2 (treatment), immersed in 10 mL of pomegranate juice for one day; Group 3 (control), immersed in 10 mL of distilled water for seven days; and Group 4 (treatment), immersed in 10 mL of pomegranate juice for seven days. Assuming approximately 15 minutes are required to consume a glass of pomegranate juice, one day of immersion (24 hours x 60 minutes, divided into 15-minute intervals per day) represented three months of simulated use, whereas seven days of immersion represented two years of simulated use.[19] The solution was replaced daily, and the containers were sealed with plastic wrap to avoid evaporation.[20] Colour measurements were repeated after one day (Groups 1&2) and seven days (Groups 3&4) of immersion.

Data analysis was performed using IBM SPSS Statistics for Windows, Version 27.0 (IBM, Armonk, New York, USA). The Shapiro-Wilk normality test was first performed to assess the normality of the data. If the data were normally distributed ($p>0.05$), a one-way ANOVA test was employed; otherwise ($p<0.05$), the Kruskal-Wallis test was applied. The one-way ANOVA and Kruskal-Wallis tests were used to compare the differences among the four groups. If the Kruskal-Wallis test revealed a statistically significant difference, the Mann-Whitney post-hoc test was performed for pairwise comparisons to determine where the differences occurred. Similarly, if the one-way ANOVA test yielded a statistically significant difference, the Tukey HSD post-hoc test was employed.

RESULTS

Figures 1 and 2 show the nanofilled composite resin before and after immersion in pomegranate juice and distilled water.

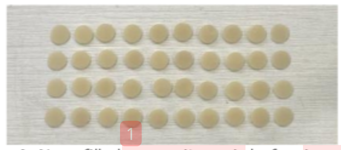

Figure 1. Nanofilled composite resin before immersion

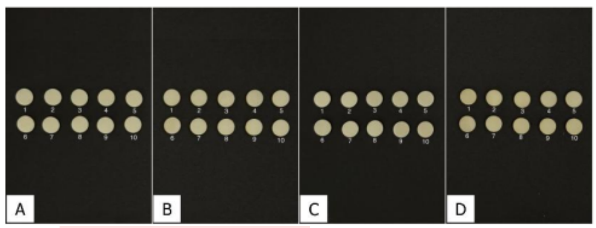

Figure 2. Nanofilled composite resin after immersion: A Group 1, B Group 2, C Group 3, D Group 4

The spectrophotometric colour measurements included the parameters E, L, C, and H. The pre-test data for E, L, C, and H and the post-test data for L and H demonstrated no significant differences (p>0.05), indicating that these datasets followed a normal distribution. In contrast, the pre-test data for C, along with the post-test data for E and C, exhibited significant differences (p<0.05), suggesting that these datasets were not normally distributed (Table 2).

Table 2. Shapiro-Wilk test on E, L, C, H pre-test and post-test data from forty samples

Group	E		L		C		H	
	Pre	Post	Pre	Post	Pre	ΔC	Pre	Post
Group 1	0.538*	0.825*	0.837*	0.853*	0.010	0.141*	0.142*	0.759*
Group 2	0.611*	0.069*	0.374*	0.074*	0.090*	0.004	0.421*	0.092*
Group 3	0.310*	0.012	0.391*	0.631*	0.129*	0.051*	0.703*	0.210*
Group 4	0.436*	0.415*	0.059*	0.207*	0.988*	0.007	0.848*	0.759*

*Shapiro-Wilk test (p>0.05)

The one-way ANOVA test showed no significant differences (p>0.05) in the pre-test E, L, and H data among the four groups, as shown in Tables 3, 4, and 6. It was then assumed that these groups started from the same baseline, allowing for continuation to the post-test. However, the pre-test C data showed a significant difference (p<0.05), indicating that the groups started from different baselines, requiring a pre-test-post-test difference analysis (Table 5).

Table 3. One-way ANOVA, Kruskal-Wallis, and Mann-Whitney tests comparing the E values of nanofiller composite resin samples
*Kruskal-Wallis test ($p<0.05$)
†Mann-Whitney test ($p<0.05$)

Group	Pre Mean$\pm$SD	Post Mean$\pm$SD	MCT			
			Group 1	Group 2	Group 3	Group 4
Group 1	1.53$\pm$0.34	1.56$\pm$0.33		0.000†	0.158	0.000†
Group 2	1.43$\pm$0.30	2.10$\pm$0.24			0.019†	0.031†
Group 3	1.62$\pm$0.35	1.80$\pm$0.36				0.002†
Group 4	1.54$\pm$0.25	2.40$\pm$0.22				
P-value	0.604	0.000*				

SD, standard deviation; MCT, multiple comparison test.

Table 4. One-way ANOVA test comparing the L values of nanofiller composite resin samples

Group	Mean$\pm$SD Pre	Mean$\pm$SD Post
Group 1	-0.13$\pm$1.03	0.03$\pm$0.70
Group 2	0.17$\pm$0.63	0.21$\pm$1.47
Group 3	0.51$\pm$0.48	0.82$\pm$0.46
Group 4	0.36$\pm$0.35	1.03$\pm$0.80
P-value	0.191	0.067

Table 5. Kruskal-Wallis and Mann-Whitney tests comparing the C values of nanofiller composite resin samples
*Kruskal-Wallis test ($p<0.05$)
†Mann-Whitney test ($p<0.05$)

Group	Pre Mean$\pm$SD	MCT (Mann Whitney)				ΔC Mean$\pm$SD	MCT (Mann Whitney)			
		Group 1	Group 2	Group 3	Group 4		Group 1	Group 2	Group 3	Group 4
Group 1	0.97$\pm$0.34		0.300	0.008*	0.026*	0.16$\pm$0.16		0.005†	0.009†	0.253
Group 2	1.14$\pm$0.35			0.117	0.237	-2.27$\pm$1.38			0.015†	0.002†
Group 3	1.38$\pm$0.31				0.731	-0.05$\pm$0.15				0.148
Group 4	1.33$\pm$0.26					0.07$\pm$1.09				
P-value	0.034*					0.001*				

Table 6. One-Way ANOVA and Tukey HSD tests comparing the H values of nanofiller composite resin samples
*One-Way ANOVA test ($p<0.05$)
†Mann-Whitney test ($p<0.05$)

Group	Pre Mean$\pm$SD	Post Mean$\pm$SD	MCT (Tukey HSD)			
			Group 1	Group 2	Group 3	Group 4
Group 1	-1.88$\pm$0.44	-1.68$\pm$0.42		0.000†	0.012†	0.000†
Group 2	-1.56$\pm$0.30	-0.40$\pm$0.46			0.000†	0.000†
Group 3	-1.70$\pm$0.30	-2.27$\pm$0.33				0.000†
Group 4	-1.58$\pm$0.19	0.83$\pm$0.39				
P-value	0.111	0.000*				

The post-test analysis showed a significant difference with a mean value of 0.000 ($p<0.05$) in the E (Table 3) and H (Table 6) across the four groups, whereas the L (Table 4) showed no significant difference ($p>0.05$) with a mean value of 0.067. Additionally, the ΔC (Table 5) demonstrated a statistically significant difference with a mean value of 0.001 ($p<0.05$) among the groups. Post-hoc tests were subsequently conducted to determine the specific differences between the groups, with the results for E presented in Table 3, ΔC in Table 5, and H in Table 6.

DISCUSSION

This study focuses on the three dimensions of colour (hue, chroma, and value) that are interdependent and cannot be assessed separately. The hypothesis that immersing nanofilled composite resin in pomegranate juice would significantly affect its colour stability was partially rejected, as some parameters exhibited significant changes while others did not. As outlined in Table 2, some data were normally distributed while others were not, necessitating both parametric and nonparametric analyses to ensure the results are accurate and appropriately reflect the characteristics of the data.

Tooth restorations are considered aesthetically pleasing if they closely resemble the colour, transparency, and morphology of natural teeth.[21] Colour stability plays an essential role in the long-term outcome of a tooth-coloured restoration. Nanofilled composite resins, characterized by their small filler size, offer a smoother finish, enhanced aesthetics, and greater colour stability over time .[22] However, discolouration may still result from exposure to the oral environment.[16]

Immersing the composite resin in neutral distilled water had no effect on the E values after one or seven days (Table 3).[23] In contrast, pomegranate juice, which contains anthocyanins, caused significant changes in E values during both immersion periods, likely due to pigment penetration over time.[24] This result aligns with the findings of Hajj et al., who observed significant colour changes in nanofilled composite resin after immersion in red wine, attributed to grape tannins and anthocyanins, while distilled water had no discernible effect.

Further analysis (Table 4) indicated no discernible changes in the resin's lightness when immersed in distilled water or pomegranate juice. Huang et al. reported that immersing various composite resins in wine and coffee caused darkening but no noticeable change in lightness when immersed in distilled water.[25] The perception of lightness is influenced by variations in illumination, optical effects, and reflection.[26]

Nonetheless, in Table 5, there was a noticeable difference in the composite resin's chromaticity when immersed in distilled water compared to pomegranate juice. Huang et al. observed a change in chromaticity that became more yellow when the various composite resins were immersed in curry but not in distilled water.[25] The difference is brought about by the pomegranate juice's anthocyanin pigment, which reflects chromatic light.[12]

Pigment concentration can affect chromaticity, and lighting conditions may further cause chromaticity shifts.[27] Differences in hue also emerged between the solutions: while distilled water is colourless, the anthocyanins in pomegranate juice exhibit varying hues under various conditions.[28] Under acidic conditions (pH 1-6), pomegranate juice appears red, while in alkaline (pH 8-14) and neutral (pH 7) conditions, it appears blue and purple, respectively.[29] The pomegranate juice used in this study has a pH of 2.6, which affects its hue.

Meshki et al. investigated the impact of pomegranate and orange juices on the colour stability of microhybrid and nanohybrid composites. Their findings indicated that both juices led to discolouration, with the microhybrid composite demonstrating greater stability compared to the nanohybrid.[16] Hajj et al. assessed the colour stability of nanofilled and monochromatic composites by immersing them in coffee, red wine, cola, and tea, with the results indicating that the nanofilled composite maintained its colour better.[30] However, these findings contradict the results reported by Poggio et al., who reported that nanofilled composites were more prone to colour changes than nanohybrid and microhybrid composites after being immersed in coffee, soda, and red wine.[31]

Both intrinsic and extrinsic factors can contribute to discoloration of nanofilled composite resins. Intrinsic factors are related to the composition of the material, including the type of resin matrix and the weight, size, and distribution of fillers.[22] The chemical structure of Bis-GMA and TEGDMA includes hydroxyl groups, which promote water absorption. Filtek Z350 XT, on the other hand, comprises Bis-EMA, which has a low water absorption due to the ethoxylation reactions that exchange hydroxyl groups with epoxy groups.[32] Filtek Z350 XT has 78.5 wt% and 63.3 vol% filler, with particles ranging in size from 0.005 to 0.02 μm.[25,33] Higher filler content and smaller filler size reduce water absorption and colour penetration.[34]

Adequate polymerization also minimizes discolouration. Previous studies have shown that polymerizing composite resins for 40 seconds resulted in less discolouration compared to 30 or 20 seconds. Inadequate light exposure during the curing process can lead to incomplete polymerization, which compromises the resin's physical properties, particularly increasing water absorption.[35] This study employed a polymerization duration of 40 seconds to ensure complete composite resin polymerization.

Extrinsic factors contributing to discolouration of nanofilled composite resins include the absorption or adsorption of pigments from food and beverages.[36] Due to a shift in their molecular structure, amphoteric natural pigments found in pomegranates, known as anthocyanins, can change colour at varying pH levels.[12] The pigment turns red when hydrogen cations (H^+) are introduced to the anthocyanin molecules in an acidic environment.[37]

These anthocyanins can infiltrate the resin matrix, reducing transparency and light transmission. Anthocyanins themselves are not powerful enough to directly damage the resin matrix. However, long-term exposure and the synergistic effects of an acidic environment, water absorption, and pigment penetration can contribute to the degradation of composite resin over time. The acidic pH of pomegranate juice promotes the release of H^+ ions, leading to matrix breakdown and disruption of polymer bonds. This process creates microcracks and microvoids between the resin matrix and filler, allowing pigments to penetrate.[24]

Microcracks are small, elliptical linear cracks, about 400 μm long and 100 μm wide, whereas microvoids are circular or irregular in shape with diameters ranging from 3 to 20 μm.[38] Rabenhorst pomegranate juice has a comparatively low concentration of anthocyanins (8.95 mg per 100 g) compared to the range of 8.96 to 76.44 mg per 100 g in pomegranates.[39] This lower anthocyanin content contributes to the minimal discolouration of the nanofilled composite resin.

The limitations of this study include its inability to fully simulate the diversity of the oral cavities, which varies among individuals. Moreover, the concentration of pomegranate juice and the type of composite resin did not vary sufficiently. Future research could include surface roughness testing to assess the degree of degradation of the resin matrix as well as microscopic analysis to evaluate the formation of microcracks and microvoids. In addition, further research could investigate the concentration of anthocyanins responsible for the colour changes seen in the composite resin.

CONCLUSION

The minimal discolouration observed in the nanofilled composite resin, as indicated by changes in E, C, and H but not in L, can be attributed to its high filler content, small filler size, and the relatively low anthocyanin content in pomegranate juice used in this study. The implication of this research is that nanofilled composite resin may be a suitable material for aesthetic restorations; however, it can still undergo subtle, perceptible colour changes over time. Therefore, caution is advised when consuming foods or beverages with high pigment concentrations.

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