

# Microhardness and color monitoring of nanofilled resin composite after bleaching and staining

Isabel Cristina G. Bandeira de Andrade<sup>1</sup>, Roberta Tarkany Basting<sup>2</sup>,  
José Augusto Rodrigues<sup>3</sup>, Flávia Lucisano Botelho do Amaral<sup>2</sup>, Cecilia Pedroso Turssi<sup>2</sup>,  
Fabiana Mantovani Gomes França<sup>2</sup>

**Correspondence:** Dr. Fabiana Mantovani Gomes França  
Email: biagomes@yahoo.com

<sup>1</sup>Department of Restorative Dentistry, Regional University of Blumenau, Blumenau, SC, Brazil,  
<sup>2</sup>Department of Restorative Dentistry, São Leopoldo Mandic Institute and Dental Research Center, Campinas, SP, Brazil,  
<sup>3</sup>Department of Restorative Dentistry School of Dentistry, Guarulhos University, São Paulo, SP, Brazil

## ABSTRACT

**Objectives:** The present study aimed to investigate the effect of staining solutions on microhardness and shade changes of a nanofilled resin composite, which had been previously in contact with bleaching agents. **Materials and Methods:** A total of 135 disk-shaped specimens (10 mm × 2 mm) were fabricated with a nanofilled resin (Filtek Supreme) and photocured with a Light Emission Diode (LED) unit and then allocated into three groups to be bleached with 10% or 16% carbamide peroxide (CP) bleaching agents or a 35% hydrogen peroxide (HP) product. Following bleaching, specimens within each group were subdivided into three groups to be immersed in coffee, red wine or distilled water. Microhardness and color were monitored at baseline, after bleaching and after staining. **Results:** Analysis of variance for split-plot design showed lower microhardness values when the composite had been in contact with HP ( $P < 0.0001$ ). The specimens immersed in red wine and coffee provided lower microhardness values than those immersed in distilled water, regardless of the bleaching agent to which the composites were previously exposed. Kruskal Wallis and Dunn tests demonstrated that the composite was lighter after bleaching with a 35% HP agent ( $P < 0.0500$ ). **Conclusion:** The composite was darker as a result of being immersed either in red wine or coffee, regardless of the bleaching agent.

**Key words:** Bleaching agents, color change, laboratory research, microhardness, staining solutions, nanofilled composites

## INTRODUCTION

Dental resin composites have borne witness to remarkable refinements in recent years, especially due to filler size reduction, which has enhanced the polishability and wear resistance of these materials.<sup>[1]</sup> An important factor driving the achievements made in advanced resin composite properties has been nanotechnology.<sup>[1]</sup>

However, despite the ongoing improvements in resin composites, a concern remains about their chemical and enzymatic degradation in the oral environment.<sup>[2]</sup>

Apart from the degradation caused by saliva,<sup>[3]</sup> by foodstuffs<sup>[4]</sup> and by beverages,<sup>[5]</sup> bleaching procedures can potentially cause softening<sup>[6,7]</sup> and increased surface roughness<sup>[8]</sup> to resin composites, depending on the resin composite and on the bleaching agent used.

Contradictory data has been reported regarding the effect of bleaching agents on microhardness and surface roughness of resin composites. Although the authors of some investigations have noticed a reduction in surface microhardness of resin composites after exposure to

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bleaching agents,<sup>[6,7]</sup> authors of other papers have described no change or increase in surface microhardness of composites after bleaching.<sup>[9,10]</sup> In regard to surface roughness, the application of bleaching agents to resin composites has been reported, in previous studies, as not changing or increasing surface roughness.<sup>[8]</sup>

Bleached resin composites have been observed to stain more easily than unbleached counterparts, probably as a result of softening and increased surface roughness.<sup>[11]</sup> Although the literature is still conflicting regarding the susceptibility of bleached and unbleached composites to staining,<sup>[12]</sup> an important issue is that staining solutions can also soften resin composites.<sup>[5]</sup> In fact, red wine and coffee, commonly used as staining solutions in the literature, have been proven to decrease microhardness of resin composites.<sup>[6]</sup> Therefore, both bleaching agents and beverages with staining capacity can decrease the microhardness of resin composites, including nanostructured materials.<sup>[6]</sup>

Considering the softening effect produced by staining solutions, it is important to evaluate whether resin composites that had been previously in contact with bleaching agents would be affected by further softening and darkening as a result of being immersed in different staining solutions.<sup>[8,12]</sup> This study aimed at elucidating this issue by evaluating the effect of staining solutions on microhardness and on the shade changes of a nanofilled composite resin previously in contact with bleaching agents.

## MATERIALS AND METHODS

Table 1 states the main materials used, the staining solutions and their compositions, pH values, manufacturers, batch numbers and manufacturers' directions. Figure 1 describes the experimental design schematically.

### Specimen preparation

A total of 135 disk-shaped specimens were

**Table 1: Nanofilled composite resin, bleaching agents and their composition, manufacturers, batch numbers, directions and pH values**

Material/Solution	Composition	Manufacturer	Batch number	Use mode	pH
Nanofilled Composite Resin Filtek Supreme XT-shade A2E-(3M ESPE, St Paul, MN, USA-FS)	Resin matrix: Bis-GMA, UDMA, TEGDMA e Bis-EMA Filler type: Nanoclusters (0.6 and 1.4 $\mu$ m), nanoparticle, zirconia, (5-20 nm) and silica (20 nm) Filler content (%): 78.5% (weight), 59.5% (volume)	3M ESPE-St Paul, MN, USA	7 EE 7 EU	Single increment 2 mm thick 20 s of photoactivation	-
Whiteness perfect 10%-FGM Produtos Odontológicos, Joinville, SC, Brazil (10% carbamide peroxide agent-10% CP)	CP 10%, carbopol, glycol, water, potassium nitrate and sodium fluoride	FGM, Joinville, SC, Brazil	7898032-323050	4 h daily for 2 weeks	Initial: 5.7 After 4 h of use: 6.13
Whiteness Perfect 16%-FGM Produtos Odontológicos, Joinville, SC, Brazil (carbamide peroxide agent 16%-16% CP)	16% CP, carbopol, glycol, water, potassium nitrate and sodium fluoride	FGM, Joinville, SC, Brazil	7898032-320080	4 h daily for 2 weeks	Initial: 5.64 After 4 h of use: 6.11
Whiteness HP 35%-FGM Produtos Odontológicos, Joinville, SC, Brazil (35% hydrogen peroxide agent-35% HP)	35% HP, thickener, dye, glycol, load and distilled water	FGM, Joinville, SC, Brazil	7898032-323036	3 sessions every 7 days, with 3 15 min applications per session	Initial: 5.51 After 15 min of use: 5.26
Coffee (Melitta São Paulo, Brazil)	Coffee grounds Water	Melitta, São Paulo, SP, Brazil	BJ612TR	16 g coffee 200 ml of water/day at 55°C	5.5
Red wine Cabernet (Concha Y Toro, Santiago, Chile)	Cabernet Sauvignon grapes Alcohol 12.5%	Concha Y Toro, Santiago, Chile	L2CA2338 L1C41269	200 ml/day at 25°C	4.2
Distilled water	-	-	-	200 ml/day at 25°C	6.8

Bis-GMA: bisphenol A diglycidyl methacrylate, MTAD: Mixture of Tetracycline isomer, citric acid and detergent, UDMA: Urethane dimethacrylate, TEGDMA: Triethyleneglycol- dimethacrylate

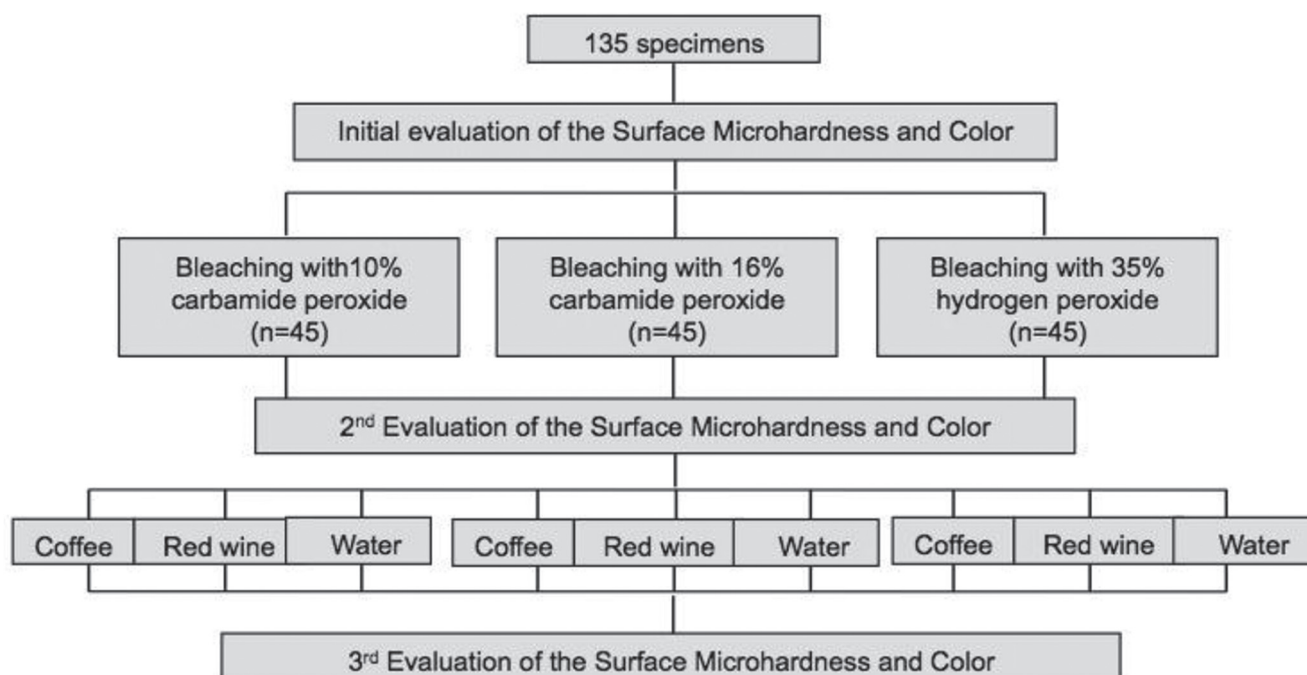


Figure 1: Experimental design

prepared with a nanofilled resin composite (Filtek Supreme XT - 3M ESPE, St. Paul, MN, USA), which was inserted with a metal spatula (Minelli no. 1 - Golgran, São Paulo, SP, Brazil) in circular acrylic molds (10 mm diameter × 2 mm thick). A mylar strip (Dentart - Polidental, São Paulo, Brazil) was placed on top of and pressed flat with a microscope slide plus a 500 g weight for 30 s. The composite was photocured with a LED unit (Radii-Cal SDI - Melbourne, Victoria, Australia - 1,200 mW/cm<sup>2</sup>) for 20 s.

Following preparation, specimens were stored in relative humidity at 37°C for 24 h. The finishing and polishing steps were performed with Al<sub>2</sub>O<sub>3</sub> abrasive disks (Sof-Lex Pop On - 3M ESPE, St Paul, MN, USA) of decreasing coarseness. The specimens were then stored at 37°C for another 24 h.

### Bleaching Treatment

The specimens were randomly divided into three groups ( $n = 45$ ) to be subjected to one of the bleaching agents. An individual acetate tray was fabricated in a vacuum-forming machine (P7/Bio-Art Equip Odontológicos Ltda., São Carlos, SP, Brazil) for each specimen, which would be then exposed to 10% or 16% carbamide peroxide (CP) bleaching agents [CP - Whiteness Perfect FGM Produtos Odontológicos, Joinville, SC, Brazil - Table 1]. These bleaching agents were applied for 4 h/day, for 14 days, simulating the at-home bleaching

technique. In regard to the group exposed to the 35% hydrogen peroxide (HP) agent (HP - Whiteness HP 35% FGM Produtos Odontológicos, Joinville, SC, Brazil), the product was applied with a disposable applicator (Microbrush - Vigodent, Rio de Janeiro, RJ, Brazil) for 45 min (three sequential 15-min applications) once a week for three consecutive weeks.

When the bleaching time was completed, the specimens were rinsed thoroughly with distilled water and stored in distilled water at 37°C.

### Immersion in staining solutions

When the bleaching procedures were completed, specimens of each group were allocated into three subgroups ( $n = 15$ ) to be immersed daily in 200 mL of either coffee (Melitta, São Paulo, Brazil) or red wine (Concha Y Toro, Santiago, Chile), as described in Table 1. Distilled water served as the negative control. Specimens were immersed in staining solutions or distilled water for 3 h/day, at room temperature, over 40 days.<sup>[10]</sup> After every 3-h period of immersion time, the specimens were rinsed thoroughly with distilled water and stored in distilled water at 37°C.

### Color testing

Color shade was measured using a spectrophotometer (VITA Easyshade - VITA Zahnfabrik, Bad Säckingen, Germany) and the data

were expressed based on the VITA shade guide and then converted into scores, as can be seen in Table 2.<sup>[13,14]</sup> Color shade was recorded on three different occasions: baseline, after bleaching and after staining.

### Microhardness testing

Three Knoop microhardness indents were carried out on the top surface of each specimen, using a HVS-1000 microhardness tester (Panambra, São Paulo, SP, Brazil), under a 50-g load, applied for 15 s. Measurements were performed at the baseline, following bleaching and after staining procedures.

### Statistical analysis

Microhardness data were analyzed using an analysis of variance (ANOVA) for split-plot design and Tukey's test, whereas the color shade recordings were submitted to Kruskal-Wallis and Dunn's tests. The significance level was set at 5%. Statistical procedures were performed with SAS 6.11 (SAS Institute Inc., Cary, NC, USA).

## RESULTS

Means and standard deviations (SD) of microhardness values and color shade recordings of the nanofilled resin composite at baseline, after bleaching and after staining, are shown in Tables 3 and 4, respectively.

In regard to the microhardness data, ANOVA for split-plot design showed no significant interaction between the bleaching agent and the staining

solution ( $P = 0.4384$ ). Bleaching agents significantly influenced the microhardness values of the nanofilled resin composite ( $P = 0.0003$ ). Lower microhardness values were noticed when the composite had been in contact with 35% HP ( $P < 0.0001$ ). No difference was noticed between the microhardness values produced by the 10% and 16% CP bleaching agent. Overall, there was a significant reduction in the microhardness values from the baseline to the post-staining condition ( $P < 0.0001$ ). The microhardness values of the nanofilled resin composite that had been previously exposed to the bleaching agents were lower when red wine or coffee were used as staining solutions, when compared to distilled water.

In terms of color change, as examined spectrophotometrically, Kruskal Wallis and Dunn tests demonstrated that the nanofilled resin composite was lighter after bleaching with the 35% HP agent ( $P < 0.0500$ ). Specimens became significantly darker after staining ( $P < 0.0500$ ). Regardless of the bleaching agent, no difference was observed for the color shade recorded for the nanofilled resin composite as a result of being immersed either in red wine or coffee, both of which produced a darker color than distilled water.

## DISCUSSION

The results of the present study showed that the bleaching agents significantly influenced nanofilled resin composite microhardness, which was reduced

**Table 2: VITA shade guide scores**

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
B1	A1	B2	D2	A2	C1	C2	D4	A3	D3	B3	A3.5	B4	C3	A4	C4

**Table 3: Mean microhardness values (SD) of nanofilled resin composite at the baseline, after bleaching and after staining**

Occasion	10% carbamide peroxide			16% carbamide peroxide			35% hydrogen peroxide		
	Coffee	Red wine	Water	Coffee	Red wine	Water	Coffee	Red wine	Water
Baseline	51.3 <sup>a</sup> (3.7)	53.7 <sup>a</sup> (2.9)	54.3 <sup>a</sup> (2.8)	54.6 <sup>a</sup> (4.4)	54.1 <sup>a</sup> (5.8)	50.7 <sup>a</sup> (5.2)	51.7 <sup>a</sup> (6.1)	54.8 <sup>a</sup> (4.7)	53.0 <sup>a</sup> (2.6)
After bleaching	48.5 <sup>b</sup> (6.8)	44.2 <sup>b</sup> (4.9)	44.6 <sup>b</sup> (4.1)	44.9 <sup>b</sup> (6.6)	44.3 <sup>b</sup> (5.7)	45.7 <sup>b</sup> (5.1)	44.2 <sup>b</sup> (4.3)	44.0 <sup>b</sup> (6.8)	42.2 <sup>b</sup> (4.8)
After staining	39.2 <sup>c</sup> (1.6)	38.7 <sup>c</sup> (3.5)	42.4 <sup>b</sup> (3.1)	38.2 <sup>c</sup> (3.1)	37.9 <sup>c</sup> (3.2)	42.8 <sup>b</sup> (2.3)	36.4 <sup>c</sup> (2.3)	35.0 <sup>c</sup> (1.2)	40.4 <sup>b</sup> (4.0)

Means followed by different lower cases are significantly different between each other within the same column. SD: Standard deviation

**Table 4: Medians and color shade ranges of nanofilled resin composite at the baseline, after bleaching and after staining**

Occasion	10% carbamide peroxide			16% carbamide peroxide			35% hydrogen peroxide		
	Coffee	Red wine	Water	Coffee	Red wine	Water	Coffee	Red wine	Water
Baseline	5 (0) <sup>a</sup>	5 (0) <sup>a</sup>	5 (0) <sup>a</sup>	5 (0) <sup>a</sup>	5 (0) <sup>a</sup>	5 (0) <sup>a</sup>	5 (0) <sup>a</sup>	5 (0) <sup>a</sup>	5 (0) <sup>a</sup>
After bleaching	5 (0) <sup>a</sup>	5 (0) <sup>a</sup>	5 (0) <sup>a</sup>	5 (0) <sup>a</sup>	5 (0) <sup>a</sup>	5 (0) <sup>a</sup>	2 (3) <sup>b</sup>	5 (3) <sup>b</sup>	2 (3) <sup>b</sup>
After staining	9 (8) <sup>b</sup>	12 (5) <sup>b</sup>	5 (0) <sup>a</sup>	9 (7) <sup>b</sup>	15 (6) <sup>b</sup>	5 (0) <sup>a</sup>	12 (8) <sup>c</sup>	11 (6) <sup>c</sup>	2 (3) <sup>b</sup>

Means followed by different lower cases are significantly different between each other within the same column



when the resin composite was exposed to the 35% HP bleaching agent. The adverse effects of this agent at high concentrations (such as 35% or 38%) have been demonstrated in previous *in vitro* studies.<sup>[7,8]</sup> It has been speculated that hydrogen-peroxide-based bleaching agents may have high oxidation and reduction capability, thus generating free radicals.<sup>[7]</sup> These radicals have been thought to degrade the resinous matrix<sup>[15]</sup> and disrupt the filler/matrix interface, effects which cause increasing water sorption and filler detachment<sup>[16]</sup> and which, in turn, augment surface roughness.<sup>[8]</sup> In addition, the oxidative capacity of peroxides may cleave the composite polymer-chains.<sup>[7]</sup> In this context, resin composites may have also impaired such physical properties as the microhardness.

The effects caused by 10% CP and CP 16% agents on the surface microhardness of the nanofilled resin composite were significantly lower than those produced by the 35% HP. It is still controversial whether these bleaching agents can affect restorative materials deleteriously.<sup>[17]</sup> While some studies have shown significant reduction in Knoop microhardness after at-home bleaching,<sup>[15]</sup> others have demonstrated the opposite.<sup>[9,10]</sup> Differences in protocols of daily use and in bleaching agents may explain such contradictory findings. For example, Malkondu *et al.*<sup>[18]</sup> in their study have reported a significant decrease in nanofilled composite after a 6 h/day bleaching period with 10% CP (as opposed to the 4-h period used in the current study). The authors here cited hypothesized that the bleaching agent had sufficient time to diffuse into the high molecular weight organic matrix of the nanofilled composite. It is worth noting, however, that these investigations were conducted in *in vitro* conditions, in which specimens are commonly stored in distilled water. An *in situ* study<sup>[19]</sup> demonstrated that bleaching with 15% CP had no effect on nanofilled composite microhardness. This may be explained by the dilution of the bleaching agent by saliva. However, this effect needs to be confirmed in further *in situ* studies. The specimens immersed in distilled water (control) showed decreased microhardness, which probably occurred because of previous exposure to bleaching agents.

Data analysis also showed a decrease in microhardness after the bleached specimens were stained. This finding may be attributed to the composition of the nanofilled resin composite,<sup>[6]</sup> the inherent characteristics of the staining solutions and the storage protocol adopted.<sup>[20]</sup> In this study, red wine and coffee produced lower

microhardness values in the resin composite than distilled water. No difference was found between the microhardness values obtained as a result of using red wine or coffee. Although the red wine had a lower pH (4.2) than the coffee (pH 5.5) and an alcoholic strength of 12.5% – overall factors (lower pH and average alcohol by volume) which seem to affect the resin matrix crosslink, the filler/matrix interface and the filler itself<sup>[21]</sup> – the coffee was used at a temperature above 37°C, a condition that may have accelerated the resin composite degradation process.<sup>[22]</sup>

Color change was evaluated with the VITA Easyshade spectrophotometer, which allows tooth shade to be determined based on the VITA Classical scale.<sup>[13]</sup> The data from this study shows that no significant difference existed between the color of the specimens at the baseline and in post-bleaching conditions, except when 35% HP was used, which made the nanofilled composite lighter. This may be attributed to a higher proportion of hydrogen and the more acidic pH of the 35% bleaching gel, as compared to the other home-use gels tested in this study. Conversely, CP agents did not change the color of the resin composite. In fact, color changes of composites have not been clinically detectable after the application of 10% CP agents.<sup>[23]</sup>

The assessment of color stability of resin composites following contact with different immersion media has shown that composites are not inert in the oral environment.<sup>[24]</sup> The results of color evaluation showed that, after immersion in coffee and red wine, specimens which had been previously exposed to bleaching agents were significantly darker than those exposed to distilled water.

It has been reported that color changes may occur in different restorative materials after bleaching followed by immersion in staining solutions, including red wine and coffee.<sup>[25]</sup> Apart from the pigments contained in red wine and coffee, the alcoholic content of the red wine (12.5% by volume) and the coffee temperature used in this study may have softened the organic matrix of the composite and allowed dye absorption.<sup>[19]</sup>

In this study, the effect on nanofilled resin caused by bleaching followed by staining was evident, i.e. microhardness decreased after bleaching and the samples became lighter when subjected to 35% HP. Immersion in red wine and coffee darkened the samples.

## CONCLUSION

Resin composite previously in contact with bleaching agents showed softening and darkening as a result of being stained in red wine and coffee.

## CLINICAL SIGNIFICANCE

Nanofilled composite resin may undergo changes in microhardness and color after bleaching and staining.

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