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Açai neither stains nor alters the mechanical surface properties of nanofill composite resin in vitro

O açaí não mancha nem altera as propriedades mecânicas de superfície da resina composta nanoparticulada in vitro

El açai no tiñe ni altera las propiedades mecánicas de la superficie de la resina compuesta de nanorelleno in vitro

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Abstract
The objective of the present laboratory-quantitative in vitro study is to evaluate the effect of açai on the color, hardness, and roughness of a nanofill composite resin. The samples were randomly assigned into three groups according to the chemical degradation (n=25): artificial saliva (control, pH 7.0) and açai sorbet (pH 3.8) and açai juice (pH 4.9). Colour (CIELa*b* scale), surface roughness (Ra), and surface hardness (SH) outcomes were analysed at baseline and after degradation. The specimens were subjected to 3 daily immersions (6 ml, 15 minutes) during 14 days at 37°C. The interval between each soak was 30 minutes. Specimens were washed with distilled water before placed in fresh saliva. After the third soak in a day, they were kept in fresh saliva overnight. ΔE* values were 1.88 for saliva/sorbet and 2.2 for juice (p=0.017). Of the solutions, only juice reduced the L* and increased a* values (p<0.001). There was no significant difference among the groups (p>0.05) for hardness and roughness. The mechanical surface properties of nanofill composite resin were not changed by açai. Thus, there is no need to discontinue the consumption of açai for patients with nanofill composite resin restorations.
Keywords: Euterpe; Composite resins; Color; Surface properties; In vitro techniques.

Resumo
O objetivo do presente artigo é avaliar o efeito do açaí na cor, dureza e rugosidade de uma resina composta nanoparticulada in vitro. As amostras foram distribuídas aleatoriamente em três grupos de acordo com a degradação química (n=25): saliva artificial (controle, pH 7,0) e sorbet de açaí (pH 3,8) e suco de açaí (pH 4,9). Os resultados de cor (escala CIELa*b*), rugosidade (Ra) e dureza de superfície (SH) foram analisados antes e após a degradação. As amostras foram submetidas a 3 imersões diárias (6 ml, 15 minutos) durante 14 dias (37°C). As amostras foram lavadas com água destilada ao final de cada imersão e nos intervalos (30 minutos) permaneceram em saliva fresca. Ao final da terceira imersão, as amostras foram colocadas em saliva fresca e nela permaneceram durante a noite. Os valores de

ΔE* foram 1,88 para saliva/ sorbet e 2,2 para suco (p=0,017). Das três soluções, apenas o suco reduziu os valores de L* e aumentou os valores de a* (p<0,001). Não foi observada diferença significativa entre os grupos (p>0,05) para dureza e rugosidade. As propriedades mecânicas da resina composta nanoparticulada não foram alteradas pelo açaí. Assim, não há necessidade de descontinuar o consumo de açaí para pacientes com restaurações de resina composta nanoparticuladas.
Palavras-chave: Euterpe; Resinas compostas; Cor; Propriedades de superfície; Técnicas *in vitro*.

Resumen
El objetivo del presente estudio in vitro cuantitativo de laboratorio es evaluar el efecto del açai sobre el color, la dureza y la rugosidad de una resina compuesta de nanorrelleno. Las muestras fueron asignadas aleatoriamente en tres grupos según la degradación química (n=25): saliva artificial (control, pH 7,0) y sorbete de açai (pH 3,8) y jugo de açai (pH 4,9). Los resultados del color (escala CIELa*b*), la rugosidad de la superficie (Ra) y la dureza de la superficie (SH) se analizaron al inicio y después de la degradación. Las muestras fueron sometidas a 3 inmersiones diarias (6 ml, 15 minutos) durante 14 días a 37°C. El intervalo entre cada remojo fue de 30 minutos. Las muestras se lavaron con agua destilada antes de colocarlas en saliva fresca. Después del tercer remojo en un día, se los mantuvo en saliva fresca durante la noche. Los valores de ΔE* fueron 1,88 para saliva/sorbete y 2,2 para jugo (p=0,017). De las soluciones, solo el jugo redujo los valores de L* y aumentó los valores de a* (p<0,001). No hubo diferencias significativas entre los grupos (p>0,05) para dureza y rugosidad. Las propiedades mecánicas de la superficie de la resina compuesta de nanorrelleno no fueron modificadas por el açai. Por lo tanto, no es necesario suspender el consumo de açai en pacientes con restauraciones de resina compuesta de nanorrelleno.
Palabras clave: Euterpe; Resinas compuestas; Color; Propiedades de superficie; Técnicas in vitro.

1. Introduction

The survival of dental restorations should contemplate patient risk factors (Opdam et al., 2018), especially for composite resin restorations. This is a timely issue because low pH colored common beverages may be a reason for failure (Ceci et al., 2016). In vitro studies proved coffee, red wine, tea, fruit juices (processed or natural), and chocolate drinks changed the optical and mechanical properties of different composite resins (Awliya et al., 2010; Nasim et al., 2010; Mundim et al., 2010; Khatri & Nandlal, 2010; Poggio et al., 2016; Khosravi et al., 2016; Abdelmegid et al., 2019).

Despite choosing the restorative material is up to the clinician (Angerame & De Biasi, 2018), the American Academy of Pediatric Dentistry (2023) recommends using Bisphenol A (BPA) free resin-based dental sealants and composites. However, a perceptive color change was observed when specimens of a BPA-free nanofill composite resin (Filtek Z350, 3M ESPE, St Paul, MN, USA) were submitted to 1-week of immersion in the grape juice (pH= 2.8) (Fontes et al., 2009). In fact, there is no evidence that nanocomposites perform clinically better than others (Alzraikat et al., 2018) and the chemical integrity of nano-aggregated particles and the organic matrix nano-filled interface are prone to staining (Lopes-Rocha et al., 2021). Besides, the effect of black açai berry (Euterpe oleracea) on the esthetic and mechanical resistance of the Z350 (3M ESPE, St Paul, MN, USA) is lack. Literature is plentiful to show the properties of açai. Its intense purple colour (Schreckinger et al., 2010) and the variable pH of commercialized pulps (from 3.76 to 5.23) (Eto et al., 2010; Carvalho et al., 2017) has led to speculation that the nanofilled composite resin mentioned above may become rougher, softer and stained by açai.

However, only industrialized açai juice was assessed so far. Borges et al. (2019) showed that the juice (pH 3.8) reduced the hardness and increased the roughness of a conventional and bulk-fill composite resins. Silva-Leite et al. (2014) found unacceptable clinical staining of nanocomposite resins after 12 weeks of immersion by the juice (pH 4.0). Alkhadim, Hulbah and Nassar (2020) also used berry juice, but there is no mention about what type of berry they used. But the açai derivative products marketed currently (sorbet, smooth, and ice cream) has been neglected by researchers. From this standpoint and based on previous research that demonstrates the potential of acidity and colored foods in failure the composites, we hypothesized sorbet and natural juice may shorter the durability of the nanofill composite resin. Thus, this laboratory-quantitative in vitro study is to evaluate the effect of açai on the color, hardness, and roughness of a nanofill composite resin aimed to evaluate the effect of sorbet and açai natural handmade juice on the staining and surface properties of a nanofilled composite resin. The null hypothesis was threefold: (1) there is no difference in the colour among artificial saliva, sorbet, and açai natural handmade juice; (2) there is no difference in the hardness among artificial saliva, sorbet, and açai natural handmade juice; and (3) there is no difference in the roughness among artificial saliva, sorbet, and açai natural handmade juice, when the nanofill composite resin was subjected to chemical degradation with these three solutions.

2. Methodology

2.1 Experimental design

The methodology of the present laboratory-quantitative in vitro study was based on the Ozera et al. (2019). The factor under study was type of solution used for the chemical degradation (three levels). A sample size of 22 specimens per group was estimated in terms of the difference among the groups (Test F; one-way ANOVA) for colour change magnitude (ΔE^*), α error level of 5%, effect size of 0.4, and β error level of 20% (software GPower 3.1.9.2, University of Düsseldorf). A 10% increase (n=25) was incorporated to account for eventual losses. Seventy-five specimens (2 mm high, 8 mm internal diameter) of Filtek™ Z350 (3M ESPE, St Paul, MN, USA) were randomly assigned into 3 groups (n=25 per group): artificial saliva (control), açai sorbet, and açai juice. The pH of the solutions was tested by SC06 electrode (Sensoglass, SensopH Ind. E Comércio de Sensores, São Paulo, Brazil) coupled to an ion 450 M analyzer (Analyser Analytical Instrumentation, São Paulo, Brazil) (de Paula et al., 2014). The chemical degradation followed the method of Ozera et al. (2019). The specimens were subjected to 3 daily immersions (6 ml, 15 minutes) during 14 days at 37°C. The interval between each immersion was 30 minutes. Specimens were washed with distilled water before placed in fresh saliva. After the third immersion in a day, they were kept in fresh saliva overnight. The response variable was L*, a*, and b* values (CIELa*b* scale), surface roughness (Ra), and surface hardness (SH), analysed at baseline and after degradation.

2.2 Specimens preparation

Filtek™ Z350 (3M ESPE, St Paul, MN, USA) (see Table 1) was handled according to the manufacturers' instructions. A single increment of material was inserted into a silicone mould (2 mm deep, 8 mm internal diameter) (Adsil Silicone Addition - Coltene, Vigodent, Rio de Janeiro, Brazil) (Ozera et al., 2019) put between two mylar strips (Quimidrol, Joinville, Brazil). The top was pressed by hand with a glass plate to expel excess material and make the specimens flat and smooth (de Paula et al., 2014). After, the specimens were light cured with Valo (Ultradent, USA) under 1,000 mW/cm2 for 20 s (manufacturer's instructions). A radiometer (Hilux Dental Curing Light Meter, Benlioglu Dental Inc., Demetron, Ankara, Turkey) was sued to monitore the irradiance (Ozera et al., 2019). After curing, the samples were removed from the mold and stored at a 100% relative humidity at 37°C for 24h (de Paula et al., 2014).

Table 1 – Information of tested composite resin.

Material	**Composition**	**Mean Filler Size, μm**	**Manufacturer / Batch No.**
Filtek Z350™ (3M ESPE)	58%-60 vol% (78.5 wt%) combination of aggregated zirconia/silica cluster filler with primary particles size of 5-20 nm and nanogglomerated 20-nm silica filler, Bis-EMA, Bis-GMA; UDMA; TEGDMA	5-20 nm 0.6-1.4 μm (clusters)	3M/ESPE 1909100498

Abbreviations: Bis-EMA, ethoxylated bisphenol-A dimethacrylate; Bis-GMA, bisphenol glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate. Source: Authors.

2.3 Roughness, hardness, and colour analysis

The values of hardness, roughness, and colour ordinates were read before and after in vitro chemical degradation. The Surfcorder SE 1700 (Kosaka Corp, Tokyo, Japan) was used to measure the roughness surface readings (Ra, mm). The mean values of each specimen were derived from three successive measurements of the center of each specimen in different directions, covering 1.25 mm in with a cut-off length of 0.25 mm, at a tracing speed of 0.1 mm/s. Surface hardness (H0) was carried out with the Future Tech-FM 800a hardness tester (Tokyo, Japan) using a Knoop indenter under 50 g load and dwell time of 5 s. For each sample, readings were taken 3× (100 µm between them) and the mean was calculated. Hardness and roughness analysis were based on de Paula et al. (2014).

The spectrophotometer (CM-2600d/2500d, Konica Minolta, Tokyo, Japan) (Ozera et al., 2019) measured the tooth colour based on the CIEL*a*b* color space system and was calibrated after three measurements It determines color in three-dimensional space. L* represents the value (lightness or darkness). The a* value is a measure of redness (positive a*) or greenness (negative a*). The b* value is a measure of yellowness (positive b*) or blueness (negative b*). The color difference (ΔE*) was calculated by applying the formula ΔE*= [(ΔL*)2 + (Δa*)2 + (Δb*)2]½. Specimens were put on a white background to prevent potential absorption effects on colour (Ozera et al., 2019). Colour analysis was based on Muhittin, Burak, Kam (2019).

2.4 In vitro chemical degradation

The protocol was adapted from Ozera et al. (2019). To simulate the ingestion of açai, all the specimens were subjected to three daily chemical immersions for 14 days by being immersed, for 15 min, in 6 mL of the solutions, at a controlled temperature of 37 °C. Table 2 shows the information of the solutions used for the chemical degradation that were daily renewed. After each immersion on a day, they were washed with distilled water and kept in fresh artificial saliva for 30 min. A single operator carried out the procedures.

Table 2 – Information of solutions groups.

Solution	Composition	Manufacturer / Batch No.
Artificial saliva	Calcium (0.1169 g of calcium hydroxide/L of deionized water); 0.9 mM of phosphorus and potassium (0.1225 g potassium phosphate monobasic/L of deionized water); 20 mM TRIS buffer (2.4280 g TRIS buffer/L of deionized water)	Pharmaderm, Cascavel-PR, Brazil.
Açai sorbet	açai pulp, water, sugar, guar gum, carboxymethyl cellulose, tara gum, natural guarana extract, citric acid acidulant, natural guarana aroma identical, glucose, artificial dye amaranth and bright blue fcf, xanthan gum	Polpa Norte, Japurá-PR, Brazil; 0136(TB)
Açai juice	100g medium açai Pulp. 100 ml	Polpa Norte, Japurá-PR, Brazil; 0430

Source: Authors.

Artificial saliva (pH 7.0) was manufactured by Pharmaderm (Cascavel-PR, Brazil) (de Paula et al., 2014). The juice (pH 3.8) was prepared by blending frozen medium açai pulp (100g) (Polpa Norte, Japurá-PR, Brazil) and water (200 ml). Frozen açai sorbet (pH 4.9) (Polpa Norte, Japurá-PR, Brazil) was only melted. The pH of the solutions was tested by SC06 electrode (Sensoglass, SensopH Ind. E Comércio de Sensores, São Paulo, Brazil) coupled to an ion 450 M analyzer (Analyser Analytical Instrumentation, São Paulo, Brazil) (de Paula et al., 2014). The chemical degradation followed the method of Ozera et al. (2019).

2.5 Statistical analysis

The significance level was 5% and the software used was Jamovi version 1.2. Data were tested by the Shapiro-Wilk (α=.05) and Levene tests and submitted to ANOVA (SH0, SH1, ΔSH, a*1, Δa*, b*1, Δb*) followed by Tukey post-hoc test and Kruskall-Wallis (Ra 0, Ra1, ΔRa, L*0, L*1, ΔL*, a*0, b*1, and ΔE*) followed by Dwass-Steel-Critchlow-Fligner post-hoc test.

3. Results and Discussion

Table 3 shows the hardness, roughness, and colour ordinates baseline and final values. ΔE* values were 1.88 for saliva/sorbet and 2.2 for juice (p=0.017). No difference between saliva and sorbet for L*1, a*1, ΔL*, and Δa* was observed (p>0.05). Only juice reduced L*1 and increased a*1 values (p<0.001). No hardness and roughness changes were found and there was no significant difference among the solutions (p>0.05) for these variables.

Table 3 – Descriptive and inferential statistics for hardness, roughness, and color coordinates according to the immersion solutions.

		Groups			
		Saliva	**Sorbet**	**Juice**	**p-values**
Hardness	**SH0**	51.15 (7.11) A	49.07(6.83) A	50.16 (6.87) A	0.570 OwA
	SH1	48.08 (7.49) A	48.13 (6.74) A	48.37 (7.41) A	0.989 OwA
	ΔSH	-3.08 (8.67) A	-0.94 (7.12) A	-1.79 (11.22) A	0.64 OwA
Roughness	**Ra0**	0.33 (0.50) A	0.20 (0.17) A	0.23 (0.19) A	0.683 KW
	Ra1	0.29 (0.49) A	0.19 (0.14) A	0.31 (0.42) A	0.655 KW
	ΔRa	-0.04 (0.40) A	- 0.01 (0.22) A	0.08 (0.41) A	0.992 KW
Color	**L*0**	61.79 (1.58) A	61.79 (1.58) A	62.01 (1.35) A	0.937 KW
	a*0	- 1.33 (0.25) A	- 1.33 (0.25) A	- 1.34 (0.22) A	1.00 KW
	b*0	3.73 (0.88) A	3.73 (0.88) A	3.57 (0.78) A	0.734 OwA
	L*1	62.48 (0.52) A	62.48 (0.52) A	60.92 (1.14) B	<.001 KW
	a*1	- 1.46 (0.25) A	- 1.46 (0.25) A	- 0.71 (0.69) B	<.001 OwA
	b*1	5.16 (1.18) A	5.16 (1.18) A	4.70 (0.76) A	0.355 KW
	ΔL*	0.69 (1.43) A	0.69 (1.43) A	-1.09 (1.16) B	<.001 KW
	Δa*	-0.13 (0.21) A	-0.13 (0.21) A	0.64 (0.54) B	<.001 OwA
	Δb*	1.42 (0.67) A	1.42 (0.67) A	1.13 (0.85) A	0.340 OwA
	ΔE*	1.88 (1.23) A	1.88 (1.23) A	2.20 (0.58) B	0.017 KW

Mean (standard deviation), n= 25. Capital letters indicate comparison among immersion solutions (horizontal). One-way ANOVA (OwA) and Tukey's test; Kruskall-Wallis (KW) and Dwass-Steel-Critchlow-Fligner post-hoc test (p<0.05). Source: Authors.

The present research has addressed the gap within staining in vitro studies and found evidence to guide patients with nanofill composite resin restorations on whether or not to discontinue the consumption of açai. We suggest no need to avoid the açai derivatives assessed here because a colour shift would be appreciated only by a skilled person (1.0 < ΔE* < 3.3) (Vichi et al., 2004). However, some study limitations should be acknowledged, and the results considered with caution.

The basis for our work does not reflect the people's real consuming behavior since the study was conducted in vitro. But, to provide credibility, we avoided potential bias as the daily renovation of saliva, sorbet and juice since the pilot study detected a significant decrease in the pH of unchanged sorbet and juice for two weeks (37° C). Moreover, sorbet and pulp were taken out in the freezer just before the experiment began, simulating a consume situation. In reference to the specimens preparation, surfaces were standardized by compressing the composite against a glass slide covered by mylar strip (Uctasli et al., 2023). Although a recent systematic review has shown a favorable result in nanofill and nanohybrid resin composites when abrasive paper disc polishers were used, we dismissed polishing because it suffers from skill and experience of operator. Besides, in the study of Soliman et al. (2021), the strip rendered relatively uniform surface topography of Filtek Z350 specimens. Images of surface topography of specimens for each dietary solution before and after treatment would show better this condition.

When considering mechanical outcomes, the material was resistant to the challenge. Neither roughness nor hardness changes of Filtek Z350 was found. Therefore, the first and second null hypothesis were accepted. It is relevant because studies show that biofilm formation depends on the material surface feature (Sterzenbach et al., 2020) and the early adhesion of S. mutans can be affected by roughness surface (Yuan et al., 2016). In line with Soliman et al. (2021), the final Ra mean values of all groups were clinically acceptable. Despite the roughness is clearly influenced by acidic solutions, we conjecture juice and sorbet would not increase a true event of plaque growth. Literature disagreements on Ra might be due to the lower pH of the açai juice together to the immersion regimen used by Silva-Leite et al. (2014) (pH=3.23, 4h/day for 12 weeks) and Borges et al. (2019) (pH=3.8, 3 × 15 min/30 days). This reasoning can also be applied to the hardness results (de Paula et al., 2014).

Although the present work is a first step towards understanding the role of the natural black açai berry on composite resins, it was not possible to compare the colour results with other (Silva-Leite et al., 2014; Borges et al., 2019) because they did not explain the shade of juice they used and the regimen. In fact, açai juices can have brownish or purple shades (Pacheco-Palencia, Hawken, Talcott, 2007). So, this information is attractive to evaluate the brownish one. Furthermore, our research is novel in describing the effect of sorbet. We believe that the full information about sorbet (as such the type and nature of the dyes and the vehicles) as well as the results from it may serve as a guide to future explorations.

The values of ΔE* shows açai produced clinical negligible staining of Filtek Z350. Therefore, the third null hypothesis was rejected. In fact, a shift was expected irrespective of the immersion media (Duc et al., 2018) even in the artificial saliva group due to resin natural intrinsic discoloration process (Ozera et al., 2019). Nevertheless, açai could have stained less by removing the composite outermost layer (Başeren et al., 2004) an issue to be further considered. When unacceptable staining after keeping nanofilled composite resin samples in açai juice for 12 weeks, repolishing or replacing the restoration would be necessary (Silva-Leite et al., 2014). In the present case (ΔE 1.88 and 2.20) a repolishing would just serve as an effective technique lowering the ΔE values. Notwithstanding, a longer term protocol or even with lower pH conditions could render rougher surfaces, deeper penetration of the dye and a more pronounced staining. In addition, water-solubility of the sorbet´s synthetic dye (amaranth and brilliant blue FCF; manufacturer information) and anthocyanin (pulp´s natural dye) seem might have not hinder their penetration on the material surface. The greater discoloration observed for juice likewise suggests anthocyanin penetrates more in the composite resin than the synthetic ones and its oily texture did not slow the dyes penetration (a theme for new work). Moreover, it was cited a dependency between low pH and colorant agent for staining (Al-Shami et al., 2023). Then, from the overhead mentioned, a new question emerges about: the green açai berry would compromise the aesthetics of the nanofilled composite resin?

4. Conclusion

Açai neither stained nor altered the surface mechanical properties of nanofill composite resin. The authors suggest future studies comparing distinct compositions of resin-based composites, which would help to better understand the effect of the evaluated solutions.

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