Assessment of the coloring capacity of the anthocyanin extract on nanocomposite dental materials

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Dental nanocomposites are promoted as most technologically advanced filling materials. Anthocyanin based food colorants are one of the main causes for the color changes of the dental composite materials. We studied whether the exposure of a nanocomposite material to anthocyanin food colorants triggers significant changes in the base color. Water immersions have a minimal effect on the color of samples. Both anthocyanin solutions have visible staining effect (ΔE>3,7). Other properties of testing solutions like acidity and alcohol content are known to generate color changes. Further research is needed to quantify the combined effect (synergic, antagonist or cumulative) of anthocyanin colorants.

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1. Introduction

The anthocyanin pigments are a group of natural colorants with potential beneficial biological effects which can be found in many food preparations (juices, drinks, foods) [1-3]. The majority of the plants (fruits, flowers, etc) have anthocyanins as pigments in their colored parts, with some exceptions, one of which being the red beet that contains betalains [4]. An interesting behaviour of the antocyanins is, that according to the pH of the solution they are capable of changing the color, switching from red to blue (one tried to apply them as pH markers) [5].

Dental composite materials are overwhelmingly used in dental practices due to their abilities to bond to enamel and dentine, their resemblance to tooth structures in color and good mechanical properties [6, 7]. The clinical assessment criteria of the sustainability of composite fillings over time include esthetical modifications: surface luster, surface staining, color match, translucency, esthetic anatomical form [8, 9]. The replacement of the colored fillings is usually performed in the visible areas of the oral cavity [10].

The change in color of the fillings is caused by intrinsic and extrinsic factors. The intrinsic factors are related to the degree of conversion of the double residual bounds, photoinitiator, inorganic filler, resin filler type, type of resin matrix, composition and size of filler particles [11 – 17]. The extrinsic factors are related to smoking, poor oral hygiene that leads to the occurrence of dental plague at the level of the fillings, the degree of finishing of the surface of the fillings, food colorants and liquids [7, 15, 16, 18- 21].

The number of the identified anthocyanin pigments is very large and thus the individual coloring capacity of dental composites could not be evaluated. It has been proven that the red wine is one of the anthocyanin pigments with strong coloring effect. We tried to find also other anthocyanin pigments with similar coloring effects.

Previous studies have shown that the greatest color change for the composites immersed in different liquids was observed in red wine [21, 22]. We also know that the blue food colors the composite materials more than other foods do [20].

We selected a nanocomposite material because the strength and esthetic properties are the characteristics of the resin-based nanocomposites [23]. Our previous studies have shown that Easy Shade spectrophotometer is able to measure color variations of the composite materials introduced in anthocyanin based food dyes [24].

The purpose of the study is to determine whether the exposure of dental materials to anthocyanin food colorants triggers significant changes in the base color. In this sense a significant change is considered to be the one, which requires the changing of fillings when there exists a difference in color measured in the CIE L*a*b of 3,7 (delta E>3,7) [25, 26].

The null hypothesis means that there will be no significant differences between delta E of water within 48 hours, delta E of wine and delta E of blueberries extract.

2. Material and method

Throughout the experiment we used the composite material Filtek Ultimate shade A2. Since the

polymerization light has an 8 mm diameter guide we decided to use specimens of this diameter.

Because in the specialist literature the influence of the background on reading the specimens is considered minimum at a thickness of the specimens of 4 mm we decided to realize specimens with this thickness. As the thickness of the composite layer exceeds 2,5 mm we had to polymerize the specimens on both sides [18, 20, 21, 27].

The polymerization was performed on each side for 20 seconds with the LED curing light Elipar Freelight 2, 3M ESPE, Ø 8 mm guide, 1000mW/cm^2, 20 sec. The top surfaces of all specimens were then polished with fine and superfine polishing disks (Sof-lex, 3M, ESPE) with a low-speed handpiece [28]. Then all prepared specimens were stored in distilled water at 37°C for 24 hours for rehydration and completion of the polymerization [20, 21, 29].

Table 1. Composition of immersing solutions.

Name	Water	Wine	Extract of anthocyanin
pH	-	3	4
Alcohol %	-	14	70
Description	Distilled water	Red wine	Blueberry extract HCI acidified 70% Ethanol

The specimens were split into three lots of 5 specimens each randomly arranged. The specimens were immersed into colorant liquids for 24 hours at 37 degrees celsius. The immersion solutions are described in the Table 1.

Color measurements were performed by positioning the specimens on a white background to prevent potential absorption effects even if the 4 mm thickness would have prevented this [18, 20, 21]. The color values were recorded using a digital spectrophotometer (Vita Easyshade, Advance, Vita, Zahnfabrik Germany). Five measurements were taken with the active point of the spectrophotometer in the center of each specimen. After 24 hours, the specimens were rinsed with distilled water and blotted dry with a tissue paper before re-measurements [24].

The independent variable (nominal) is the type of solution in which samplings have been immersed and the dependent variable is ΔE. In order to obtain the values for ΔE the measurement of the parameters L*a*b of the samplings is required before and after the immersion in colorant liquids. The measurements have been performed by means of the Easyshade (Vita Zahnfabrick) device. Delta E was calculated for each sampling of study starting from the measured values L*a*b according to the formula

$$\Delta E_{mn}=\sqrt{\Delta L^2+\Delta a^2+\Delta b^2}$$

whereby

- m is the index of the measured values after the immersion in coloring liquids or distilled water
- n is the index of the base values measured
- $\Delta L=L_m-Ln$ is the variation on the L axis between the two measurements
- $\Delta a=a_m-a_n$ is the variation on the *a axis between the two measurements
- $\Delta b=b_m-b_n$ is the variation on the *b axis between the two measurements [30]

3. Results

The tabular processing of data, the calculation of the mean and median of the ranges of values ΔE has been performed via Excel 2010 (Microsoft Corp). The statistical tests have been performed over the statistical program SPSS 21 (IBM Corp.).

Because we distributed the samplings randomly, ΔE could not be calculated specifically for each individual sample. Hence, we calculated ΔE for each of the samplings subjected to the test (coloring or witness) compared to all base values measured. Next, for every range of values corresponding to a sampling we calculated a medium value. These medium values were then used in a statistical study.

Normal distribution was confirmed with Kolmogorov-Smirnov test. Following a skewness and kurtosis analysis (Table 2) we decided to do the same statistical analysis using median values for the same probe calculated ΔE string. Doing so we tried to reduce the influence of extreme values on mean ΔE values.

Table 2. Skewness and kurtosis for mean ΔE values.

Skewness and kurtosis for mean ΔE values (mean of ΔE strings)			
Mean ΔE values	Water	Wine	Extract of anthocyanin
Skewness	,561	1,278	,919
Kurtosis	-,876	,581	,094

A comparative statistical analysis of median and mean values was done using a paired nonparametric test, (Wilcoxon Signed Ranks test). There were revealed significant differences just for water at 48h and wine. (Table 3).

Table 3. Median versus mean statistics.

Test Statistics[a]

Median vs Mean	Water	Wine	Extract of anthocyanin
Z	-3,332[b]	-2,869[c]	-,475[c]
Asymp. Sig. (2-tailed)	,001	,004	,635

a. Wilcoxon Signed Ranks Test

b. Based on positive ranks.

c. Based on negative ranks.

Clinical and colorimetric significance of differences between mean and median values can be observed in Table 4. The table reveals that no matter what calculation method is used for ΔE if the samplings are kept in distilled water the initial color does not change significantly however if kept in coloring liquids significant changes in color will be observed.

Table 4. ΔE Colorimetric significance of ΔE values.

	ΔE Mean	ΔE Median	Colorimetric significance	Clinical significance
Distilled water	0,9642	0,9372	Less than 50% of specialist observers can distinguish a difference in color	Changing of the filling is not recommended
wine	7,9531	7,9626	A difference of color from the initial one can be observed	Changing of the filling is recommended
Anthocyanin extract	10,8036	10,8081	A difference of color from the initial one can be observed	Changing of the filling is recommended

Despite the differences between the two ranges of values their colorimetric significance has no value, because a difference between the values ΔE of maximum 0,03 is far beyond the segregation of human eye. The distribution of the values ΔE according to the immersed solution is represented graphically in Fig. 1.

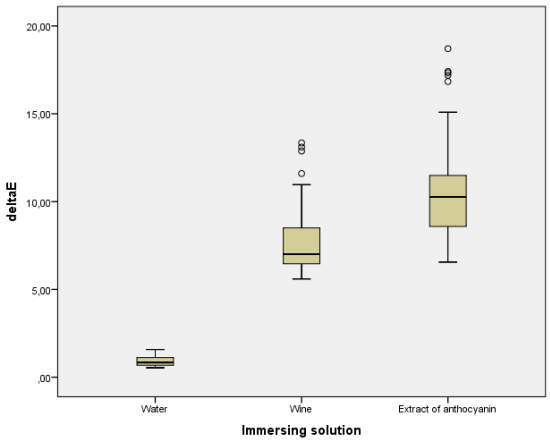

Fig. 1. Graphic representation of ΔE values grouped by immersing solutions.

We studied comparatively if there are differences between ΔE for the samplings immersed in wine or food colorant and the ΔE for the samplings preserved in water (witness) also if exist any differences between the ΔEs for the two colorants. For the statistical study we used a parametric test (t test) recommended for scalar values. For the confirmation of the result we used a non-parametric test (Mann-Whitney). Regarding the use of the t-test, testing the equality of variances between the lots has been performed with Levene's Test for Equality of Variances.

The results of the statistical test reveal, that regardless of the type of calculation of values ΔE (mean or median) significant differences occur between the coloring capacity of water compared to wine and food colorant as well as between wine and food colorant (Table 5).

Table 5. Comparative statistics for Mean ΔE and Median ΔE.

		ΔE Mean P (0,05 sig)		ΔE Median P (0,05 sig)	
Solution 1	Solution 2	T test	Mann-Whitney	T test	Mann-Whitney
Distilled water	Wine	0,000	0,000	0,000	0,000
Distilled water	Food colorant	0,000	0,000	0,000	0,000
Wine	Food colorant	0,000	0,000	0,000	0,000

4. Discussions

Within the limits of our study the coloring solutions with antocyanin pigments (wine and food colorant) can determine significant color changes of the composite materials but the influence of the cofactors on the amplitude of the coloring effect is not known.

The difference between the samples preserved in water and the ones immersed in colorant was, as expected, significant. We identified a significant difference between the two solutions marking a stronger effect for the food colorant (see Table 4 and Table 5). This stronger effect of the colorant can be caused by the alcohol concentrate (70%), the anthocyanin pigments and the acids from the solution, however it is not possible to specify exactly their importance.

The color change after the staining immersion treatment was measured by using ΔE parameter of CIEL*a*b* system. According to the individual ability of the human eye to appreciate differences in colors, different intervals are:

ΔE ab = 1 detectable difference of 50% of the normal

ΔE ab $\leq$ 2 clinically acceptable match

ΔE ab = 2.7 clinically acceptable match of 50%, the other 50% requires the replacement of esthetic dental materials,

ΔE ab = 3.7 the value can be easily observed by the experienced observers, but it can remain unobservable for the regular observers, however over this value it is required to change the color of the filling [25, 26].

Other studies take into consideration higher values than ΔE, considering only the clinical tolerance of the changes in color ΔE<2.6 as not perceptible, ΔE>2.6 as perceptible and clinically acceptable, and ΔE>5.5 as clinically not acceptable [31].

The changes produced by water on the composite material after 24 hours of immersion have the value ΔE< 1. Thus the changes produced by water on the composite material are clinically undetectable through direct visual examination. The changes produced by the extract of blueberries and wine on the composite material after 24 hours from the immersion have the values of ΔE> 5,5. This modification of ΔE ab will be observed by all examiners through visual analysis, the value being clinically inacceptable.

The values ΔE after the immersion of the various composites in wine, for a longer period of time determine accentuated changes, but our study reveals that even in case of an immersion for a shorter period of time the values are clinically detectable. (32)

It was demonstrated that an increased amount of alcohol in the immersing solution (a concentration of 75% ethanol/ /water) had greater influence on dental composites properties as: sorption, solubility, flexural strength, and flexural elastic modulus. (33) Allthough, both colored solutions used in our study determine clinically detectable changes, the accentuated coloring of the immersed samples in the blueberry extract could be due to the increased amount of alcohol.

One of the limitations of the present study is the in vitro methodology which partially stimulate the conditions from the mouth cavity.

5. Conclusions

Anthocyanin based food colorants are one of the main causes for the color changes of the dental composite materials. Other properties of testing solutions like acidity and alcohol content are known to generate color changes. Further research is needed to quantify the combined effect (synergic, antagonist or cumulative) of anthocyanin colorants.

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