Accepted manuscripts are the articles in press that have been peer reviewed and accepted for publication by the Editorial Board of the Vojnosanitetski Pregled. They have not yet been copy edited and/or formatted in the publication house style, and the text could still be changed before final publication.

Although accepted manuscripts do not yet have all bibliographic details available, they can already be cited using the year of online publication and the DOI, as follows: article title, the author(s), publication (year), the DOI.

Please cite this article: **DISCOLORATION OF RESIN BASED COMPOSITES IN NATURAL JUICES AND ENERGY DRINKS**

**PREBOJAVANJE KOMPOZITA PRIRODNIM SOKOVIMA I ENERGETSKIM PIĆIMA**

Authors: **Milica Antonov*, Lea Lenhardt*, Dragica Manojlović*†, Bojana Miličević*  Miroslav D. Dramičanin*; Vojnosanitetski pregled (2016); Online First December, 2016

UDC:

DOI: 10.2298/VSP161018394A

When the final article is assigned to volumes/issues of the Journal, the Article in Press version will be removed and the final version appear in the associated published volumes/issues of the Journal. The date the article was made available online first will be changed, too.
Correspondence to: Dragica Manojlović, University of Belgrade, School of Dental Medicine, Rankeova 4, Belgrade, 11000, Serbia Tel/fax: +381 11 2443 366. E-mail: dragica.manojlovic@stomf.bg.ac.rs

Running title: RBCs discoloration in drinks
Abstract

Background/Aim. The aim of this study was to evaluate changes of color and fluorescence of resin-based composites (RBCs) exposed to natural juices and energy drinks. Methods. Microhybrid composite Gradia Direct™ Extra Bleach White disc-shaped specimens (n=35) were immersed in three different natural juices and four different energy drinks. Absorption spectra of natural juices and energy drinks, diffuse reflection and fluorescence of composite samples were measured prior and after seven days immersion by Spectrophotometer Thermo Evolution 600 and Fluorolog-3-221 spectrofluorometer. Composite’s color was calculated from diffuse reflection spectra and expressed in CIELAB (Commission International de l’Eclairage) color space. Results. All natural juices and energy drinks induced color change of resin based composites, but to the different extent. Only aronia and carrot juices induced total color change considerably higher than clinically acceptable threshold, 9.3 and 6.2, respectively. All energy drinks and aronia juice induced notable decrease in fluorescence; the highest change of 28% was evidenced in the case of aronia juice. Conclusion. Change of color and fluorescence will appear differently with various solutions due to different chemical composition and concentration of colorant species in different beverages. Solutions with higher optical absorption induced higher total color change. Discoloration of composites in aronia and carrot juices are similar to those earlier reported for red wine, tea and coffee.

Key words:

composite; discoloration; fluorescence; staining
Apstrakt

Uvod/Cilj. Cilj ove studije bio je da se ispita promena boje i fluorescencije kompozita nakon izlaganja prirodnim sokovima i energetskim pićima. Metode. Uzorci mikrohibridnog kompozita Gradia Direct™ (n=35) potopljeni su u tri različita prirodna soka i četiri različita energetska pića. Apsorpcioni spektri prirodnih sokova i energetskih pića, difuzni refleksioni spektri i fluorescencija kompozitnih uzoraka izmereni su pre i nakon sedmodnevno potapanja na spektrofotometru Thermo Evolution 600 i Fluorolog-3-221 spektrofluorometru. Iz difuznih refleksionih spektara izračunata je boja kompozita i izražena u CIELAB sistemu. Rezultati. Svi prirodni sokovi i energetska pića doveli su do promene boje kompozita, ali u različitom obimu. Ukupna promena boje značajno veća od klinički prihvatljivog praga bila je samo kod sokova od aronije i šargarepe (ΔE = 9.3 i 6.2). Sva energetska pića i sok od aronije izazvali su primetno smanjenje fluorescencije; najveća promena od 28% zabeležena je u slučaju soka od aronije. Zaključak. Promene boje i fluorescencije razlikuju se u različitim rastvorima zbog različitog hemijskog sastava i koncentracije prebojavajućih supstanci u različitim pićima. Rastvori sa većom optičkom apsorpcijom pokazali su veću ukupnu promenu u boji. Prebojavanje kompozita u sokovima od aronije i šargarepe slično je ranije već zabeleženom u slučaju crvenog vina, čaja i kafe.

Ključne reči: kompozit; diskoloracija; fluorescencija; prebojavanje
Introduction

Resin-based composites (RBCs) should mimic the aesthetic characteristics of natural teeth and possess a color stability throughout the functional lifetime of the restoration. However, RBCs are prone to discoloration when exposed to saliva, food and beverages, and different stains in the oral environment. Discoloration of dental restorations makes them aesthetically unacceptable and is a frequent reason for replacement of composite restorations, with 16.9% incidence – coming second after secondary caries. Regularly consumed food and beverages sever restoration’s nature of being tooth colored and in recent times many literature reports have addressed stain-causing effects and problems.

In the majority of reports changes in the color of restorations after storage in different food and beverages have been assessed by the total color change (ΔE) of CIELAB Color System coordinates. So far, red wine, tea, and coffee have been demonstrated as frequently consumed beverages which may cause a significant discoloration of teeth and restorations. However, literature data on the deterioration of fluorescence is scarce, even though the contribution of fluorescence to the visual appearance of teeth and restorations should not be neglected. Also, limited data have been presented on the biochemical constituents of food and beverages that are responsible for stain-causing effects despite the fact that the proper knowledge of biochemistry behind the staining may aid and improve the effectiveness of stain removal.

Thus, the aim of this study was to thoroughly investigate changes in optical properties of resin composites exposed to some popular natural juices and energy drinks by evaluating changes in both their color and fluorescence and to identify colorant species responsible for observed effects.

Natural juices and energy drinks are gaining increased attention of customers in the last years; recent data (reviews and meta-analyses) indicate a current trend of increased consumption of fruit and vegetable juices and energy drinks. Though many scientific studies analyzed the influence of these beverages on overall health, less work has been done on their effects on the color stability of dental restorations. Herein, we analyzed in vitro staining effects of tree natural juices (beet, carrot and aronia) and four
energy drinks (Guarana Kick®, Red Bull®, Energi·s® and Burn®) on color and fluorescence of microhybrid commercial composite. Biochemical constituents of these beverages that are responsible for staining were recognized from optical absorption and reflection measurements.

The aim of this study was to thoroughly investigate changes in optical properties of RBCs exposed to some popular natural juices and energy drinks by evaluating changes in both their color and fluorescence and to identify colorant species responsible for observed effects. The null hypotheses tested were: (1) there are no differences in color among RBC samples stained in energy drinks and natural juices and non-stained samples; (2) there are no differences in fluorescence among RBC samples stained in energy drinks and natural juices and non-stained; and (3) immersion of composites in different-type energy drinks and natural juices produce similar effects on the optical properties of composites.

**Methods**

*Specimen preparation and staining procedure*

Disc shaped specimens of Gradia Direct™ (GC Corp. Tokyo, Japan) extra bleach white composite (n=40) were prepared in silicon molds, 2 mm thick and 13 mm in diameter. The molds were placed on a glass slab, filled with composite material and gently pressed with a glass slide to extrude excess material. Polymerization was performed for 20 s with a polywave LED light-curing unit (bluephase G2, Ivoclar Vivadent, Schaan, Lichtenstein) with light intensity of 1100 mW/cm². The distance between the light source and the specimen was standardized by the use of 1 mm glass slide. After polymerization, the samples were removed from the mold and polished under wet conditions with a series of Super-Snap Buff disks (medium, soft, super soft) and Super-Snap SuperBuff disks (Shofu Dent Cor, San Marco, Japan) and stored in distilled water at 37°C for 24 h. Specimens were divided in equal groups and immersed in following fresh natural juices: beet juice (Rote-Bete-Saft®, SchneeKoppe, Germany), carrot juice (Mohrensaft®, SchneeKoppe, Germany), aronia (Aronia®, Aroniada-Agro,
Bulgaria) and energy drinks: Guarana Kick® (Knjaz Miloš, Serbia), Red Bull® (Red Bull, Austria), Energi·s® (Frutti, Serbia (Sinalco International, Germany)), Burn® (Coca Cola HBC, Hungary), as shown in Table 1. Storage time was seven days at 37°C to simulate the mouth environment. All solutions were renewed daily to prevent bacterial contamination. After that specimens were rinsed with tap water and blotted dried with a tissue paper before measurements.

Diffuse reflection measurements

Spectrophotometer Thermo Evolution 600 (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an integrated sphere (Labsphere RSA-PE-19) was used for diffuse reflection measurements in the 220–900 nm range with 1 nm step.

Fluorescence measurements

Fluorolog-3 Model FL3-221 spectrofluorometer (Horiba JobinYvon) was used for obtaining excitation-emission matrices (EEMs) of samples utilizing a 450-W Xenon lamp as the excitation source and R928 photomultiplier tube as a detector in the front-face configuration. Excitation range was from 270 to 550 nm and the emission range 300 and 650 nm, with 5 nm and 1 nm step, respectively. Excitation and emission slits were set at 3 nm with acquisition time set to 0.07 s. Fluorescence was measured before and after seven-day immersion in staining solutions.

Digital imaging

Digital images were acquired with Canon digital camera EOS 1200D and Intel QX3 Computer Microscope before and after specimen staining.
Data analysis

All color testing were carried out according to the CIE-Lab-color system which uses the three dimensionless colorimetric measurements ($L^*$, $a^*$ and $b^*$):

$$\Delta L^* = L^*_{sample} - L^*_{reference},$$

$$\Delta a^* = a^*_{sample} - a^*_{reference},$$

$$\Delta b^* = b^*_{sample} - b^*_{reference}.$$

CIE $L^*a^*b^*$ color coordinates were calculated from diffuse reflection measurements, relative to standard illuminant (D65), against a white background (barium sulfate). The total color difference ($\Delta E^*$) and chroma ($\Delta C^*$) for each disk sample was calculated using the following equation \(^{18}\).

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{\frac{1}{2}}.$$  

$$C_{ab}^* = (a^{*2} + b^{*2})^{\frac{1}{2}},$$

$$\Delta C^* = C_{ab, sample}^* - C_{ab, reference}^*.$$  

Total fluorescence emission (TF) is calculated as a volume under the fluorescence intensity surface of the excitation-emission plane:

$$TF = \sum_{\lambda_{EX}=170\text{nm}}^{550\text{nm}} \sum_{\lambda_{EM}=300\text{nm}}^{650\text{nm}} I(\lambda_{EX}, \lambda_{EM}).$$

Differences if fluorescence were quantified as percentage of TF change compared to TF of the reference sample:

$$\Delta TF (\%) = \frac{TF_{sample}}{TF_{reference}} \times 100\%.$$

Results

Figure 1 present absorption spectra in the 220–900 nm spectral range of staining solutions used in this study. All solutions display strong absorption in the UV spectral
Energy drinks show well-resolved absorption peak at ~280 nm, Figure 1b. In the visible spectral range fresh natural juices showed moderate absorption, Figure 1a, while energy drinks showed quite low (see inset at the Figure 1b). Comparing overall absorption, among fresh natural juices aronia solution showed the strongest absorption and beet juice the lowest. Between energy drinks, Burn® had the highest absorption. Distilled water was used as a reference and showed no absorption.

Diffuse reflection spectra of composite samples were measured in 350-850 nm spectral region before and after staining. Spectra of samples stained in natural juices are displayed in Figure 2a and spectra of samples stained in energy drinks are given in Figure 2b. In both cases spectra were obtained by averaging data obtained from measurements on all samples from each group; spectrum of samples immersed in distilled water is presented as a reference.

Figure 2a shows a considerable decrease of reflection of samples stained in natural juices when compared to reflection of reference samples; the largest decrease was observed for samples exposed to aronia juice, then for those exposed to carrot juice and the smallest, but still of significant magnitude, for samples stained with beet juice. Changes of reflection were considerably lower with samples stained in energy drinks; the largest was found for staining in Burn® and smallest for staining in Guarana Kick®.

Color coordinates (in Lab color system) were calculated from diffuse reflection spectra and are given in the Table 2 along with the values of total color change (ΔE*) and change of chroma (ΔC*) calculated with respect to the reference samples. Staining with natural juices lowered the lightness (L*) and altered color coordinates (a* and b*) of composites. The total color change was therefore comprised from the change in lightness and change in chroma, and was the largest (ΔE*=9.3) for staining in aronia juice. Staining in energy drinks slightly changed color coordinates, but did not change lightness. The most pronounced color changes of ΔE*=2.8 were seen with Burn®.

Changes of fluorescence of resin composites after staining in natural juices and energy drinks were assessed from fluorescence excitation-emission matrices (EEM’s), which are composed of series of emission spectra measured for different excitation energies. Contour plots (projection of emission intensity into excitation-emission plain) of fluorescent EEM spectra recorded with samples stained in natural juices and energy
drinks are presented in Figure 3. For all samples two strong excitation bands can be observed, the first from 270 nm to 340 nm, and the second from 360 nm to 470 nm. Both excitations produced emissions in the 350–550 nm spectral region, with the most intense blue emission around 450 nm.

Staining-induced changes in fluorescence of composites were quantified as a relative difference of the total fluorescence of the stained sample over the fluorescence of a reference sample, Table 3. Among staining with natural juices, only composite exposed to aronia juice showed significant decrease in fluorescence (28%). On the other hand, staining in all types of energy drinks led to the large decrease of fluorescence, the largest value of 25% was observed with Red Bull®.

Changes in the appearance of resin composites (color and fluorescence) after staining is illustrated on the Figure 4. Images were recorded by digital camera and optical microscope (60× magnification) under daylight and under UV illumination.

**Discussion**

Results show that staining of composites is more intense in solutions that have higher absorption in the visible spectral range; therefore, the third hypothesis was rejected. Natural juices have larger absorption than energy drinks, Figure 1, and, as a consequence, reflection of samples exposed to natural juices was lower than reflection of samples exposed to energy drinks, Figure 2. Having in mind that color coordinates are calculated from the diffuse reflection spectra, the total change of color and change of chroma showed the same effect, Table 2. One should note that total color change larger than 2.7 (clinically acceptable threshold 19) was observed on samples stained in aronia juice (9.3) and carrot juice (6.2). The degree of discoloration is comparable to those recently assessed for the staining of the same resin composite in tea, coffee and red wine 12. Total color changes of samples stained in Guarana® (0.5), beat juice (2.2) and Red Bull® (2.3) were below clinically acceptable threshold, while the values for Energie·s® (2.7) and Burn® (2.8) were just on the threshold value, and would surpassed it for the longer staining time. Based on these results, the first hypothesis could not be rejected or confirmed, since different staining solutions produced different effects. One should also
note that in the case of staining in energy drinks total change of color is mainly due to the change in chroma (no changes in the lightness), while staining in natural juices significantly reduced lightness of samples and moderately altered chroma.

Aronia juice was only among the tested juices which caused a decrease in the fluorescent response of composite samples; this decrease of 28% was the highest among all tested solutions in this study and similar to ones found for several types of beer 20. Regarding energy drinks, Red Bull® and Guarana Kick® showed considerable decrease in fluorescence, much higher than Burn® and Energi-s®. Therefore, the second hypothesis was not confirmed nor rejected. In all cases shapes of fluorescence spectra were not changed and only intensity of the fluorescence was affected.

The changes of color and fluorescence of RBCs after seven days immersion in natural juices and energy drinks are of such magnitude that can be easily evidenced on microscope images obtained with 60 times magnifications. The loss of white appearance of composite samples is illustrated on digital camera images taken under daylight illumination, Figure 3.

Having in mind matching results of absorption and diffuse reflection measurements, Figures 1 and 2, it is possible to state that changes in color and fluorescence of resin composites upon exposure to natural juices and energy drinks was a consequence of adsorption and absorption of colorant species. Chemical composition and concentration of colorant species are different in various beverages; therefore, discoloration and change of fluorescence will appear differently with different staining solutions as evidenced from results depicted in Tables 2 and 3. Main colorant constituents of carrot juice are carotenoids (lycopene and β-carotene) which have characteristic absorption maximum in 400–500 nm spectral range and retinol (vitamin A) which absorbs around 330 nm 21. First absorption band of aronia juice is typical for polyphenolic (flavonoids) compounds that absorb at about 330 nm 22, while the other peak (400–600 nm) is due to the presence of anthocyan 23. Regarding beet juice, the peak at 270 nm originates from proteins (tryptophan and thyrosine). The absorption of proteins was also present in two other juices, but protein absorption peaks were of high intensity to be clearly resolved without considerable dilution of juices. Peak at a 470–550 nm in absorption spectrum of beet juice corresponds to a group of betalains pigments 24, 25 and is an overlapped
absorption of: 1) betaxanthins (yellow pigments) which have a characteristic absorption maximum at 260 and 474 nm, 2) betanin – type betacyanins (red-violet pigments) with a characteristic absorption at 538 nm.26

Energy drinks - Guarana Kick®, Red Bull®, Energi-s®, Burn® showed strong absorption in the 190–350 nm spectral range (Figure 1b). The difference in the absorption of tested energy drinks comes from the difference in the concentration of actual energizers (caffeine, taurine and vitamins B). The UV absorption spectrum of caffeine exhibits a pair of absorption bands peaking at 205 nm and 273 nm with a characteristic shoulder between them.27,28 Strong yellowish color change with Energi-s® may be caused by the presence of riboflavin (E101) which absorbs at 450 nm.29

**Conclusion**

Within the limitations of this *in vitro* study, it can be concluded that after seven-day immersion in natural juices and energy drinks RBCs change color and fluorescence. Magnitudes of color and fluorescence changes depend on the concentration and chemical composition of colorant species in natural juices and energy drinks. Strong absorbing aronia and carrot juices induce total color change considerably higher than clinically acceptable threshold. All energy drinks and aronia juice induce notable decrease in RBC fluorescence. This study has identified biochemical compounds responsible for RBC staining in natural juices and energy drinks which should clarify staining mechanisms and improve the effectiveness of stain removal.

**Conflict of Interest Statement**

The authors do not have any financial interest in the companies whose materials are included in this article.

**Acknowledgements**

Financial support for this study was provided by the Ministry of Education, Science, and Technological Development of the Republic of Serbia (grant numbers 45020 and 172007).
REFERENCES:


Table 1

<table>
<thead>
<tr>
<th>Product</th>
<th>Composition</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rote-Bete-Saft®</td>
<td>Beet juice (99%), lemon juice (1%), antioxidant, ascorbic acid</td>
<td>SchneeKoppe, Germany</td>
</tr>
<tr>
<td>Mohrensaft®</td>
<td>Carrot juice (99%), lemon juice (1%), vitamin A</td>
<td>SchneeKoppe, Germany</td>
</tr>
<tr>
<td>Aronia®</td>
<td>100% pressed aronia berries juice (no sugar, no additives, no preservative)</td>
<td>ARONIADA-AGRO, Bulgaria</td>
</tr>
<tr>
<td>Guarana Kick®</td>
<td>Caffeine (max.32mg/100ml), water, sugar, CO₂ (min.5g/l), citric acid, taurine</td>
<td>KNJAZ MILOŠ, Serbia</td>
</tr>
<tr>
<td></td>
<td>Caffeine (max.32mg/100ml), taurine (400mg/100ml), citric acid</td>
<td></td>
</tr>
<tr>
<td>Red Bull®</td>
<td>acid, CO₂, water, sugar, sodium carbonate, magnesium, colors (caramel, riboflavin), vitamins</td>
<td>Red Bull, Austria</td>
</tr>
<tr>
<td></td>
<td>Caffeine, taurine (400mg/100ml), citric acid, CO₂</td>
<td>FRUTTI, Serbia</td>
</tr>
<tr>
<td>Energi-s®</td>
<td>(min.4g/l), water, sugar, preservative (E211 max.150mg/l), inositol (19.5mg/100ml), colors (E150c, E101), vitamins</td>
<td>(Sinalco International, Germany)</td>
</tr>
<tr>
<td>Burn®</td>
<td>acid, CO₂ (min.2g/l), water, sugar, preservative: sodium benzoate isopotasium sorbate, colors (E150d), inositol (max.200mg/l), vitamins, guarana extract, ascorbic acid</td>
<td>Coca Cola HBC, Hungary</td>
</tr>
</tbody>
</table>
Table 2

Mean values of CIELAB color coordinates for resin composites after seven-day staining in natural juices and energy drinks; total color change ($\Delta E^*$) and chroma change ($\Delta C^*$).

<table>
<thead>
<tr>
<th></th>
<th>$L^*$</th>
<th>$a^*$</th>
<th>$b^*$</th>
<th>$\Delta E^*$</th>
<th>$\Delta L^*$</th>
<th>$\Delta C^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>89.9</td>
<td>-1.9</td>
<td>6.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Beet juice</td>
<td>88.8</td>
<td>-1.9</td>
<td>8.4</td>
<td>2.1</td>
<td>1.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Carrot juice</td>
<td>84.7</td>
<td>-3.1</td>
<td>9.5</td>
<td>6.1</td>
<td>5.2</td>
<td>3.1</td>
</tr>
<tr>
<td>Aronia juice</td>
<td>81.1</td>
<td>-1.3</td>
<td>9.2</td>
<td>9.1</td>
<td>8.8</td>
<td>2.3</td>
</tr>
<tr>
<td>Guarana</td>
<td>89.9</td>
<td>-2.1</td>
<td>7.1</td>
<td>0.4</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>Red Bull</td>
<td>90.8</td>
<td>-2.2</td>
<td>8.9</td>
<td>2.4</td>
<td>-0.9</td>
<td>2.2</td>
</tr>
<tr>
<td>Energi-s</td>
<td>89.6</td>
<td>-1.8</td>
<td>9.2</td>
<td>2.6</td>
<td>0.3</td>
<td>2.5</td>
</tr>
<tr>
<td>Burn</td>
<td>89.5</td>
<td>-1.9</td>
<td>9.3</td>
<td>2.7</td>
<td>0.4</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Table 3
Decrease of total fluorescence of resin composites after seven-day exposure to natural juices and energy drinks.

<table>
<thead>
<tr>
<th></th>
<th>Decrease of fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aronia juice</td>
<td>28 %</td>
</tr>
<tr>
<td>Beet juice</td>
<td>~ 0 %</td>
</tr>
<tr>
<td>Carrot juice</td>
<td>~ 0 %</td>
</tr>
<tr>
<td>Red Bull®</td>
<td>25 %</td>
</tr>
<tr>
<td>Guarana Kick®</td>
<td>20 %</td>
</tr>
<tr>
<td>Burn®</td>
<td>14 %</td>
</tr>
<tr>
<td>Energi-s®</td>
<td>13 %</td>
</tr>
</tbody>
</table>
Fig 1. - Absorption spectra of: a) fresh natural juices: beet juice (brown line), carrot juice (orange line) and aronia juice (purple line); b) energy drinks: Guarana Kick® (green line), Red Bull® (red line), Energi·s® (blue line) and Burn® (black line).

Fig 2. - Diffuse reflection spectra of: a) fresh natural juices: water (reference, blue line), beet juice (purple line), carrot juice (orange line) and aronia juice (pink line); b) different energy drinks: water (reference, blue line), Guarana Kick® (green line), Red Bull® (red line), Energi·s® (dark blue line), Burn® (black line).
Fig 3. - Fluorescence EEM spectra of specimens after 7-days staining in natural juices and energy drinks, with immersion in distilled water as a reference: sample immersed in a) distilled water, b) beet juice, c) carrot juice, d) aronia juice, e) Guarana Kick®, f) Red Bull®, g) Energy®, h) Burn®.
Fig 4. - Images of samples immersed in distilled water, fresh natural juices and different energy drinks for 7 days, recorded by digital camera and optical microscope under different illumination (daylight and ultraviolet).