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Discoloration of resin based composites in natural juices and energy drinks

Prebojavanje kompozita prirodnim sokovima i energetskim pićima

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Abstract

Background/Aim. Discoloration of dental restorations makes them aesthetically unacceptable and is a frequent reason for replacement of composite restorations. 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- day immersion by spectrophotometer Thermo Evolution 600 and spectrofluorometer Fluorolog-3-221. Composite's color was calculated from diffuse reflection spectra and expressed in CIELAB color space (*Commission International de l'Eclairage*). **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 is similar to those earlier reported for red wine, tea and coffee.

Key words:
dental materials; composite resins; materials testing; color; energy drinks; fruit and vegetable juices; fluorescence.

Apstrakt

Uvod/Cilj. Diskoloracija zubnih nadoknada čini ih estetski neprihvatljivim i često je razlog za njihovu zamenu. 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 sedmodnevnog potapanja na spektrofotometru Thermo Evolution 600 i spektrofluorometru Fluorolog-3-221. Iz difuznih refleksionih spektara izračunata je boja kompozita i izražena u CIELAB sistemu (*Commission International de l'Eclairage*). **Rezultati.** Svi prirodni sokovi i energetska pića doveli su do promene boje kompozita, ali u različitom obimu. Ukupna promena boje je bila značajno veća od klinički prihvatljivog praga samo kod sokova od aronije i šargarepe (ΔE = 9.3 i 6.2, redom). 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 već ranije zabeleženom u slučaju crvenog vina, čaja i kafe.

Ključne reči:
stomatološki materijali; smole, kompozitne; materijali, testiranje; boje; energetski napici; sokovi od voća i povrća; fluorescencija.

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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% of incidence – coming second after secondary caries [1]. Regularly consumed food and beverages may affect color stability of teeth and RBCs and in recent times many literature reports have addressed on their stain-causing effects and problems [2–5].

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 (*Commission International de l'Eclairage*). So far, red wine, tea and coffee were demonstrated as frequently consumed beverages which may cause a significant discoloration of teeth and restorations [6–10]. However, literature data on the deterioration of fluorescence is scarce [11], even though the contribution of fluorescence to the visual appearance of teeth and restorations should not be neglected. Also, limited data were available regarding the biochemical constituents of food and beverages responsible for stain-causing effects [12] despite the fact that the proper knowledge of biochemistry behind the staining may aid and improve the effectiveness of stain removal.

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 [13]. Though many scientific studies analyzed the influence of these beverages on overall health, less work was put in the investigation of their effects on the color stability of dental restorations [14–17]. Thus, the aim of this study was to thoroughly investigate changes in the optical properties of resin composites exposed to some popular natural juices and energy drinks by evaluating changes both in their color and fluorescence as well as to identify colorant species responsible for observed effects.

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 the beverages which are responsible for staining were recognized on the basis of optical absorption and reflection measurements.

The null hypotheses tested were: 1) there are no differences in color among the RBC samples stained in energy drinks and natural juices and non-stained samples; 2) there are no differences in fluorescence among the 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/cm^2. 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®, Schnee-Koppe, 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.

Table 1

Staining solutions used in this study

Product	Composition	Manufacturer
Rote-Bete-Saft®	Beet juice (99%), lemon juice (1%), antioxidant, ascorbic acid	SchneeKoppe, Germany
Mohrensaft®	Carrot juice (99%), lemon juice (1%), vitamin A	SchneeKoppe, Germany
Aronia®	100% pressed aronia berries juice (no sugar, no additives, no preservative)	ARONIADA-AGRO, Bulgaria
Guarana Kick®	Caffeine (max. 32 mg/100 mL), water, sugar, CO_2 (min.5 g/L), citric acid, taurine	KNJAZ MILOŠ, Serbia
Red Bull®	Caffeine (max.32 mg/100 mL), taurine (400 mg/100 mL), citric acid, CO_2, water, sugar, sodium carbonate, magnesium carbonate, colors (caramel, riboflavin), vitamins	Red Bull, Austria
Energi·s®	Caffeine, taurine (400 mg/100 mL), citric acid, CO_2 (min. 4g/L), water, sugar, preservative (E211 max. 150 mg/L), inozitol (19.5 mg/100 mL), colors (E150c, E101), vitamins	FRUTTI, Serbia (Sinalco International, Germany)
Burn®	Caffeine (max.32 mg/100 mL), taurine (4000 mg/L), citric acid, CO_2 (min. 2g/L), water, sugar, preservative: sodium benzoate ipotassium sorbate, colors (E150d), inositol (max. 200 mg/L), vitamins, guarana extract, ascorbic acid	Coca Cola HBC, Hungary

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 the 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 nm to 550 nm and the emission range 300 nm 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 CIELAB color system defined by *Commission International de l' Eclairage* (CIE) 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 (ΔE^*) and chroma (ΔC^*) for each disk sample was calculated using the following equation [18]:

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

$$C_{ab}^* = (a^{*2} + b^{*2})^{1/2}$$

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

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

$$TF = \sum_{\lambda EX = 270\ nm}^{550\ nm} \sum_{\lambda EM = 300\ nm}^{650\ nm} I(\lambda_{EX}, \lambda_{EM})$$

Differences in fluorescence were quantified as percentage of TF change compared to TF of the reference sample by using the following equation:

$$\Delta TF\ (\%) = \frac{TFsample}{TFreference} \times 100$$

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 range (< 400 nm). Energy drinks showed well-resolved absorption peak at ~280 nm (Figure 1a). In the visible spectral range fresh natural juices showed moderate absorption (Figure 1b) while energy drinks showed quite low (see inset in the Figure 1b). Comparing overall absorption, among fresh natural juices aronia solution showed the strongest absorption and beet juice the lowest. Among energy drinks, Burn® had the highest absorption. Distilled water was used as a reference and showed no absorption.

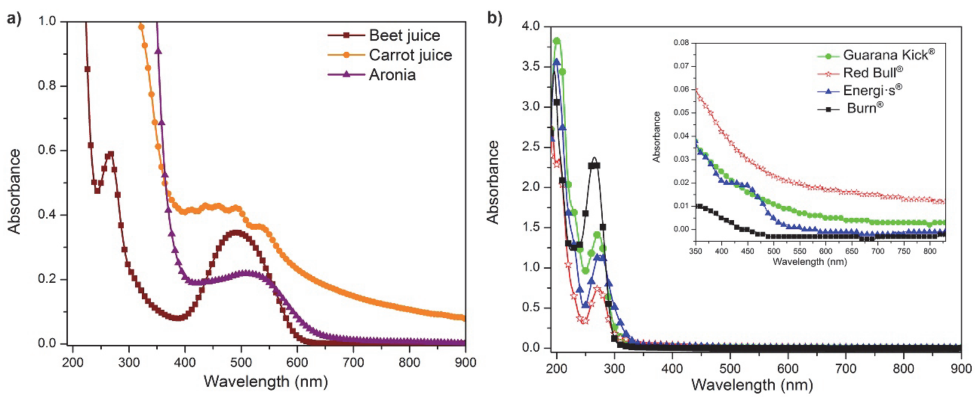

Fig 1. – Absorption spectra of: a) fresh natural juices – beet juice (brown line), carrot juice (orange line) and aronia juice (purple line); b) energy drinks – Guarana Kick® (green line), Red Bull® (red line), Energi·s® (blue line) and Burn® (black line).

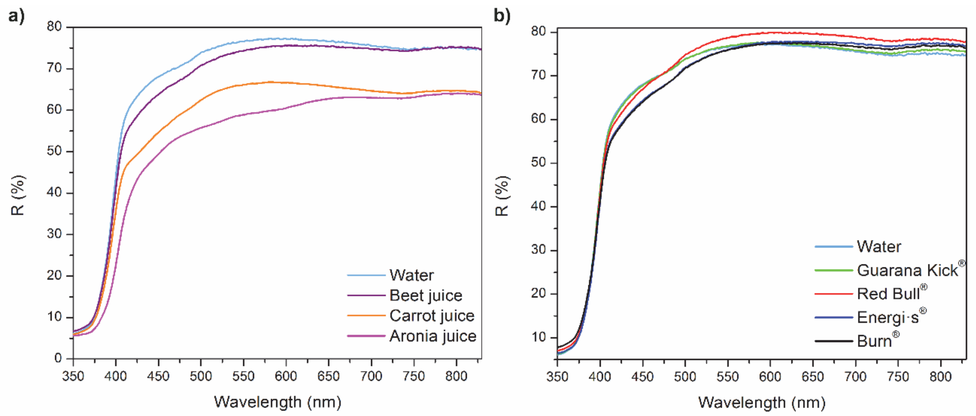

Fig 2. – Diffuse reflection spectra of: a) fresh natural juices – water (reference, blue line), beet juice (purple line), carrot juice (orange line) and aronia juice (pink line); b) different energy drinks – water (reference, blue line), Guarana Kick® (green line), Red Bull® (red line), Energi·s® (dark blue line), Burn® (black line).

Table 2

Mean values of *Commission International de l'Eclairage* (CIELAB) color coordinates for resin composites after seven-day staining in natural juices and energy drinks; total color change (ΔE^*) and chroma change (ΔC^*)

Product	L^*	a^*	b^*	ΔE^*	ΔL^*	ΔC^*
Water	89.9	-1.9	6.7	-	-	-
Beet juice	88.8	-1.9	8.4	2.1	1.1	1.7
Carrot juice	84.7	-3.1	9.5	6.1	5.2	3.1
Aronia juice	81.1	-1.3	9.2	9.1	8.8	2.3
Guarana	89.9	-2.1	7.1	0.4	0	0.4
Red Bull	90.8	-2.2	8.9	2.4	-0.9	2.2
Energi·s	89.6	-1.8	9.2	2.6	0.3	2.5
Burn	89.5	-1.9	9.3	2.7	0.4	2.5

Diffuse reflection spectra of the composite samples were measured in 350–850 nm spectral region before and after staining. Spectra of the samples stained in natural juices are displayed in Figure 2a and spectra of the 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 the samples stained by natural juices when compared to reflection of the reference samples; the largest decrease was observed in samples exposed to aronia juice, then in these exposed to carrot juice and the smallest, but still of significant magnitude, in the samples stained with beet juice. Changes of reflection were considerably lower in the samples stained in energy drinks; the largest one was found in the samples exposed to Burn® and the smallest exposed to 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 of the change in lightness and change in chroma and was the largest ($\Delta E^* = 9.3$) in aronia juice. Staining in energy drinks slightly changed the color coordinates, but did not change the lightness. The most pronounced color changes of $\Delta E^* = 2.8$ were seen in a case of Burn®.

Changes of fluorescence of the resin composites after staining in natural juices and energy drinks were perceived in 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 the fluorescent EEM spectra recorded with the 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 the 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®.

Table 3

Decrease of total fluorescence of resin composites after seven-day exposure to natural juices and energy drinks

Product	Decrease of fluorescence (%)
Aronia juice	28
Beet juice	~ 0
Carrot juice	~ 0
Red Bull®	25
Guarana Kick®	20
Burn®	14
Energi·s®	13

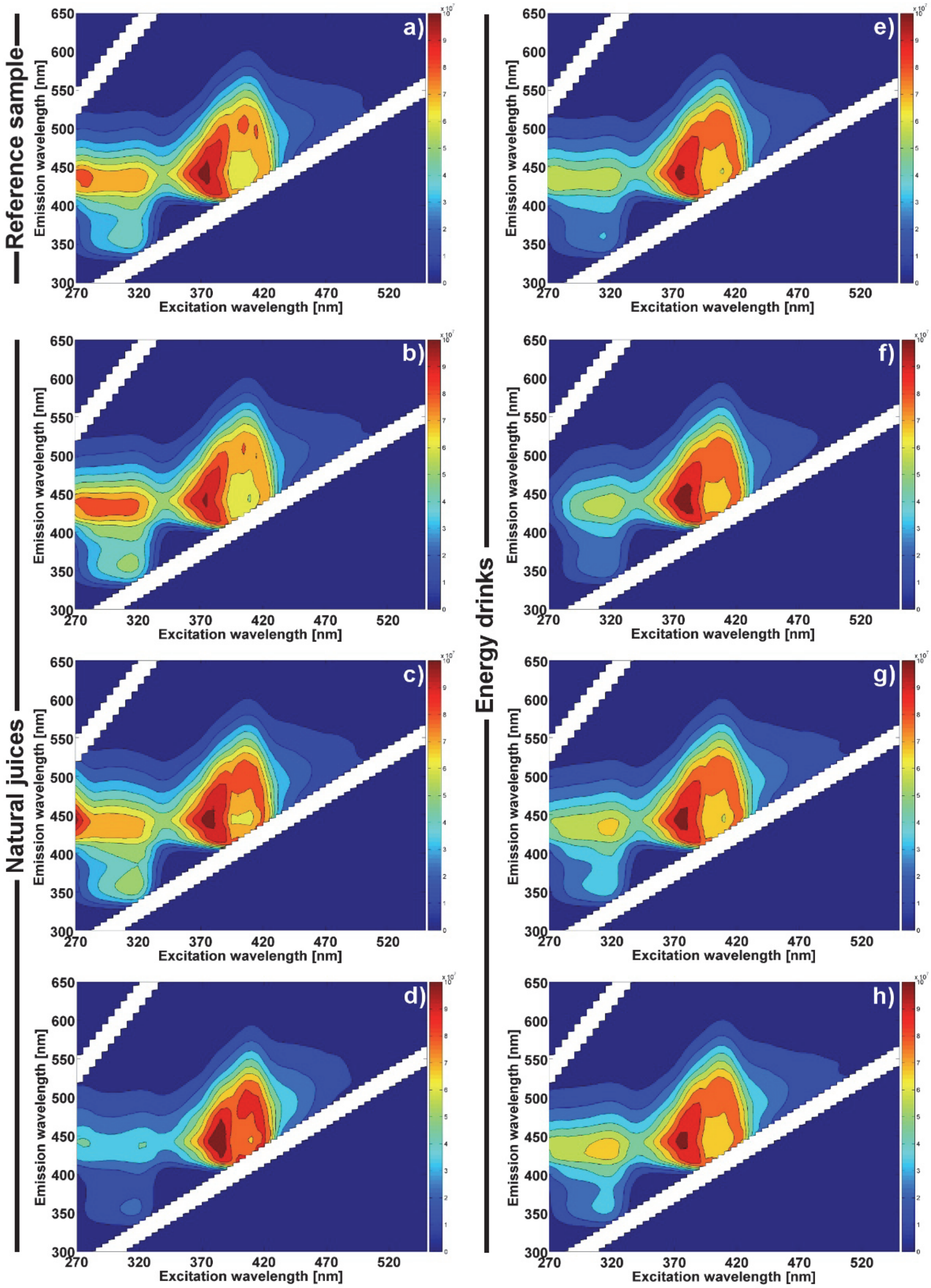

Fig 3. – Fluorescence excitation-emission matrices (EEM) spectra of specimens after 7-days staining in natural juices and energy drinks, with immersion in distilled water as a reference: sample immersed in a) distilled water, b) beet juice, c) carrot juice, d) aronia juice, e) Guarana Kick®, f) Red Bull®, g) Energy®, h) Burn®.

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

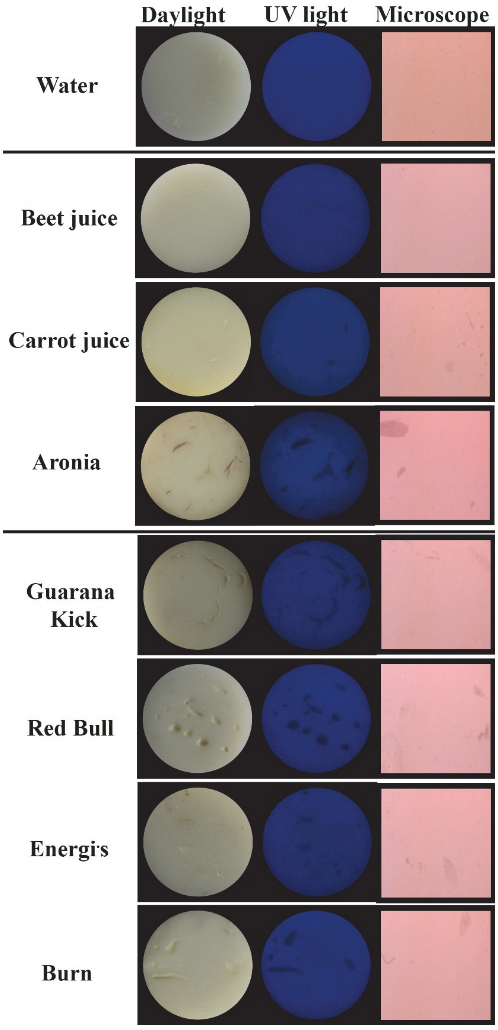

Fig 4. – Images of samples immersed in distilled water, fresh natural juices and different energy drinks for 7 days, recorded by digital camera and optical microscope under different illumination [daylight and ultraviolet (UV)].

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 the samples exposed to natural juices was lower than reflection of the 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 the total color change larger than 2.7 (clinically acceptable threshold [19]) was observed on the samples stained by aronia juice (9.3) and carrot juice (6.2). The degree of discoloration is comparable to those recently assessed for staining of the same resin composite in tea, coffee and red wine 12. Total color changes of the samples stained by Guarana® (0.5), beet 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 exceed it if the staining time was longer. 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 the samples and moderately altered chroma.

Aronia juice was the only tested juice in this research which caused a decrease in the fluorescent response of the composite samples; this decrease of 28% was the highest among all tested solutions in this study and similar to ones found in 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 intensity that can be easily proved by microscope images obtained by 60 times magnification power. The loss of white appearance of the 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 the 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 presented 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 from clinically acceptable threshold. All energy drinks and aronia juice induce notable decrease in RBC fluorescence. This study 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

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

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