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## Spektrofotometrijska procjena promjene boje zuba dugotrajno izloženih različitim uvjetima

### *Spectrophotometric Evaluation of Color Alterations of Teeth Exposed to Different Conditions in Time*

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#### Sažetak

Ljudski zubi građeni su od organskih i anorganskih tkivnih komponenata sličnih kostima. Može se pretpostaviti da strukturalna promjena boje zuba u različitim prirodnim uvjetima može poslužiti u forenzično tafonomiji kao, na primjer, u slučaju kad se potvrđuje okoliš i uvjeti ukopa te vrijeme provedeno nakon smrti (PMI-a). Danas se uočavaju i vrlo male promjene u nijansi zuba zahvaćajući dobro razvijenim dentalnim spektrofotometrima. Poznato je da zubi, ako su izloženi utjecaju zraka, brzo dehidriraju, ali stupanj i način tih promjena u vremenskom intervalu dosad nije objektivno izmjeren. **Svrha istraživanja:** Svrha ovog istraživanja bila je odrediti stupanj i obrazac promjene boja zuba izloženih određeno vrijeme trima različitim uvjetima. **Materijal i postupci:** Za istraživanje su odabrani impaktirani treći kutnjaci zato što do tada nisu bili izloženi okolišnim uvjetima. Njihove vrijednosti CIE L\*a\*b\* nakon ekspozicije u suhim uvjetima, natrijevu kloridu (NaCl) i umjetnoj slini (kontrola) izmjerio je iskusni stručnjak dentalnim spektrofotometrom u razmacima od 1 sat, 24 sata, 1 tjedan, 3 tjedna, 5 tjedana i 7 tjedana. **Rezultati:** Vrijednosti koordinata za L\*-lightness (svjetlost) i b\*-blue-yellow (plavo-žutu boju) bile su znatno povišene u suhim uvjetima ( $p < ,001$  za svjetlost i  $p \leq ,050$  za boju), a koordinate za a\*-green-red (zeleno-crveno) nisu pokazale statistički značajan pomak. Izloženost ekstrahiranih trećih kutnjaka suhim uvjetima prouzročila je veliko povećanje koordinata L\* i b\* prema svjetlijem i žućem dijelu, u usporedbi s izloženošću zuba umjetnoj slini i natrijevu kloridu, u svim testiranim uvjetima koordinata a\* pokazala se kao vrlo promjenljiva. **Zaključak:** Izloženost ekstrahiranih impaktiranih umnjaka suhim uvjetima uzrokovala je znatno uzastopno povećanje vrijednosti L\* i b\* u usporedbi s izloženošću zuba natrijevu kloridu i umjetnoj slini.

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#### Adresa za dopisivanje

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#### Ključne riječi

forenzička stomatologija; smrt; vlaga;  
zub; odontometrija; kolorimetrija

#### Uvod

Boja zuba određena je njegovom unutarnjom i vanjskom strukturom te spektralnom distribucijom upadne zrake svjetla. Jakost svjetlosti u zubu slabi zbog rasipanja i apsorpcije u caklini i dentinu. Resorpcija u caklini vrlo je ograničena zbog male količine organske komponente (aromatske aminokiseline) (1). Zbog male apsorpcije u caklini, žuti i crveni dio spektra prolazi kroz caklinu i odbija se od caklinsko-dentinskog spojišta ponovno na površinu (2). Rasipanje svjetlosne zrake u dentinu uzrokuju cilindrična struktura dentinskih tubulusa (3,4) i, u manjem opsegu, peritubularna kolagena vlakna koja i apsorbiraju veću količinu svjetla (5–8). Zato na danjem svjetlu zubi imaju boju bjelokosti.

Dentin uglavnom određuje boju zuba, a caklina modificira njegov izgled rasipajući kraće valne duljine u vidljivom spektru plave boje (9). Ustanovljeno je da kristali hidroksila-

#### Introduction

Tooth color is determined by the internal and external tooth structure and spectral distribution of light, which enters the tooth. Attenuation of light within the tooth is caused primarily by scattering and absorption in enamel and dentine. Absorption in enamel is very limited because of the small amount of organic component (aromatic aminoacids) responsible for it (1). Due to this low absorption, yellow and red lights are highly transmitted through enamel and can reflect from dentin-enamel junction back to the tooth surface (2). Scattering in dentin is predominantly caused by the cylindrical structure of dentine tubules (3, 4) and, to a lesser extent, by peritubular collagen fibers which also absorb large portion of light (5-8). Hence, in the normal daylight tooth color appears ivory.

While tooth color is determined mainly by dentin, enamel plays a significant role in modifying tooth appear-

patita znatno pridonose rasipanju svjetlosti, više nego struktura caklinske prizme (7,8). Zbog toga rastapanje kristalita ili njihova nepotpuna formacija utječe na rasipanje zraka svjetlosti. Što je caklina poroznija, to više rasipa kraće (plave) valne duljine svjetlosti. Ako više rasipa plavu boju, izgleda svjetlije (9).

Caklina normalno sadržava 7 do 10 posto vode u volumenu, koja je odgovorna za apsorpciju i rasipanje svjetlosti. Naime, ispod njezine površine postoje mikroskopske šupljine ispunjene vodom nastale zbog nepotpune mineralizacije. Poznato je da isušivanje cakline zrakom uzrokuje svjetliju boju cakline baš zbog isušivanja ispodpovršinskog sloja vlage (10). Time se objašnjava hipoteza da ekstrahirani zubi postaju svjetliji nakon što ih izložimo zraku zbog isparavanja vode.

Tijekom stomatološkog zahvata zubi postanu svjetliji i manje zasićeni, ali vrate svoju boju nakon rehidracije u slini (11). No, nikad nije ustanovljeno kakav utjecaj na boju zuba ima dugotrajna dehidracija u usporedbi s izloženošću vlazi. Boja ljudskih zuba može biti zanimljiva forenzičarima zbog pretpostavke da se na zubima pojavljuju karakteristične promjene u boji nakon izlaganja različitim utjecajima u okolišu. Forenzični znanstvenici već se koriste ispitivanjem boje za usporedbu vrsta tla, boje i vlakana te za analizu kostiju i zuba (12). Promjene u boji kostiju korisne su ako se tumači tijekom taloženja i struktura kostiju (13) i izloženosti fizikalnim agensima (14), ili ako se traže razlike između perimortalnih i postmortalnih ozljeda kostura (15). Svojstvo zuba da mijenja boju proporcionalno je godinama (16) i može se iskoristiti u istraživanju kod procjenjivanja dobi. Autori ovog teksta vjeruju da se, osim za određivanje dobi, promjene boje zuba mogu iskoristiti i za istraživanje raspadanja organizma u specifičnim okolišnim uvjetima te određivanja postmortem intervala (PMI-a).

### Mjerenje boja u stomatologiji

Suvremeni stomatološki spektrofotometri mogu precizno nekoliko puta izmjeriti spektralne vrijednosti zuba (17, 18). Kako je količina spektralnih podataka velika, mogu se definirati tri kordinate koje opisuju točnu poziciju boje u tri dimenzijama. Najčešći trodimenzionalni koordinatni sustav koji se upotrebljava za opisivanje boje u stomatologiji jest Commission Internationale de L'Eclairage ( $L^*a^*b^*$ ) sustav kolorimetrije (19).  $L^*$  os opisuje ljestvicu svijetlo-tamno i počinje od 0 (crno) do 100 (bijelo);  $a^*$  os označava spektar boja između zelene i crvene, a  $b^*$  os spektar boja između plave i žute.  $L^*$ ,  $a^*$  i  $b^*$  vrijednosti kvantitativne su mjere i zato se mogu izračunati brojčane razlike između dviju točaka boje. Ekvilibrjska udaljenost između njih procjenjuje se kao  $\Delta E$  i može se izračunati formulom:  $\Delta E = \Delta L^* + \Delta a^* + \Delta b^* \times 1,5$ .  $\Delta E$  razliku od 1 može vizualno percipirati 50 posto promatrača (20). Spektrofotometar može točno zabilježiti vizualno neuočljive razlike u boji. Te se visoko osjetljive

ance by scattering the shorter wavelengths in the visible blue range (9). It has been established that hydroxyapatite crystals themselves contribute significantly to scattering, rather than the prism structure (7, 8). For this reason, dissolution of crystallites or their incomplete formation will affect the scattering of light. The more porous the enamel, the more it scatters short (blue) wavelengths of light. The more the enamel scatters blue light, the lighter it appears (9).

7-10% of enamel volume is made of water as one of its normal constituents and this water affects its ability to both absorb and scatter light. Microscopic subsurface water-voids are commonly present because enamel is never perfectly mineralized. It is known that air-drying causes lighter appearance of enamel due to removal of subsurface moisture (10). This provides a rationale for hypothesis that extracted teeth become brighter when exposed to air due to evaporation of water.

During dental treatment, teeth color becomes lighter and less saturated but after being rehydrated in saliva, teeth color is reestablished (11). However, the effect of long-term dehydration comparing to a moisture exposure on teeth color has not been investigated. Assuming that the exposure of teeth to different environmental conditions in time scale can result in characteristic color alterations, the human tooth color could be considered to be of substantial interest to forensics. Color examination is already being widely used by forensic scientists for comparison of soil, paint, fibers, as well as for the analysis of bones and teeth (12). Bone color changes have been used to interpret depositional history and skeletal assemblage (13), exposure to physical agents (14), or to make a distinction between perimortal and postmortal skeletal injuries (15). Given that specific color change of teeth is proportional with the increase of age (16), this property could be used in age estimation studies. Therefore, the authors believe that besides attesting age, teeth color changes may also prove useful within forensic taphonomy field, for example, in verification of burial environment and postmortem interval (PMI).

### Color measurement in dentistry

Contemporary dental spectrophotometers can accurately and reproducibly measure spectral data of teeth (17, 18). From a bulk of spectral data, the three color coordinates which describe the exact position of color in three-dimensional color space can be defined. The most commonly used three-coordinate system for description of color in dentistry is the Commission Internationale de L'Eclairage ( $L^*a^*b^*$ ) system of colorimetry (19). The  $L^*$  axis describes the lightness-darkness scale and it starts from 0=black to 100=white; the  $a^*$  axis describes the color specter between green and red ends, and the  $b^*$  axis describes the color specter between blue and yellow ends. The  $L^*$ ,  $a^*$  and  $b^*$  values are quantitative measures, hence it is possible to calculate the numerical differences between two color points. The equilibrium distance between two color points is estimated as  $\Delta E$  and it can be calculated according to formula:  $\Delta E = \Delta L^* + \Delta a^* + \Delta b^* \times 1.5$ . The  $\Delta E$  difference of 1 can be perceived visually by 50% of human observers (20). However, spectrophotometer

kvantitativne mjere mogu upotrijebiti pri utvrđivanju vrlo malih promjena u boji prouzročenih različitim okolišnim uvjetima.

Stomatološki spektrofotometri primjenjuju se za određivanje boje zdravih zuba (obično onih do zuba koji treba restaurirati) kako bi se vjerno reproducirala boja restauriranog zuba. Spektrofotometri su konstruirani za upotrebu u stomatologiji te imaju ili vrlo mali otvor (kontaktni spektrofotometri) ili prijenosni držak s instaliranom fokalnom optikom (nekontaktni spektrofotometri). Integrirani izvor svjetla obično je danje svjetlo (D-65), a optička geometrija je uglavnom  $0^{\circ}$ - $0^{\circ}$  ili  $45^{\circ}$ - $0^{\circ}$  u svrhu prevencije gubitka svjetla (rubni gubitak) koliko god je moguće (12, 13). To znači da spektar svjetlosti izlazi iz naprave kroz otvor i ulazi u zub pod kutom od 0 ili 45 stupnjeva, a resultantna zraka upada pod kutom od 0 stupnjeva. Refleksija spektra svjetla, rasipanje i apsorpcija specifični su za svaki zub te rezultiraju trima vrijednostima boja koje su određene kao  $L^*$ ,  $a^*$  i  $b^*$  vrijednosti. Te tri kvantitativne vrijednosti nakon toga se uspoređuju s već postojećim vrijednostima  $L^*$ ,  $a^*$  i  $b^*$  pohranjenima u bazi podataka spektrofotometra. Najčešće korišteni ključ boja koji se rabi za vizualno određivanje jest Vita Classical (Vita, Bad Sackingen, Njemačka). Sadržava 16 ključeva boje čije su  $L^*$ ,  $a^*$  i  $b^*$  vrijednosti pohranjene u bazi podataka spektrofotometra. Nakon snimanja određenog zuba na LCD-zaslону uređaja pojavljuje se najbližnja boja koja se nalazi u memoriji i uspoređuje se sa snimljenim vrijednostima. Tehničari koji rade s keramikom koriste se tim vrijednostima kako bi odabrali odgovarajuću boju i dobili potrebnu.

Digitalno određivanje boje zuba postaje sve češće zato što se u vizualnom postupku mogu potkrasti mnoge pogreške. To znači da sve više pacijenata ostavlja svoj potpis „specifičnih kvantitativnih vrijednosti boja zuba“ u bazama podataka raznih spektrofotometara diljem svijeta. Imajući na umu tu činjenicu, autori predlažu da se te informacije iskoriste i u forenzične svrhe. Određivanje promjene boje zuba dentalnim spektrofotometrom nije se prije smatralo forenzičnim poslom, te nema eksplicitne zaštite ljudskih prava za tu proceduru. Zbog sve češćeg mjerenja boje zuba tim metodama, moralo bi se razmisliti o korištenju digitalnih podataka i u ostale svrhe a ne samo kad je riječ o određivanju boje za restaurativne postupke.

Svrha ovog istraživanja je spektrofotometrijska analiza *in vitro* promjena boja tvrdoga zubnog tkiva nakon dugotrajnog izlaganja različitim okolišnim uvjetima.

Hipoteza je da dugotrajna izloženost ekstrahiranih zuba suhim uvjetima uzrokuje znatne promjene u trima bojama, za razliku od izloženosti vlažnim uvjetima, odnosno natrijevu kloridu i slini (kontrola).

can precisely record color differences which are not even visually perceivable. These highly sensitive quantitative measures can be used to establish very slight color alterations due to teeth exposure to different environmental conditions.

Dental spectrophotometers are used to establish tooth color of a sound tooth (usually adjacent to the tooth that needs to be restored) in order to produce a copy of that appearance. They are constructed for use in dentistry and, therefore, they have either a very small aperture (contact spectrophotometers) or a portable handpiece with focal optics included (non-contact spectrophotometers). The integrated light source is usually day-light (D-65) source and the optical geometry is usually  $0^{\circ}$ - $0^{\circ}$  or  $45^{\circ}$ - $0^{\circ}$  geometry to prevent loss of light (edge-loss) as much as possible (12, 13). This means that the light spectrum leaves the device through the aperture and enters the tooth either at 0 or at 45 degrees and that the resultant light is collected at 0 degrees. The spectral reflection, scattering and absorption, specific for each individual tooth structure will result in three color values specified earlier as  $L^*$ ,  $a^*$  and  $b^*$ . Those three quantitative measures will subsequently be compared to the  $L^*$ ,  $a^*$  and  $b^*$  values of certain amount of colored examples that already exist in the device's database. A widely used shade guide for the purpose of visual color selection is Vita Classical (Vita, Bad Sackingen, Germany). This shade guide has 16 color tabs whose  $L^*$ ,  $a^*$  and  $b^*$  values are incorporated in the database of the spectrophotometer. After acquiring the tooth color measurement, the closest color match between the tooth and the shade tab is defined and the specific code of that colored example appears to the dentist in the LCD screen of the device. The porcelain technician uses this code to select the porcelain powder for color production.

Digital method for tooth color measurement is becoming very popular because the visual color selection is prone to many errors. This means that an increasing number of patients is leaving their signature in terms of “specific quantitative tooth color values” in databases of different dental spectrophotometers worldwide. Acknowledging this fact, the authors provide a rationale for consideration of these data for the purpose of forensic science. The evaluation of teeth color change using dental spectrophotometer has not been considered a forensic issue previously and, therefore, the explicit protection of human rights has not been established for this procedure. Nevertheless, increased use of this color measurement method and the utilization of digital color data for other purposes than dental restorative procedures should be well thought-out.

The objective of this study was to spectrophotometrically analyze the *in-vitro* color changes of dental hard tissues in time, during their preservation in different environmental conditions.

The hypothesis is that the exposure of extracted teeth to dry conditions will cause a considerable change in three color parameters over time comparing to the exposure of extracted teeth to moisture conditions, NaCl and saliva (control).



## Materijali i metode

### Zubi

Istraživanje je provedeno u Zavodu za oralnu kirurgiju Stomatološkog fakulteta Sveučilišta u Sarajevu (Bosna i Hercegovina). Korišteni su potpuno impaktirani treći kutnjaci. Ti su zubi odabrani zato što prije toga nisu bili izloženi nikakvim vanjskim utjecajima koji bi im mogli promijeniti boju (npr. kromogeni utjecaji iz hrane, duhan, vodice za usta, lijekovi). Svim sudionicima objašnjeni su uvjeti istraživanja i potpisali su informirani pristanak. Za ekstrakciju od 32 pacijenta bio je planiran 51 impaktirani zub (sudjelovalo je 15 žena i 17 muškaraca, prosjek godina = 28, sd = 4,2). Izvađeni su standardno klijestima u razdoblju od travnja 2010. do veljače 2011. Osobito se pazilo da se izvađeni zubi odvoje od parodonta u jednom komadu, kako bi se sačuvao biomaterijal. Nakon što su pregledani zubi dobiveni od 31 sudionika, za istraživanje je odabrano 45 (prosjek godina = 27, sd = 4,2). Kriterij je bio intaktna površina ekstrahiranog zuba. Šest zuba bilo je isključeno zbog oštećene površine ili zato što su bili premaleni u odnosu prema otvoru spektrofotometra. Odmah nakon ekstrakcije zubi su isprani hladnom vodom kako bi se uklonili ostaci krvi i debris s površine te su odmah izmjereni spektrofotometrom Easy Shade (Vita, Bad Sackingen, Njemačka) serijskog broja 303256. Nakon toga su nasumce stavljeni u jedan od triju testnih uvjeta. Prvo mjerenje boje označeno je kao *osnovna vrijednost* i ta vrijednost predstavljala je nepromijenjenu, vitalnu boju.

Od 45 zuba, 15 je bilo izloženo suhim uvjetima (tamni spremnik na 25°C), 15 vlažnim uvjetima (0,9 % NaCl na 25°C u tamnom spremniku) i 15 (kontrolna skupina) zuba je sačuvano u uvjetima sličnim onima u ljudskim ustima (umjetna slina na 37°C u tamnom spremniku).

### Mjerenje boje

Zbog mjerenja boje zubi su stavljeni u crnu kutiju kako bi se uskladio gubitak svjetla zbog rasipanja svjetlosti. Korišćeni su trima parametrima boje Easy Shade ( $L^*$ ,  $a^*$  i  $b^*$ ) iskusni stručnjak ih je uzastopce mjerio nakon 1 sat, 24 sata, 1 tjedan, 3 tjedna, 5 tjedana i 7 tjedana.

Spektrofotometar Easy Shade ručni je uređaj koji se usmjeri na određenu točku te zato mora biti u doticaju s površinom zuba dok se obavlja mjerenje. Optički vrh dug je 5 milimetara u promjeru i koristi se presudocirkularnom 0°–0° mjernom geometrijom (15). Svako mjerenje obavljeno je na vestibularnoj strani zuba, otprilike milimetar do dva od okluzalne plohe i milimetar do dva iznad caklinsko-cementnog spoja te u sredini između mezijalne i bukalne plohe (slika 1.). Zubi pohranjeni u natrijevu kloridu i slini najprije su obrisani, a zatim su izmjerene njihove vrijednosti. Posebna pozornost posvećena je dosljednosti u pozicioniranju spektrofotometra kod mjerenja svakog zuba.

Rezultati koji su se statistički analizirali predstavljali su prosjek triju mjerenja  $L^*$ ,  $a^*$  i  $b^*$  parametra boja.

## Materials and methods

### Teeth

The study was conducted at the Department of Oral Surgery, Faculty of Dentistry (University of Sarajevo, Bosnia and Herzegovina). For this study, fully impacted third molars from both jaws were used, because they were not previously exposed to any extrinsic factors, which could have had an influence on their color (eg. chromogenes from dietary sources, tobacco smoking, some mouthrinses, medications). All participants were asked to sign the written consent, which explained the conditions of the study. 51 impacted wisdom teeth, planned for the extraction, from 32 participants (15 female and 17 male), (mean age = 28, sd = 4.2) were removed in a standard way using forceps in the period between April 2010 and February 2011. Good care was taken to detach teeth from the periodontium in one piece in order to keep the biomaterial intact. After the inspection, 45 teeth from 31 participants (mean age = 27, sd = 4.2) with undamaged surface were selected for this study. Six teeth were excluded because their surface was damaged, or they were too small to be able to position the aperture of the instrument properly. Immediately upon their removal the teeth were rinsed in cold water in order to remove the blood and debris from their surface, then measured with spectrophotometer, Easy Shade (Vita, Bad Sackingen, Germany), serial number 303256, and randomly placed in one of the three different environmental conditions. The first color measurement was specified as a *baseline color*, representing the unaltered, vital tooth color.

From 45 teeth, 15 were exposed to dry conditions (dark container at 25°C), 15 to humid conditions (0.9% NaCl at 25°C in dark container), and 15 (control group) were preserved in conditions similar to the human mouth (artificial saliva at 37°C in dark container).

### Color measurement

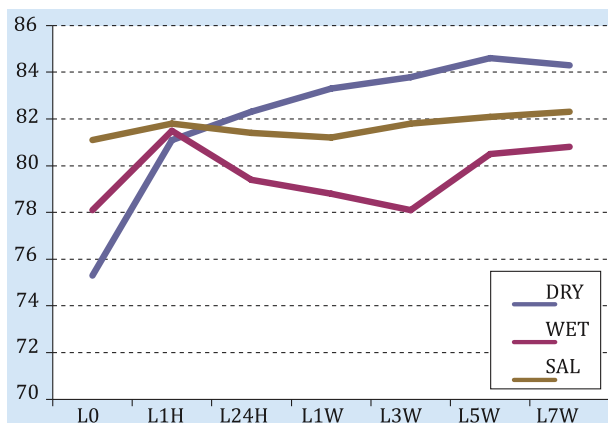
For the purpose of color measurements, the teeth were positioned in a black box, in order to uniform the light loss due to scattering for all tested teeth. Using Easy Shade, the three color parameters ( $L^*$ ,  $a^*$  and  $b^*$ ) were consecutively measured after 1 hour, 24 hours, 1 week, 3 weeks, 5 weeks and 7 weeks by one trained operator.

Easy Shade spectrophotometer is a handheld spot measurement device and needs to be brought into direct contact with the tooth surface when a measurement is being made. The fiberoptic tip is 5 mm in diameter and uses a pseudo-circular 0°–0° measuring geometry (15). Each measurement was performed on the vestibular side of each tooth approximately 1–2 mm under the cutting edge and 1–2 mm above the cemento-enamel junction in the middle between the mesial and distal curves of the teeth (Figure 1). The teeth stored in NaCl and saliva were first dabbed and then measured. Care was taken to reposition the aperture of the instrument in the same place by each consecutive measurement of each tooth as described above.

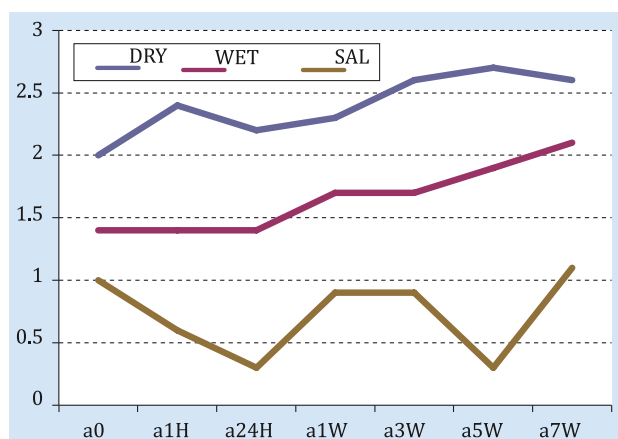
The data included in statistical analysis were the average of three measurements of  $L^*$ ,  $a^*$  and  $b^*$  color parameters.



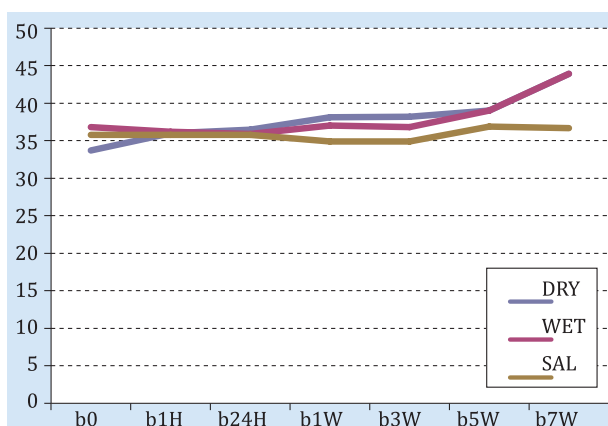
**Slika 1.** Spektrofotometrijsko mjerenje ekstrahiranog trećeg kutnjaka  
**Figure 1** Spectrophotometric measurement of extracted third molar



**Slika 2.** Obrasci promjene svjetline (L\*) u vremenskim intervalima: L0 (osnovna vrijednost), L1H (jedan sat), L24H (pet sati), L1W (jedan tjedan), L3W (tri tjedna), L5W (pet tjedana), L7W (sedam tjedana) izmjereni za tri skupine – suhu, mokru (NaCl) i onu s umjetnom slinom  
**Figure 2** The patterns of lightness (L\*) change in time intervals: L0 (baseline), L1H (one hour), L24H (five hours), L1W (one week), L3W (three weeks), L5W (five weeks), L7W (seven weeks) measured for three groups, dry, wet (NaCl) and artificial saliva



**Slika 3.** Obrasci promjene zeleno-crvenog (a\*) parametra u vremenskim intervalima: a0 (osnovna vrijednost), a1H (jedan sat), a24H (pet sati), a1W (jedan tjedan), a3W (tri tjedna), a5W (pet tjedana), a7W (sedam tjedana) izmjereni za tri skupine – suhu, mokru (NaCl) i s umjetnom slinom  
**Figure 3** The patterns of green-red parameter (a\*) changes in time intervals: a0 (baseline), a1H (one hour), a24H (five hours), a1W (one week), a3W (three weeks), a5W (five weeks), a7W (seven weeks) measured for three groups, dry, wet (NaCl) and artificial saliva



**Slika 4.** Obrasci promjene plavo-žutoj (b\*) parametra u vremenskim intervalima: b0 (osnovna vrijednost), b1H (jedan sat), b24H (pet sati), b1W (jedan tjedan), b3W (tri tjedna), b5W (pet tjedana), b7W (sedam tjedana) izmjereni za tri skupine – suhu, mokru (NaCl) i s umjetnom slinom  
**Figure 4** The patterns of blue-yellow parameter (b\*) changes in time intervals: b0 (baseline), b1H (one hour), b24H (five hours), b1W (one week), b3W (three weeks), b5W (five weeks), b7W (seven weeks) measured for three groups, dry, wet (NaCl) and artificial saliva

**Statistička analiza**

Za sve zube u svakoj od triju skupina izračunate su srednja vrijednost i standardna devijacija vrijednosti prvih mjerenja L\*, a\* i b\* parametra. T-testom su se procjenjivale razlike između vrijednosti svih intervala (IBM SPSS program, verzija 19,0).

**Rezultati**

Na tablicama 1., 2. i 3. predstavljene su promjene u svjetlini (L\*), zeleno-crvenoj (a\*) i plavo-žutoj (b\*) boji zuba čuvanih u suhim uvjetima, natrijevu kloridu i umjetnoj slini.

**Statistical analysis**

For all teeth within each of three groups the mean and standard deviation of L\*, a\* and b\* color parameters, measured at the baseline, were calculated. Paired T-tests were used to evaluate the differences between all time intervals (IBM SPSS program, version 19.0).

**Results**

Tables 1, 2 and 3 show the change of lightness (L\*), green-red (a\*) and blue-yellow (b\*) respectively of teeth preserved in dry conditions, NaCl and artificial saliva. The base-

**Tablica 1.** Trendovi varijacije L\* parametra u vremenskim intervalima u trima skupinama zuba čuvanih u različitim uvjetima  
**Table 1** The trends of variation of L\* parameter in time intervals among three teeth groups conserved in different conditions

Varijabla • Variable		Srednja vrijednost • Mean difference	Standardna devijacija • Standard Deviation of the difference	t	df	Značajnost • Sig. (2-tailed)
Suhi uvjeti • Dry conditions	L početak - L 1 sat • L baseline - L 1 hour	-5.82	2.76	-8.15	14	<0.001
	L početak - L 24 sata • L baseline - L 24 hours	-6.97	2.92	-9.24	14	<0.001
	L početak - L 1 tjedan • L baseline - L 1 week	-8.03	3.85	-8.07	14	<0.001
	L početak - L 3 tjedna • L baseline - L 3 weeks	-8.53	4.60	-7.17	14	<0.001
	L početak - L 5 tjedana • L baseline - L 5 weeks	-9.33	3.07	-11.75	14	<0.001
	L početak - L 7 tjedana • L baseline - L 7 weeks	-9.03	5.04	-6.92	14	<0.001
NaCl	L početak - L 1 sat • L baseline - L 1 hour	-3.39	2.55	-5.13	14	<0.001
	L početak - L 24 sata • L baseline - L 24 hours	-1.30	4.51	-1.11	14	0.284
	L početak - L 1 tjedan • L baseline - L 1 week	-0.69	4.20	-.63	14	0.534
	L početak - L 3 tjedna • L baseline - L 3 weeks	-0.03	4.69	-.02	14	0.983
	L početak - L 5 tjedana • L baseline - L 5 weeks	-2.46	5.82	-1.63	14	0.124
	L početak - L 7 tjedana • L baseline - L 7 weeks	-2.70	7.03	-1.48	14	0.159
Umjetna slina • Artificial saliva	L početak - L 1 sat • L baseline - L 1 hour	-0.59	1.56	-1.36	12	0.198
	L početak - L 24 sata • L baseline - L 24 hours	-0.18	1.22	-.54	12	0.597
	L početak - L 1 tjedan • L baseline - L 1 week	-0.01	1.12	-.02	12	0.981
	L početak - L 3 tjedna • L baseline - L 3 weeks	-0.54	1.34	-1.44	12	0.174
	L početak - L 5 tjedana • L baseline - L 5 weeks	-1.07	1.45	-2.65	12	<0.021
	L početak - L 7 tjedana • L baseline - L 7 weeks	-1.26	2.18	-2.07	12	0.060

**Tablica 2.** Trendovi varijacije a\* parametra u vremenskim intervalima u trima skupinama zuba čuvanih u različitim uvjetima  
**Table 2** The trends of variation of a\* parameter in time intervals among three teeth groups conserved in different conditions

Varijabla • Variable		Srednja vrijednost • Mean difference	Standardna devijacija • Standard Deviation of the difference	t	df	Značajnost • Sig. (2-tailed)
Suhi uvjeti • Dry conditions	a početak - a 1 sat • a baseline - a 1 hour	-.84	1.61	-2.03	14	0.061
	a početak - a 24 sata • a baseline - a 24 hours	-.81	1.75	-1.79	14	0.095
	a početak - a 1 tjedan • a baseline - a 1 week	-.73	2.37	-1.19	14	0.252
	a početak - a 3 tjedna • a baseline - a 3 weeks	-.91	2.44	-1.44	14	0.171
	a početak - a 5 tjedana • a baseline - a 5 weeks	-1.06	2.46	-1.66	14	0.118
	a početak - a 7 tjedana • a baseline - a 7 weeks	-1.12	2.57	-1.68	14	0.114
NaCl	a početak - a 1 sat • a baseline - a 1 hour	.16	.81	.79	14	0.439
	a početak - a 24 sata • a baseline - a 24 hours	-.08	.98	-.31	14	0.757
	a početak - a 1 tjedan • a baseline - a 1 week	.16	1.03	.62	14	0.544
	a početak - a 3 tjedna • a baseline - a 3 weeks	.51	1.05	1.89	14	0.079
	a početak - a 5 tjedana • a baseline - a 5 weeks	-.20	1.14	-.70	14	0.495
	a početak - a 7 tjedana • a baseline - a 7 weeks	-.03	1.04	-.12	14	0.904
Umjetna slina • Artificial saliva	a početak - a 1 sat • a baseline - a 1 hour	.63	1.22	1.85	12	0.088
	a početak - a 24 sata • a baseline - a 24 hours	1.04	1.25	3.01	12	<0.011
	a početak - a 1 tjedan • a baseline - a 1 week	1.13	1.28	3.18	12	<0.008
	a početak - a 3 tjedna • a baseline - a 3 weeks	.83	.80	3.73	12	<0.003
	a početak - a 5 tjedana • a baseline - a 5 weeks	.95	1.11	3.08	12	<0.009
	a početak - a 7 tjedana • a baseline - a 7 weeks	.63	1.53	1.50	12	0.159

Osnovna vrijednost L\* parametra u skupini zuba čuvanih u suhom mediju jako se razlikovala od srednjih vrijednosti parametra L\* drugih vremenskih intervala ( $p \leq 0,001$ ). Promijenjeni uzorak u toj skupini pokazao je dugotrajno kontinuirani rast L\* parametra od srednje vrijednosti L\* = 75,3 za prvo mjerenje do L\* = 84,6 nakon pet tjedana te neznatni pad vrijednosti na kraju sedmog tjedna – L\* = 84,3 (slika 2.).

U skupini tretiranoj natrijevim kloridom parametar L\* znatno se razlikovao od vrijednosti osnovnog mjerenja već na-

line L\* in dry group was significantly different from the L\* means at all other time intervals ( $p \leq 0.001$ ). The pattern of the change in this group showed continuous increase of L\* parameter over time, from mean L\*=75.3 at the baseline to L\*=84.6 at five weeks and a slight drop at week seven (L\*=84.3), see Figure 2.

In the NaCl group, the L\* was significantly different from baseline at 1 hour interval, whereas in the artificial saliva group the L\* was significantly different at 1 hour and after 5 weeks.

**Tablica 3.** Trendovi varijacije b\* parametra u vremenskim intervalima u trima skupinama zube čuvanih u različitim uvjetima  
**Table 3** The trends of variation of b\* parameter in time intervals among three teeth groups conserved in different conditions

Varijabla • Variable		Srednja vrijednost • Mean difference	Standardna devijacija • Standard Deviation of the difference	t	df	Značajnost • Sig. (2-tailed)
Suhi uvjeti • Dry conditions	b početak - b 1 sat • b baseline - b 1 hour	-2.36	4.32	-2.11	14	0.053
	b početak - b 24 sata • b baseline - b 24 hours	-2.83	3.83	-2.85	14	<0.013
	b početak - b 1 tjedan • b baseline - b 1 week	-4.39	5.43	-3.13	14	<0.007
	b početak - b 3 tjedna • b baseline - b 3 weeks	-4.48	4.82	-3.60	14	<0.003
	b početak - b 5 tjedana • b baseline - b 5 weeks	-5.32	4.22	-4.87	14	<0.001
	b početak - b 7 tjedana • b baseline - b 7 weeks	-10.19	7.03	-5.61	14	<0.001
NaCl	b početak - b 1 sat • b baseline - b 1 hour	.65	2.31	1.09	14	0.293
	b početak - b 24 sata • b baseline - b 24 hours	.94	3.46	1.05	14	0.308
	b početak - b 1 tjedan • b baseline - b 1 week	-.20	4.79	-.16	14	0.874
	b početak - b 3 tjedna • b baseline - b 3 weeks	-.01	4.17	-.01	14	0.990
	b početak - b 5 tjedana • b baseline - b 5 weeks	.00	3.96	.00	14	0.995
	b početak - b 7 tjedana • b baseline - b 7 weeks	-.28	3.77	-.29	14	0.773
Umjetna slina • Artificial saliva	b početak - b 1 sat • b baseline - b 1 hour	.38	2.02	.68	12	0.506
	b početak - b 24 sata • b baseline - b 24 hours	.43	2.26	.68	12	0.506
	b početak - b 1 tjedan • b baseline - b 1 week	.58	1.80	1.16	12	0.266
	b početak - b 3 tjedna • b baseline - b 3 weeks	.86	1.62	1.91	12	0.080
	b početak - b 5 tjedana • b baseline - b 5 weeks	-.93	2.66	-1.26	12	0.231
	b početak - b 7 tjedana • b baseline - b 7 weeks	-.67	2.36	-1.03	12	0.322

U skupini sa suhim uvjetima 15 je uzoraka (n, uzorak). Treba odrediti jedan parametar (srednju vrijednost L). To ostavlja n-1 stupnjeva slobode za određivanje varijabilnosti (15-1 stupnjeva slobode = 14df za skupinu u suhim uvjetima). • There are 15 observations (n, samples) in dry condition group. There is one parameter (the mean L) that needs to be estimated. This leaves n-1 degrees of freedom for estimating variability (meaning 15-1 degrees of freedom =14 df for dry condition group).

U skupini s mokrim uvjetima 15 je uzoraka (n, uzorak). Treba odrediti jedan parametar (srednja vrijednost L). To ostavlja n-1 stupnjeva slobode za određivanje varijabilnosti (15-1 stupnjeva slobode = 14 df za skupinu u mokrim uvjetima). • There are 15 observations in wet condition group. There is one parameter (the mean L) that needs to be estimated. This leaves n-1 degrees of freedom for estimating variability (meaning 15-1 degrees of freedom =14 df for wet condition group).

U skupini s umjetnom slinom 13 je uzoraka (n, uzorak). Treba odrediti jedan parametar (srednja vrijednost L). To ostavlja n-1 stupnjeva slobode za određivanje varijabilnosti (13-1 stupnjeva slobode = 14 df za skupinu s umjetnom slinom). • There are 13 observations in artificial saliva group. There is one parameter (the mean L) that needs to be estimated. This leaves n-1 degrees of freedom for estimating variability (meaning 13-1 degrees of freedom =14 df for artificial saliva group)

kon jednog sata, dok se u kontrolnoj osnovna vrijednost parametra L\* znatno razlikovala nakon jednog sata i pet tjedana.

Zeleno-crveni parametar boje zuba nije se znatno razlikovao u suhim uvjetima i u skupini dugotrajno tretiranoj natrijevim kloridom, dok se parametar a\* jako promijenio u kontrolnoj skupini nakon 24 sata, 1 tjedan, 3 tjedna i 5 tjedana (tablica 2.). Promijenjeni uzorak bio je kao dosta varijabilan pokazujući manje pomake a\* parametra (slika 3.).

Plavo-žuti parametar boje zuba u suhoj skupini znatno se povisio s vremenom od b\* = 33,7 do b\* = 43., a u skupini tretiranoj natrijevim kloridom i u kontrolnoj b\* parametar nije značajno fluktuirao (slika 4.).

## Rasprava

Promjene boja zuba zbog istrošenosti i degeneracije tvrdog zubnog tkiva poseban su pokazatelj procesa starenja. Opće je poznato da su zubi kod mlađe populacije svjetliji i manje žuti, a kod starijih osoba postaju žući i s godinama posive. Promjene u boji nastaju zbog trošenja cakline čija su debljina i glatkoća odgovorne za svijetli izgled zuba.

Na temelju tih fizioloških promjena može se ustanoviti je li ekstrahirani zub pripadao mlađoj ili starijoj osobi. Osim fizioloških procesa starenja, tu su i patološki procesi koji se

The green-red parameter of the teeth did not change significantly in dry and NaCl group over time, however, in the saliva group the a\* has changed significantly at 24 hours, 1 week, 3 weeks and 5 weeks (Table 2). The pattern of that change was variable showing minor shifts of a\* parameter (Figure 3).

The blue-yellow parameter of the teeth in dry group increased significantly over time from b\*=33.7 to b\*=43.9, whereas in the NaCl and saliva group the b\* color parameter did not fluctuate significantly (Figure 4).

## Discussion

Color alterations of teeth, due to wear and degeneration of hard tissues, are a unique indicator of aging over time. It is common knowledge that color of teeth in the majority of young population is brighter and less yellow, whereas in elderly population teeth become yellowish and grayish over time. This is due to wear of enamel whose thickness and smoothness is responsible for the bright look of the teeth.

Due to these physiological changes, it could be possible to indicate whether the extracted teeth belong to a young or



moгу prepoznati na osnovi promjene boje zuba. Na primjer, kongenitalna sistemska bolest, kao što je amelogenesis imperfecta, može se dijagnosticirati na temelju promjena u boji cakline. Osim toga, upotreba lijekova, kao što su tetraciklinski antibiotici tijekom zrenja cakline ili povećana koncentracija flora u vodi za piće (floroza), pokazuju jasnu kliničku sliku promjene boje zuba. Te specifične diskoloracije mogu pomoći u dijagnostici uzroka promjene boje. Uz sve navedeno treba spomenuti i način života koji također utječe na boju zuba. Tako nije teško razlikovati pušača od nepušača. Može se također pretpostaviti da konzumacija raznih vrsta hrane i pića u kombinaciji sa specifičnim zemljopisnim položajem prebivališta isto tako utječe na boju zuba. Uz sve rečeno, sve promjene boje zuba još uvijek nisu izmjerene precizno i nekoliko puta u određenim intervalima kako bi se ustanovilo bi li mogle biti izvor promjene boje.

Glavni zadatak forenzične stomatologije jest dobiti informacije o mogućem uzroku, vremenu i mjestu smrti. U forenzici se primjenjuju razna optička svojstva zuba i stomatoloških materijala kako bi se ustanovilo je li tijelo bilo izloženo visokim temperaturama (21, 22). Već je ustanovljeno da korijen zuba može imati ružičastu točku ako je žrtva umrla zbog gušenja (23). No, dosad nije bilo istraženo pokazuju li promjene boje zuba u postmortem stadiju predvidljivi obrazac promjena u usporedbi s istim zubima *in vivo*. Ti obrasci mogli bi upućivati na to kakva su svojstva mjesta gdje je pokopano tijelo i koliko dugo je bilo izvrgnuto specifičnim uvjetima.

Rezultati ovog istraživanja pokazuju da zubi sačuvani u suhom mediju postaju tijekom godina svjetliji i žući. Značajna statistička razlika dobivena je za  $L^*$  i  $b^*$  parametar boja zuba koji su bili dugo izloženi suhim uvjetima (tablica 1. i 2.).

U skladu s prijašnjim *in vitro* istraživanjima (11) i u ovom je istraživanju potvrđen prvi uspon vrijednosti  $L^*$  parametra zuba u suhim uvjetima samo nakon jednog sata. Nakon pet tjedana počeo je opadati, što se može pripisati stabilizaciji boje (slika 2.). Parametar  $b^*$  povećavao se od prvog do posljednjeg mjernog intervala, a parametar  $a^*$  nije se znatnije mijenjao u suhoj skupini. Povećana vrijednost žute boje može se pripisati dehidraciji dentina koji je požutio zbog gubitka vlage. Hidratizirana tkiva obično imaju odsjaj te se zato maskira njihova izvorna boja zbog rasipanja svjetlosti. Zbog toga suhi zubi izgledaju žuće.

Vrijednosti parametra  $L^*$  znatno su povišene i u skupini tretiranoj natrijevim kloridom jedan sat nakon mjerenja. To se može pripisati prestanku cirkulacije i isušivanju pulpne komorice, što uzrokuje unutarnje odbijanje svjetlosti. Nakon što se ekstrahirani zub rehidrirao u otopini, snizile su se vrijednosti  $L^*$  parametra te su poslije bile stabilne.

U kontrolnoj skupini zabilježene su na početku male promjene u vrijednostima  $L^*$  parametra, da bi se nakon pet tjedana jako povisile. Iako je tu pojavu teško objasniti, pretpostavlja se da su za te zakašnjele visoke vrijednosti  $L^*$  parametra odgovorni proteini iz umjetne sline (tablica 1.).

Parametar  $a^*$  najmanje je pridonio sveukupnom optičkom izgledu. Na njega najviše utječu pulpa i okolna gingiva. Ovo istraživanje pokazalo je male varijacije u vrijednostima  $a^*$  parametra, osim u kontrolnoj skupini u kojoj su razlike bile znatne, ali upitne.

to an elderly person. However, apart from the physiological changes, there are some pathological processes which can be visually recognized due to color deviation in teeth. For instance, a congenital systemic process such as amelogenesis imperfecta can be diagnosed by color alterations of enamel. Furthermore, use of medication, such as tetracycline antibiotics during maturation of enamel, and excessive presence of fluoride in drinking water (fluorosis) present as specific color alterations. These specific discolorations can help to diagnose the origin of the color change. Nevertheless, the lifestyle of the individuals influences teeth color as well. It is not difficult to distinguish a heavy smoker from a non-smoking person when it comes to teeth color. It is also assumable that the consumption of different food and beverages in combination with the geographic location of the habitat can influence teeth color as well. However, all these color changes have not been measured in reproducible and precise manner in order to determine whether they can be a predictor of the origin of the change.

The main goal of forensic dentistry is to provide information about the possible cause, time and place of death. Different optical and mechanical properties of teeth and dental materials have been used in forensic dentistry to indicate whether the cadaver was exposed to high temperatures (21, 22). It has been evaluated that tooth root can exhibit a pink spot in correlation with death caused by asphyxia (23). However, whether the alterations of teeth color in post mortal stadium show a predictable pattern when compared to the same teeth in vital conditions has not been previously studied. Those color-change patterns could possibly reveal the burial environment of the corpse and the duration of exposure to specific environmental conditions.

The results of this study show that the teeth preserved in dry conditions become lighter and more yellow over time. Significant statistical differences were obtained for  $L^*$  and  $b^*$  color parameters among teeth exposed to dry conditions over time (Table 1 and 2).

According to previous *in vitro* findings (11) in this study, the first increase of lightness ( $L^*$ ) in teeth exposed to air could be measured already after 1 hour. After five weeks, the lightness started to decrease, which could be explained with color stabilization (Figure 2). The blue-yellow ( $b^*$ ) increased from the first to the last measured interval whereas greenness-redness ( $a^*$ ) did not change significantly in the dry group. The increase in yellow could be explained by dehydration of dentine, which appeared more yellow due to lost of moisture. Moisturized tissues usually gleam and, therefore, the original color, created by light scattering could be masked. Therefore, due to scattering, dry teeth could appear more yellow.

The lightness increase was statistically significant after one hour also in the NaCl group, which can be explained by the initial increase of  $L^*$  parameter due to the cut-off of blood vessels, drying out of the pulp chamber and resultant change of the internal light reflection. After re-moisturizing, the  $L^*$  parameter dropped again and remained more or less stable in NaCl group.

In the saliva group, small variations in  $L^*$  parameter were present but only after five weeks, the lightness increased con-

U nastavku ovog istraživanja bilo bi dobro dulje pratiti promjene u boji zuba u suhim uvjetima. Ako se pokaže da u tim uvjetima slijede određeni predvidljivi uzorak, taj bi se uzorak mogao kvantificirati te primjenjivati za određivanje vremena nakon smrti. Današnji patolozi uglavnom se usmjeravaju na proces dekompozicije mekih tkiva u određivanju vremena nakon smrti. Iako se dekompozicija ljudskih trupala (algor, livor, rigor mortis) događa u određenim vremenskim intervalima, kasnija dekompozicija nema predvidiv obrazac (24) zato što ovisi o nizu čimbenika kao što su temperatura, vrsta tla, pH, habitus, strvinari i uzrok smrti, a svi mogu usporiti ili ubrzati proces raspadanja (14). Zato forenzičar može samo pretpostavljati o post mortem intervalu. Kako zube manje zahvaćaju promjene iz okoliša nekoliko tjedana nakon smrti, negoli mekana tkiva i unutarnje organe, novi obrazac postmortem dugotrajnih promjena boje zuba može pridonijeti vjerodostojnosti informacija o vremenu poslije smrti. U nastavku istraživanja trebalo bi dehidrirane zube rehidrirati kako bi se ispitalo do koje im se mjere može vratiti izvorna boja te koje bi vrijednosti parametra bile zabilježene na ponovnom očitovanju.

Uz sve to, bilo bi korisno istražiti i procijeniti utjecaj raznih okolišnih uvjeta na boju zuba, kao što su obojena pića, razne vrste tla i količina svjetla. Obojenje zuba koje rezultira odlaganjem ili pokapanjem tijela u određenim uvjetima također se može izmjeriti spektrofotometrom te na osnovi objektivnih podataka povezati specifična obojenja sa specifičnim tlom. Analiza boja već se primjenjuje u forenzici kako bi se identificirale i razlikovale različite vrste tla (25, 26). Iako se promjene boje na kostima već rabe u određivanju tla ili materijala s kojim je kost bila u doticaju (27), ta se metoda odnosi isključivo na opažanje promjena okom, tj. vizualno, te usporedbu s kartom Munsell Color. Takav način obrade podataka subjektivan je i često vodi do pogrešnih interpretacija dokaza (28). Ograničena sposobnost ljudskog oka u usporedbi sa spektralnim mjerenjem razlog je zašto se promjene na kostima vide tek nakon duljeg izlaganja okolišnim uvjetima (13, 29). Rezultati ovog istraživanja, koje se oslanja na kvantitativnu i objektivnu tehniku detektiranja vrlo malih i vizualno nepredvidivih promjena u boji, pokazali su da velike promjene u boji zuba nastaju već nakon jedan sat. Takve pouzdane spektrofotometrijske metode mogu se primijeniti i na uzorke kostiju (30). Ulaganje u novi istraživački princip može poboljšati interpretaciju forenzičnih ukopa izoliranih slučajeva i masovnih grobnica.

Unatoč svemu, kako bi znanost mogla profitirati od tih informacija, mora se riješiti nekoliko problema. Činjenica je da kvantitativni podaci o boji zuba izraženi u vrijednostima triju boja nisu uvijek dostupni. Iako je uporaba stomatoloških spektrofotometara sve češća, još su preskupi većini stomatologa. U slučaju da i postoje informacije u bazi podataka spektrofotometra o premortem boji zuba umrle osobe, upitna je kvaliteta te informacije jer zubi mijenjaju boju kako osoba stari. S druge strane postoje pokazatelji da promjena boje zuba jedne osobe tijekom godina prati određeni obrazac (21, 31) ako ne postoje ekstremne situacije ili loše navike. Zato se regresijskom analizom može ponovno izračunati boja zuba. Zbog toga će biti, budu li se daljnja istraživanja po-

siderably. Although this finding is difficult to explain, an assumption can be made that some saliva proteins could be responsible for this late rise of lightness (Table 1).

The green-red parameter in teeth is the least contributing factor to the overall optical appearance. It is probably caused by presence of pulp and gingiva in the near surrounding. In this study, greeness-redness varies in very small intervals, and although the changes of  $a^*$  color parameter in saliva group were significant, the importance of those patterns could be doubted.

In further studies, it is required to additionally track color changes in the dry group further. If a further research would show that teeth color alterations over longer time intervals are showing predictable patterns, then a quantitative method of time-since-death determination could be developed relying on such results. Nowadays, to attest time-since-death, forensic pathologists strongly rely on observable changes in soft tissues caused by decomposition processes. Although initial human decomposition (algor, livor, rigor mortis) follows fairly well established time intervals, the later decomposition does not follow any predictable pattern,(24) because it is conditioned by a number of varying factors, such as: temperature, soil, pH, body habitus, scavenging and cause of death, which can slow down or accelerate the decomposition process (14). Therefore, a forensic pathologist can give only a rough estimate on the possible post mortem interval. As teeth should be less affected by fluctuating environmental conditions in the first few weeks from death than soft tissue and inner organs, a known pattern of postmortem changes in teeth color over time could yield more reliable information regarding time-since-death. In continuation of this study, dehydrated teeth should be re-moisturized in order to evaluate to what extent the original color would reestablish itself to the original color values and which patterns of this color reestablishment would be denoted for three color parameters.

Nevertheless, it would be beneficial to evaluate the effect of different environmental conditions, such as colored beverages, diverse soil or lighting conditions on teeth color. Staining of teeth that would result from deposition or burial in a specific environment could be measured with the spectrophotometer, thus providing objective data that could link the detected staining to a specific type of soil. Color analysis has been used in forensics to allow identification and discrimination of soils (25, 26). Although staining of bone has already been used to interpret the contact of bone with soil or other materials (27), this method relies exclusively on color changes that are observable to the naked eye and then compared with Munsell Color Charts, which leads to subjectivity and sometimes erroneous interpretation (28). The limited eye sensitivity, comparing to spectral measurements, is probably the reason why the occurrence of bone color changes is described only after a prolonged exposition to environment (13, 29). The results of this study, which rely on a quantitative and objective technique that detects very slight and visually unperceivable color alterations, showed that significant color changes in teeth could occur already after one hour interval. Such reliable spectrophotometric investigations could also be applied on bone specimens (30). Investment in this

kazala korisna u određivanju boje zuba u forenzičnoj tafonomiji i identifikaciji, mnogo zainteresiranih za bazu podataka koja će sadržavati boju zuba.

### Ograničenja

Iako su u ovoj studiji tri mjerenja istog zuba pokazala malu standardnu devijaciju, u sljedećoj studiji trebalo bi se koristiti individualiziranim ključem za svaki zub kako bi se standardiziralo postavljanje vrha spektrofotometra na površinu zuba. Trebalo bi također povećati broj uzoraka zuba (uzimajući u obzir jednaku distribuciju spola te mali raspon godina u uzorku) kako bi se poboljšala vrijednost metode.

### Zaključak

Izlaganje ekstrahiranih impaktiranih kutnjaka suhim uvjetima prouzročilo je uzastopni porast vrijednosti svijetlog ( $L^*$ ) i plavo-žutog ( $b^*$ ) parametra, kada uspoređujemo one iz skupina zuba koji su bile izloženi natrijevu kloridu i umjetnoj slini.

Strukturalne promjene u svjetlini ( $L^*$ ) i plavo-žutom ( $b^*$ ) parametru opažene u ovom istraživanju dopuštaju obradu zuba iz navedenih okolišnih okolnosti.

new research principle could improve the interpretation of forensic burials both for isolated cases as well as for mass graves.

However, in order to be able to use this information, the science will need to overcome a few problems first. It is a fact that quantitative color information of teeth, expressed in three color values is not always available. Although their popularity increases, the dental spectrophotometers are still too expensive for dentists. And even if these original teeth color values of a dead person would be available in databases of a spectrophotometer, the question is how accurate this color information would be considering the fact that color alters with aging. On the other hand, there are indications that color changes in one individual over the years follow a predictable pattern (21, 31), unless some extreme life events or bad habits are present. Therefore, it might be possible to recalculate the color of the particular person using regression analysis. For this reason, if further research shows usefulness of teeth color to forensic taphonomy and identification, building a teeth-color database could be of a huge interest.

### Limitations

Although in this study the three measurements at the same tooth area have shown small standard deviation, in the follow-up study an individual surface jig could be the appropriate method to alleviate and highly standardize the repositioning of the spectrophotometer tip on the tooth surface. Moreover, an increased number of teeth (taking in consideration equal sex distribution and narrow age range of participants) could improve the validity of the present method.

### Conclusions

The exposure of extracted impacted wisdom teeth to dry conditions caused considerable consecutive increase of lightness ( $L^*$ ) and blue-yellow parameter ( $b^*$ ) compared to the exposure of teeth to NaCl and artificial saliva.

The structural changes in lightness ( $L^*$ ) and blue-yellow parameter ( $b^*$ ) observed in this study allow for locating of tooth in one of the studied environmental circumstances.

#### Abstract

Human teeth consist of organic and anorganic tissue components similar to bones. Therefore, it is assumable that the registration of structural changes of teeth color in different environmental conditions may prove useful within forensic taphonomy field, for example, for verification of burial environment and postmortem interval (PMI). Registration of very small alterations of teeth color is nowadays possible due to well developed dental spectrophotometers. It is known that teeth exposed to air quickly dehydrate, but the degree and the pattern of these color changes in time intervals have not been objectively measured. **Purpose:** The objective was to determine the degree and pattern of color alterations of teeth exposed to three different conditions in time. **Material and Methods:** The impacted third molars were used in this study because they were not previously exposed to any environmental conditions. Their CIE  $L^*a^*b^*$  values after exposure to dry conditions, NaCl and artificial saliva (control) were measured with dental spectrophotometer in time intervals of 1 hour, 24 hours, 1 week, 3 weeks, 5 weeks and 7 weeks by one trained operator. **Results:** The  $L^*$ -lightness and the  $b^*$ -blue-yellow color coordinate increased in dry conditions ( $p < .001$  and  $p < .050$  respectively), which was statistically significant, whereas  $a^*$ -green-red color coordinate showed shifts of color in this condition which were not statistically significant. The exposure of extracted third molars to dry conditions caused significant increase of  $L^*$  and  $b^*$  color coordinate towards more light and more yellow in comparison to the exposure of teeth to artificial saliva and NaCl. The  $a^*$  color coordinate displayed high variability of its alteration in all tested conditions. **Conclusions:** The exposure of extracted impacted wisdom teeth to dry conditions caused a considerable consecutive increase of lightness ( $L^*$ ) and blueness-yellowness ( $b^*$ ) in comparison with the exposure of teeth to NaCl and artificial saliva.

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#### Key words

Forensic Dentistry; Death; Humidity; Tooth; Odontometry; Colorimetry

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