

many minor hypoplasia lines are trivial. They might not necessarily be related to clear-cut episodes of developmental disruption, while the stress signal may be clear when attention is restricted to the palpably indented major growth arrest lines (Corruccini et al., 1985). Second, the most affected individuals were missing from the sample, as they did not survive the famine. Thus, recovery from LEH-inducing stress ironically may be a sign of increased adaptability during the famine.

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CRYSTALLOGRAPHIC AND COLORIMETRIC ANALYSIS OF DENTAL ENAMEL

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ABSTRACT Tooth color and the correlation of the composition of dental enamel with color were investigated in samples of teeth from two medieval Serb cemeteries. Differences in the composition of apatite crystals in the dental enamel of the two samples were found. Color ranges of teeth from the two samples differ in hues and chromas. This result suggests that enamel composition may have an influence on the color of teeth. The prevalence of chlorapatite in enamel causes tooth color to be closer to red and of higher chroma than teeth whose enamel consists of hydroxylapatite. No evidence indicated that soil ingredients were incorporated into the dental enamel of either sample.

INTRODUCTION

In this study we investigated tooth color and the correlation of tooth composition and color. The main inorganic elements of dental enamel are found in the form of apatite crystal, which comprises more than 90 percent of the enamel. Inorganic components significantly determine the color of teeth.

The color of teeth is not, of course, solely dependent on the optical properties of enamel. One of the main optical characteristics of enamel is translucency. Therefore, the layer of dentin situated under the enamel, which has its own optical properties, also influences the color of a tooth. Dentin is characterized by about 40 percent organic component (Arwill et al., 1969). In the case of a skeletal sample, the influence of dentin on tooth color is not important for two reasons. First, skeletal dentin does not have an organic component because the

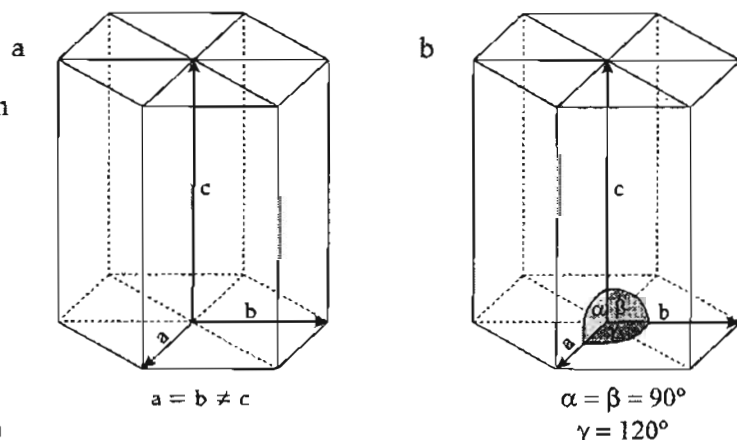


Fig. 1. Hexagonal structure of the apatite crystal.

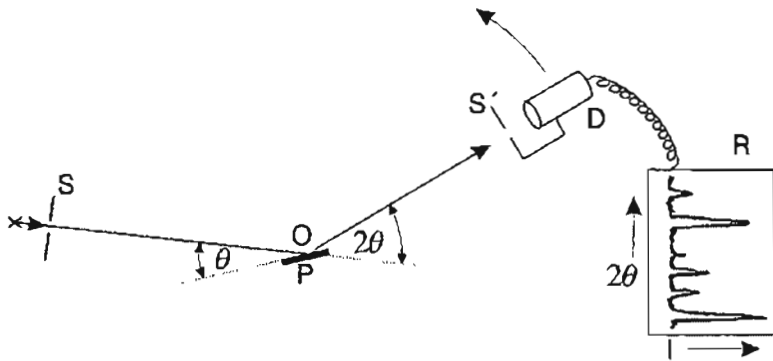


Fig. 2. Scheme of the Röntgen diffractometer.

apatite may be present in a test sample of enamel. The hydroxyl ion may be substituted by the chlorine ion in chlorine apatite, by the fluorine ion in fluorapatite, and by the carbonate ion in dentil.

Contrary to bone in which the constituent elements of hydroxylapatite can be replaced chemically by other ions under various geochemical and hydrolytic conditions (Garland, 1987) of the soil, enamel undergoes little change. This property is due to the high mineralization of hydroxylapatite (Aiello and Dean, 1990).

The measurements of the crystal apatite unit cell, the a and c axes (Fig. 1), are important because the presence of hydroxylapatite, chlorapatite, fluorapatite or some other form of apatite in large quantity in a sample of enamel can be determined on the basis of their sizes. Whether ion substitution occurred from the solution whose diffusion is controlled by ions from the apatite crystal itself can be determined by calculating the values of the measurements of the hexagonal-shaped crystal apatite unit cell.

The unit cell of the apatite structure has two axes (a -axis and b -axis) of the same length under a 120° ($\gamma=120^\circ$) angle and a third axis (c -axis) under a right angle relative to these two a -axes ($\alpha=\beta=90^\circ$) (Fig. 1). The axes of the unit cell with apatite structure, which is hexagonal-shaped, are: $a = b = c$. The angles formed by these axes are $\alpha=\beta=90^\circ$ and $\gamma=120^\circ$. The dimensions of these cells are particularly important in crystallographic research (Tables 1 and 2), yet the changes of the apatite structure are difficult to measure and require the implementation of extremely accurate research methods.

RESEARCH OBJECTIVE

The principal aims of this paper were: 1) to determine the composition of the apatite crystals in the dental enamel of exhumed medieval human skeletons; 2) to assess the color range of medieval teeth; and 3) to evaluate the correlation between the composition of the enamel and the color of the teeth.

MATERIAL

The investigations were done on 22 extracted human upper incisors: 11 from each of two cemetery sites, Žiža and Stara Torina. The results of the analysis of 107 adult skeletons have already been published (Đurić-Srejić et al., 1992).

METHODS

Crystallographic analysis (X-ray diffraction) and digital image analysis were the methods employed. These have not been previously used in anthropological research. Before measurements were taken the samples were cleaned ultrasonically. In the laboratory, the enamel was pulverized in an agate mortar. Identical quantities of pulverized enamel were placed into tubes, coded, and subjected to crystallographic testing.

Crystallographic Analysis

Crystallographic analysis of soil samples from the cemeteries was performed to assure that no ingredients from the soil were present in the dental enamel. Thus, factors determining the enamel composition could have been only dietary habits and water.

X-ray radiation on a grid was used to measure the size of the basic crystal. Whether the binding of the organic and inorganic molecules occurred in the crystal grid structure can be determined based on the changes of the values of the size of the basic enamel crystal cell.

X-ray diffraction is based on the phenomenon of X-ray diffraction that appears in the passing of X-rays through crystal, if the conditions of Bragg's law have been met. The powder diffractometer was used

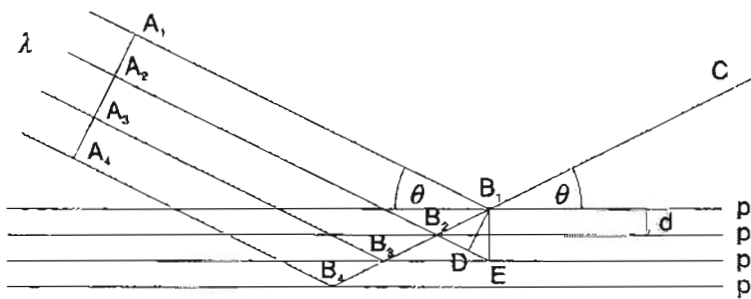


Fig. 3. Diffraction of equidistant X-rays.

to obtain data on X-ray diffraction on the particles of the crystal matter. An X-ray tube with a copper anode (anti cathode) was used as a radiation source. The wave length of the radiation specific for copper is $\lambda_{CuK\alpha} = 0.154178$ nm. Since copper has two characteristic lines, $CuK\alpha 1 = 0.154040$ nm and $CuK\alpha 2 = 0.154434$ nm, of which the first is twice as intensive as the second, the wave length of the radiation for $CuK\alpha$ was obtained according to the formula:

In order to accomplish the emission of specific radiation with an intensity sufficient for diffractometry, voltage U_a of about 40kV was brought to the tube anode and a current I_a of about 20 mA was passed. An X-ray generator often

$$\lambda_{CuK\alpha} = \frac{-2\lambda_{CuK\alpha 1} + \lambda_{CuK\alpha 2}}{3}$$

has a stabilized source of anode current. The beam of X-ray was directed to the powder goniometer. The width of the X-ray beam was limited by crevice S shown in Fig. 2. This collimated beam was dropped on the preparation P.

The preparation P was made by pressing the powder of the test material into an aluminum frame with an opening of 20x10x2 mm. The sample was placed in the center of the goniometer so that the upper surface of the test powder was in the axis of the goniometer O (Fig. 2). The beam diffracted from the preparation passed through the crevice S' and dropped on the X-ray detector D. During the acquisition of diffraction data the detector moved at a constant rate around the axis O, whereas the preparation moved at half this rate. The usual rate for the detector movement is 2°/min.

Intensified and formed impulses were led from the X-ray detector to the integrator which was used to give the number of impulses in the unit of time as voltage values. A recorder logged the changes of the voltage on a paper strip. The paper strip moved at a constant rate that was synchronous with the movement of the detector on the goniometer. Thus the diagram of the abscissa formed an angle 2θ , and the ordinate, the intensity of the diffracted ray (the number of impulses per second) (Fig. 2).

One diffractogram was composed of the basic line (phon or background) for the time without diffraction and maximum (reflection) of different heights with different angles 2θ . Each of these angles represented one family of grid planes whose position in space was indexed by Miller indices (hkl) (Fig. 3). The intensity I and angles 2θ were determined from each diffractogram. The reflection intensity was determined in units 1/100 of the height of maximum (reflection) of the highest intensity on the diagram. Thus, all other values had an intensity of less than 100.

The values of the diffraction angle were measured by lowering the normal on the abscissa from the middle of the maximum (of reflection) measured at half of its height. According to the equation:

where d is the distance of the grid planes of Miller indices (hkl); λ is the wavelength of X-ray radiation, and θ is the angle formed by the X-ray with a family of parallel planes. Assuming that $n=1$, the interlayer distances are

$$d = \frac{\lambda}{2 \sin\theta}$$

calculated for all reflections.

The X-ray diagrams of both groups of sample preparations were filmed on a Philips diffractometer, type 1820, with a Philips generator, type 1729. The operating voltage of the X-ray was 40 kV, and the current power of 30 mA. The powder diagrams were recorded in a range from 4-130°. The Philips APD system with a computer, which automatically calculated the interlayer distance and determined the intensity, was used for data processing.

Digital Image Analysis

Digital image analysis was used for colorimetric evaluation of the teeth (Miličević, 1994). Since color measurement and specification relative to some known color (differential colorimetry) has an advantage over identification of color in absolute terms, measurements were made using a physical standard as a reference. The standard selected was an artificial ceramic tooth with the shade guide Lumin Vacuum (Vita, Bad Saeckingen, Germany) labelled D3. This shade guide is widely accepted as a referent and differs the least from the other shades on the guide. Digitization of the sample teeth and Vita shade D3 was done using a Hewlett Packard SJ IIcx scanner at 400 dots per inch resolution. Analysis of the digitized images was performed by the commercial graphic software package, Corel Photo Paint V6.0 (Fig. 4).

Although color phenomena rely upon the spectral characteristic of the reflected light, colors could be specified through the relationship of the three primary

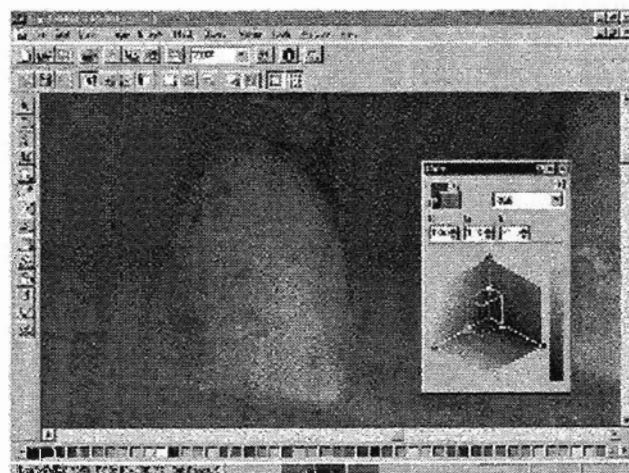


Fig. 4. Color analysis of the digitized image of a tooth.

TABLE 1. Dimensions of unit cells of enamel crystals - data from the literature.

Type of apatite	a-axis (nm)	c-axis (nm)	$V_a(\text{nm}^3)$
Hydroxylapatite	0.941	0.688	52.705
Hydroxylapatite	0.942	0.688	52.892
Fluorapatite	0.937	0.688	52.258
Fluorapatite	0.936	0.688	52.183

TABLE 2. Volume of unit cells - standard.

Hydroxylapatite	Chlorapatite	Fluorapatite
$V_o=52.88\text{nm}^3$	$V_o=53.76\text{nm}^3$	$V_o=52.42\text{nm}^3$

TABLE 3. Parameters of unit cells of enamel crystals - experimentally obtained data.

Specimen	a-axis (nm)	c-axis (nm)	$V_o(\text{nm}^3)$
Žiča	0.9425 (6)	0.6868 (4)	52.85 (8)
Stara Torina	0.9439 (9)	0.6887 (4)	53.10 (1)

TABLE 4. Color differences relative to the physical standard.

	Total difference ΔE units	Value ΔE units	Spectral position (*)	Chroma ΔE units
Žiča	8.82	7.82	-8.28	0.26
Stara Torina	12.49	7.43	-26.13	6.30

TABLE 5. ANOVA - Average value difference relative to the physical standard.

Source of Variation	SS	df	MS	F	P-value	F criteria
Between Groups	0.826957	1	0.826957	0.06529	0.800931	4.35125
Within Groups	253.3176	20	12.66588			
Total	254.1446	21				

TABLE 6. ANOVA - Average color difference relative to the physical standard.

Source of Variation	SS	df	MS	F	P-value	F criteria
Between Groups	73.70711	1	73.70711	17.64562	0.00044	4.35125
Within Groups	83.54152	20	4.177076			
Total	157.2486	21				

TABLE 7. ANOVA - Average hue difference relative to the physical standard.

Source of Variation	SS	df	MS	F	P-value	F criteria
Between Groups	1752.659	1	1752.659	93.28198	5.66E-09	4.35125
Within Groups	375.7766	20	18.78883			
Total	2128.436	21				

TABLE 8. ANOVA - Average chroma difference relative to the physical standard.

Source of Variation	SS	df	MS	F	P-value	F criteria
Between Groups	200.0051	1	200.0051	69.79966	5.93E-08	4.35125
Within Groups	57.30833	20	2.865416			
Total	257.3134	21				

colors. Maximal intensity of all three primary colors makes pure white; absence of all three primary colors corresponds to black; while any other color can be made combining the primary colors in different intensities.

Analysis of color by a computer was based on the principal that a digital image contains numeric information about the specification of color for every element of a picture (dot or pixel). Color was measured analyzing an area of approximately one square mm in the middle third of the labial surfaces of the teeth. Results were obtained from the image as the relative presence of the three additive primary colors. The results were numerically transformed into the CIELab system with equal color space, because that system includes physical, physiological, and psychological aspects of the color phenomena (Trussel, 1993).

The CIELab system has three parameters. Parameter L interprets value (the quantity of light contained in the color, independent of chromatic attributes), while the other two parameters, a and b, show chromatic properties of color. Parameter a interprets the balance between green and red, while parameter b is a measure of the blue-yellow balance in color. The values of the CIELab parameters correspond to the abilities of an average observer to perceive colors. For example, a color whose value is 60 seems twice as light to an observer than a color with a value of 30, independent of the physical properties of light.

The distance between two colors in the CIELab system corresponds to the ability of an observer to differentiate colors. The distance between colors is measured in ΔE units, where the difference of one ΔE unit represents a limit for discrimination between two colors. As parameters a and b are not commonly used, the results are interpreted in psychometric determinants of color (value, hue, and chroma), but using the metrics of CIELab system.

Value is a parameter of color which determines the quantity of light present in color. Depending on value, we talk about relatively light and dark colors. Hue is related to the spectral position of color, and indicates the part of the spectra which is dominantly present in the color observed. Commonly, this represents the name of the color (blue, red, orange, greenish-yellow, etc.). Chroma determines the purity of the dominant color or the degree of saturation of color.

Therefore, with higher chroma we can easily recognize a dominant color, where lower chroma indicates a more neutral color, which is closer to gray, than high chroma. In this article, value and chroma were interpreted in ΔE units, while hue has been shown as an angular position in the color spectra.

Diffractiongrams were made, analyzed, and compared in both test groups. The sizes of unit cells were calculated by the least square methods and reflections were indexed using the LSUCR program. Statistical processing of data was performed using analysis of variance.

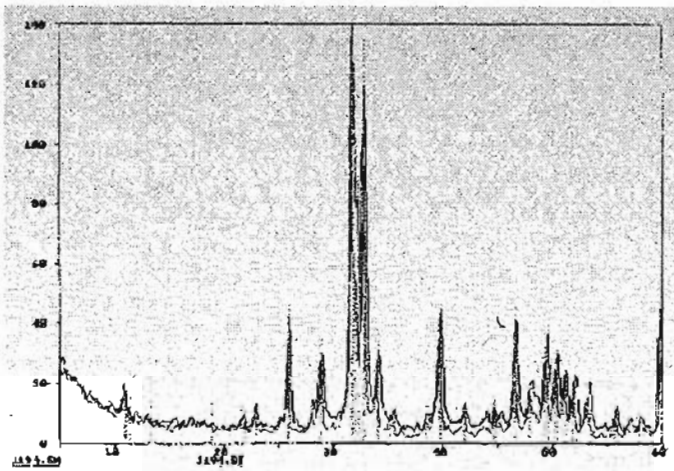


Fig. 5. Diffractograms of dental enamel from Žiča (solid line) and Stara Torina (dotted line) samples.

RESULTS

Crystallographic analysis showed the composition of soil from both cemetery sites. The sample soils were mainly composed of quartz, feldspar, liscunes, pherphidites, and traces of other ingredients. The values of the measurements and the volumes of the unit cells of the dental enamel crystals from Žiča and Stara Torina are shown in Table 3. Diffractograms for both samples are shown comparatively in Fig. 5. The results of the colorimetric analysis of both groups of samples are given in Table 4. All values were interpreted relative to the standard, Vita shade D3.

Analysis of variance showed that the average total color differences from the physical standard vary significantly ($P=0.00044$) between the two samples (Table 5). The average differences in value relative to the physical standard differ slightly between groups, but the results lack statistical significance (Table 6). However, the differences in hues (Table 7) and chroma (Table 8) relative to the physical standard are highly statistically significant.

The difference in the parameters of the elementary cells (a- and c-axis) need not be a precise indication of true

relations of crystal dimensions. Possibly, the different crystals of enamel have in one case a shorter and wider habitus than another, where the habitus is narrower and more elongated than the other. In such seemingly different crystals of the enamel, the volumes of the elementary cells may be identical (Tihacek-Sojoc, 1996).

In order to determine the accuracy of the unit cell using the existing angles and measurements, the volume of the enamel crystal unit cells was calculated according to the formula: $V = a^2 \cdot c \cdot \cos 30^\circ$, where: V is the unit cell volume; a is the α -axis of the unit cell; and c is the c-axis of the unit cell.

DISCUSSION

Results of the crystallographic analysis of the enamel sample from Žiča indicate that the volume of the unit cell is close to the values characteristic for hydroxylapatite. These results closely match the values for hydroxylapatite from the Table of Minerals, as well with the experimentally obtained data of Beevers and Joung and Elliot (Elliot, 1986). Those results were obtained by assessing natural teeth. Therefore, we were not surprised that our values differ slightly, and are closer than Elliot's (1986) to those from the Table of Minerals (Đurić, 1996).

The values of the volume of the unit cell of enamel from Stara Torina indicate the prevalence of chlorapatite. Comparison of the Stara Torina unit volume (Table 3) with the values in Table 2 shows that the chlorapatite are more similar than those of Žiča. The differences between our data and values published in the Table of Minerals (Fig. 7) indicate that other minerals were also present in the sample.

Comparing the diffractogram of soil with diffractograms of enamel from both cemetery sites, we concluded that no ingredients from the soil, such as quartz, feldspars, liscunes, pherphidites, or traces of other elements, had been incorporated into the enamel. Factors that determine the composition of the enamel could have been solely dietary habits and water.

Some authors (Ferguson and Chestnut, 1978) disagree about the vitality of human dental enamel. A number of articles deal with assessment of the enamel structure and the dynamic processes in it. One group of authors (Doi, 1986; Callens et al., 1986) assumes that enamel is alive because of intensive dynamic processes and the presence of remineralization. In contrast, other authors (Carlstrom et al., 1983) accent the disproportion of inorganic and organic components of enamel. They argue that enamel probably cannot be considered alive because no other human tissue has such a high percentage of inorganic components and a very low amount of water.

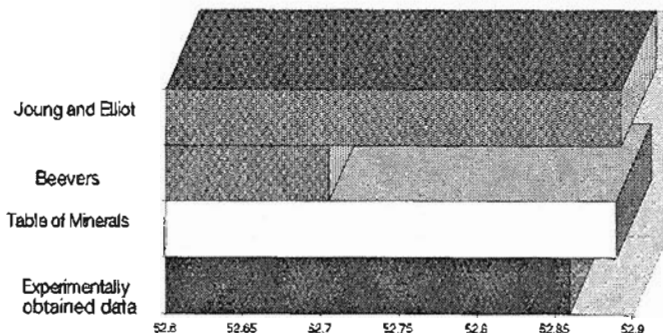


Fig. 6. Žiča (black bar): comparison of values of unit cell of hydroxylapatite crystal from different sources.

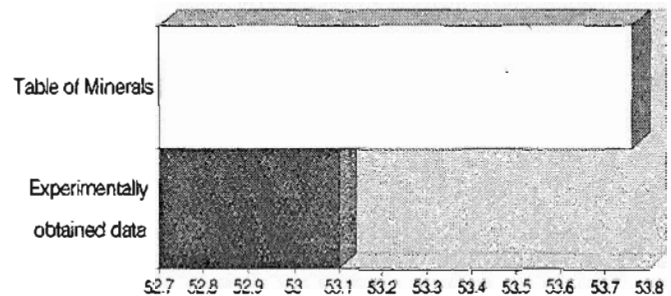


Fig. 7. Stara Torina (black bar): comparison of values of unit cell of chlorapatite crystal from different sources.

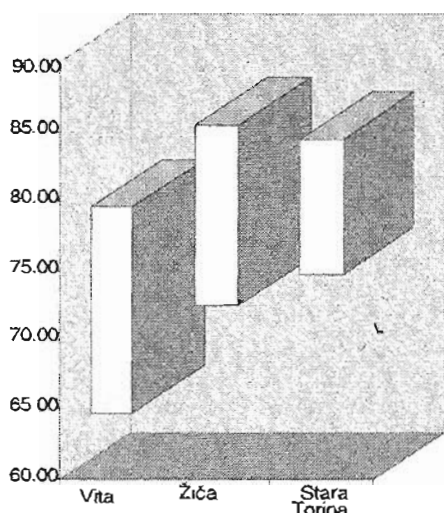


Fig. 8. Comparison of ranges of values for teeth from Žiča, Stara Torina, and the Vita shade guide.

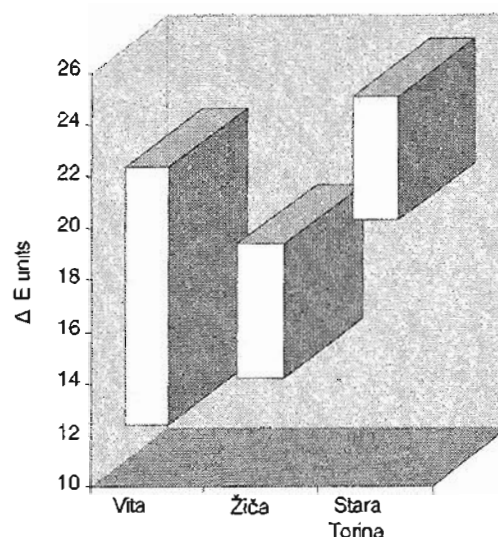


Fig. 9. Ranges of chromas for teeth from Žiča, Stara Torina, and the Vita shade guide.

Results presented in this article show differences between the enamel from two populations, caused by different diet habits and water. Therefore, vital enamel must be subject to changes under the influences of various chemical agents, both external and internal. This result argues that the dental enamel in our sample had once been alive.

Comparison of the color ranges of the teeth shows the similarity of the values in the two samples (Fig. 8). Compared with the color range which represents the tooth colors of a modern population (Vita), the average value of both groups of samples are higher than that of the Vita range. Moreover, the ranges in values of both groups overlap the range of contemporary tooth colors (Vita), which means that shades with equivalent values can also be found in the modern population.

Chroma of teeth from Žiča are completely within the range of chromas found in Vita, mainly covering its lower segment. Colors of teeth from Stara Torina are of significantly higher chroma than those of Žiča. Some of chroma were even beyond the maximum found in the referent shade guide (Vita) (Fig. 9).

The sample groups differ by hues. The range of hues of Vita is located in the part of spectra to the left of both Žiča and Stara Torina, with the range of hues from Žiča situated between those of Vita and Stara Torina.

From the results of this study, the hypothesis that the composition of enamel affects the color of teeth has been confirmed. The samples with a prevalence of chlorapatite had colors with completely different hues and significantly higher chromas than did those with hydroxylapatite. However, the differences were not significant because of the sizes of the samples.

CONCLUSIONS

The compositions of apatite crystal from dental enamel of two populations of medieval Serbs differ from one another. Pure hydroxylapatite occurs in the enamel from Žiča, whereas chlorapatite prevails in the enamel from Stara Torina. No evidence exists that ingredients from the soil had been incorporated into the enamel from either sample.

The color ranges of teeth from Žiča and Stara Torina differ from one another in hues and in chromas. Hues of teeth from Stara Torina are in the reddish-orange part of spectra. Hues of Žiča teeth are in the orange region and closer to the hue range of modern teeth than that of Stara Torina. Yet, the Stara Torina sample had significantly higher chromas than those from Žiča.

The results indicate that enamel composition may have an influence on the color of a tooth. The prevalence of chlorapatite in enamel causes tooth color to be closer to red and of higher chroma than teeth whose enamel consists of hydroxylapatite.

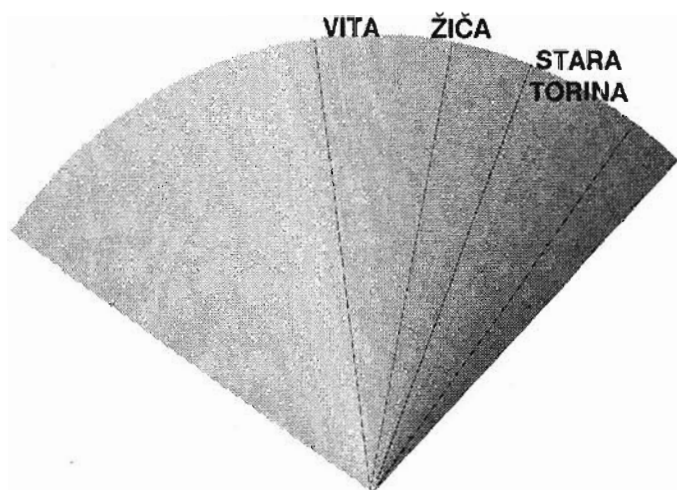


Fig. 10. Ranges of hues for teeth from Žiča, Stara Torina, and the Vita shade guide.

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DIAGNOSTIC CHARACTERISTICS OF HUMAN BITE MARKS: A REVIEW OF SOLVED CASES

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ABSTRACT Despite their non-standardized documentation and interpretation, human bite marks are very useful in the legal arena. A literature review of case histories was undertaken to search for some basic dental traits that characterize bite marks successfully used to identify suspects in forensic cases. Information for eighteen cases indicated that the two dental traits that occurred most frequently, diastema and malposition of teeth, were usually sufficient to identify a suspect. The relative simplicity of these findings suggests questioning the necessity and cost-effectiveness of lengthy and complex analyses in many forensic cases.

INTRODUCTION

Human bite marks have been accepted as evidence in courts in the United States since the 1870s. Their utility is based on the distinctiveness of individual dentitions (Rothwell, 1995). The legal value of bite mark evidence lies not only in its uniqueness, but also in its frequent occurrence in crimes. Bite marks are found on victims or perpetrators of sex crimes, child abuse, assault, and homicide (American Board of Forensic Odontology, 1986).

Regardless of the specificity of bite mark evidence, many legal, clinical, and forensic authorities question its accuracy. Sources of error can be numerous and vary with the many techniques (e.g., computer tomography scan, scanning electron microscopy, dusting and various overlay and casting procedures, photographic techniques) used for preservation and analysis. Training and verification of odontological expert witnesses is haphazard and non-standard. Disagreement occurs even among respected authorities (Weigler, 1992).

The American Board of Forensic Odontology (ABFO) devised standards for bite mark analysis in 1986, but no general agreement yet exists about national or international standards for bite mark comparison (Rothwell, 1995). The ABFO system employs information about demographics of the victim, anatomical location and shape of the body surface involved, the shape of the bite, and other soft tissue observations, such as the presence of abrasions and lacerations. An important part of the system is the ABFO score sheet, a lengthy itemized list of tooth-by-tooth matches between traits of the bite mark and the suspect's dentition. Discrepancies between the bite mark and the suspect's dentition are noted in three categories of "gross features." These are presence of each tooth in the suspect and consistent size, and consistent shape of arches. In addition, "tooth position" (in labiolingual position, in rotational position, and in terms of spacing between tooth margins) and "interdental features" (e.g., mesiodistal and labiolingual lengths of each tooth, distinctive curvatures of any teeth) are compared between the bite mark and the individual teeth in the suspect. This format leaves a category for other features. With this scoresheet, the odontologist may score eight or ten characteristics for each tooth in the suspect's dentition (ABFO, 1986:386).

Despite some research showing great reliability (Rawson et al., 1986), much criticism has been levelled at the ABFO method for its scorecard style approach. Some experts have questioned the reliability of some of its criteria, such as the measurements of the bite mark (Ebert, 1988). Other researchers could not reproduce the high reliability reported by Rawson et al. (1986) when different techniques of evidence preservation were used (Rothwell, 1995).