ECOLOGICAL CHEMISTRY AND ENGINEERING S

Vol. 17, No. 4 2010

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ESTIMATION OF INTRINSIC AND EXTRINSIC ENVIRONMENT FACTORS OF AGE-RELATED TOOTH COLOUR CHANGES

OCENA CZYNNIKÓW WEWNĘTRZNYCH I ZEWNĘTRZNYCH POWODUJĄCYCH ZMIANY BARWY ZĘBÓW ZWIĄZANE Z WIEKIEM

Abstract: Age-related colour changes of biological objects are the results of changes in structure and properties which may reflect the influence of the extrinsic and intrinsic environment. The method able to discriminate these two factors was not published yet. Non-erupted teeth which are isolated from the environment of the oral cavity (intrinsic factors) have not yet been explored too. A device for research the dentist's chair-side measurements of vital erupted teeth as well as extracted impacted teeth (immediately after extraction) was built from commercially supplied components (fiber optic spectrometer) coupled with specific custom-made parts and a specific software driver. The measurement method related tooth for total colour changes in system CIELAB 1976 was evaluated to be inadequate. For more precise method of the ΔE_{CMC}(l:c) were theoretical trichromatic coordinates of standard tooth and the ratio of extrinsic and intrinsic factors for vital erupted and impacted teeth modelled by multivariate 3D-mathematical regression models. The rate of complex discolouration caused by the total factors decreases over the life of humans. The rate of colour changes caused by intrinsic factors is nearly constant over the life-time. Age estimation of the vital erupted teeth 21 (inversion exponencial function of $CMC_{(2:1)}$) will be only approximate (s.d. 6.2 years). More convenient for approximate age estimation are the impacted teeth immediately after extraction (inversion linear function of $CMC_{(2:1)}$), significant correlation with the known real age p-value < 0.001, (s.d. 3.1 years). Correlation between the subjects age and the yellowness of b* values of skulls is significant ($r^2 = 0.80$). The similar correlation between the subjects age and yellowness of b* values of impacted teeth ($r^2 = 0.79$) suggests a presumably similar mechanism of colour changes in bone and impacted teeth. These teeth are relatively available biological samples and can be obtained without any difficult medical or ethical issue.

Keywords: age-related colour changes of teeth, intrinsic and extrinsic factors, 3D mathematical regression models, estimation of real age

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The colour of teeth is determined by combined effects of intrinsic and extrinsic colourations. Age related colour changes have proved useful in clinical dental practice, concerned with prosthetic treatment (especially traumatogenic). Intrinsic tooth colour is associated with the light scattering and absorption properties of the enamel and dentine. Extrinsic colour is associated with the absorption of materials at the surface of enamel and in particular the pellicle coating which ultimately causes extrinsic stains etc. [1, 2].

Many colour scales has been developed for this purpose Berns [3]. Most frequently used system CIELAB was designed in 1976 year. In this three-dimensional colour space the colour co-ordinates values L*, a*, b* are calculated from the X,Y,Z tristimulus values for both the reference and specimen as follows:

$$L^* = 116\left(Y/Y_n\right)^{1/3} - 16 \tag{1}$$

$$a^* = 500\left[\left(X/X_n\right)^{1/3} - \left(Y/Y_n\right)^{1/3}\right] \tag{2}$$

$$b^* = 200\left[\left(Y/Y_n\right)^{1/3} - \left(Z/Z_n\right)^{1/3}\right] \tag{3}$$

where X/X_n, Y/Y_n, $Z/Z_n > 0.008856$
or:

$$L^* = 903.3\left(Y/Y_n\right) \tag{4}$$

$$a^* = 3893.5\ \left[\left(X/X_n\right) - \left(Y/Y_n\right)\right] \tag{5}$$

$$b^* = 1557.4\left[\left(Y/Y_n\right) - \left(Z/Z_n\right)\right] \tag{6}$$

where X/X_n, Y/Y_n, $Z/Z_n \leq 0.008856$.

Tristimulus X_n, Y_n, Z_n values are of the iluminant/observer combination.

Evaluation of total colour changes E^*_{CIELAB} (shortened symbol E*) are simple, but too much imprecise.

$$E^* = \left[\left(a^*\right)^2 + \left(b^*\right)^2 + \left(L^*\right)^2\right]^{1/2} \tag{7}$$

The L^* value is lightness (black 0, reflecting 100), the a^* value is redness ($+a^*$) or greenness ($-a^*$), and the b^* value is yellowness ($+b^*$) or blueness ($-b^*$). The a^* and b^* co-ordinate values near zero correspond to neutral colours (white, greys) and their values increase in for more saturated or intense colours.

CIELAB colour space has been modified to a more precise CMC system and standardised in International Standard ISO 105-J03:1995(E): Calculation of colour differences [4]. Evaluation of total colour changes ΔE_{CMC} (l:c), (shortes symbol $CMC_{(l:c)}$) is calculated

$$CMC_{(l:c)} = \left[\left(\frac{\Delta L^*}{l \cdot S_L}\right)^2 + \left(\frac{\Delta C^*}{c \cdot S_C}\right)^2 + \left(\frac{\Delta H^*}{S_H}\right)^2\right]^{\frac{1}{2}} \tag{8}$$

The three separate components of the colour differences ΔE_{CMC}(l:c), (shortened symbol $CMC_{(l:c)}$) lightness ΔL^*, chroma ΔC^*, and hue ΔH^* are weighted by weighting factors S_L, Sc, S_H calculated from the chromatic coordinates of a standard L^*_0, a^*_0, b^*_0. Other weighting factors (1:c) may be numerically optimised to suit the desired purposes. The calculation CMC of colour differences was used in several studies published recently. These articles usually compare teeth and selected filling or prosthetic materials and use $CMC_{(l:c)}$ calculation just as an indicator of perceptibility or acceptability. The purposes of this study were to evaluate the $CMC_{(1:1)}$, $CMC_{(2:1)}$ and $CMC_{(3:2)}$ formulas to identify which of them provides the best indicator for acceptability of small colour differences in the esthetic dental restorative materials. Trichromatic coordinates of third standards are defined from these materials [5-7].

The third standard is used for the calculation of weighting factors. These values were not yet determined for human teeth (theoretical standard tooth) and so another aim of this study was to set them and consider the benefits of using the spectral parameter (eg the colour change compared with a standardised tooth instead of the white standard and establishing weighting factors, the rest is used for dark signal). $CMC_{(l:c)}$ values had not yet been used as a spectral parameter for age-related correlations of teeth. Age-related colour changes of impacted teeth which are isolated from the environment of the oral cavity have not yet been explored. These teeth are not affected by the ambient conditions, the change in colour is only caused by physiological processes inside the human body and so they are ideal for determination of the ratio of intrinsic and extrinsic factors that cause the change in tooth colour. The concept of a custom-made instrument, new use of the CMC system for calculating colour differences and 3-D mathematical models of the ratio of extrinsic and intrinsic factors for vital and impacted teeth and age estimation using inverse functions of colour-age relationship has been demonstrated and are described in the following text.

Materials and methods

A special instrument was built from commercially supplied components. The designed device consisted of an Avantes S 2000 fibre optics spectrometer coupled with an XE 2000 xenon light source and an FCR 7 UV 200-2-ME reflectance probe, which contained six 200 μm optical fibres for the light source around one 200 μm sampling fibre (AVANTES, Eerbeek, Netherlands). These components were completed with custom-made parts, for example a special holder for the measurement of extracted teeth or spectrometric standards or special plastic probe shield for chair-side measurements, which was designed for frequent resterilisations. The same geometric configuration, 45/8, was found optimal for all types of determinations (chair-side measurements, extracted teeth). A special software-driver (called VIS) was supplied with the spectrometer for Object oriented Design Lab view $TM^{5.0}$. This unique software was programmed using VIS to control the spectrometer, the xenon light source, collect data and calculate trichromatic coordinates X, Y, Z, colour co-ordinates L*, a*, b*, E* and $CMC_{(l:c)}$ values.

The spectrometer Avantes S 2000 was Tzerny-Thurner design spectrophotometer with CCD detector with 2048 diodes. Just 1902 pixels are used for data collection, the rest is used for electric dark signal correction function. The output of the VIS is Spectral Output table containing 1902 values of subject reflectance. The spectrometer was calibrated with white and black standards before each measurement. It was programmed to switch off the light for black standard calibration. The following spectrometer parameters were set: integration time 100 ms, delay before flashes 0 ms, averaged samples 10. It was necessary to perform one cycle of ten flashes to stabilize the xenon light source before every measurement.

The following groups of patients were collected with the agreement of the ethical committee of Central Army Hospital in Prague, CZ (according to the Helsinki Declaration):

The group for the determination of Total factors of vital erupted teeth discolourations consisted of 69 probands (35 males and 34 females), 20-75 years old. All of them were non-smokers, in good overall health and their oral hygiene was satisfactory (standard hygiene indices PBI, CPITN, API, DMF > 10). This teeth was was polished with a mechanical brush with DepuralNeo paste (Dental a.s., CZ) and rinsed with deionised water. This tooth was measured in its central area in all subjects.

The group for the determination of Intrinsic factors of extracted impacted teeth discolourations (measured immediately after extraction) consisted of 23 probands (12 males and 11 females), 20-69 years old, non-smokers, in good overall health with satisfactory oral hygiene (standard hygiene indices PBI, CPITN, API, DMF > 10). The tissue and blood residues of these extracted teeth were removed carefully and the coronal part was polished with a mechanical brush with DepuralNeo paste and rinsed with deionised water.

The Extrinsic factors were determined by a differential method using data of extracted impacted teeth (immediately after extraction) and vital erupted teeth of the some probands before surgery. This group of differential measurement was collected over more than five years, because the patient had to have not only a extracted impacted tooth, but also a vital and intact erupted tooth 21.

The SubVISs for calculating X, Y, Z, and L^*, a^*, b^* and CMC were created. The computation of CMC was based on predefined values of the trichromatic coordinates L_0^*, a_0^*, b_0^* of third standard (theoretical standard tooth and weight factors l, c). Another additional module was created for approximate age determination using inverse functions of colour-age relationship [8, 9].

Results and discussion

Parameters of all used mathematic-statistical models mentioned above were obtained by linear and nonlinear regression methods, depending on the model type. Data were fitted using the least squares method (LSM). Since the LSM requires normal distribution, all residual errors were tested for normality. Statistical significance of all parameters was tested using t-test and normal standard deviations were used to quantify uncertainty of parameters.

Figure 1 shows the age dependence of the mean trichromatic coordinates $L^*(21)$, $a^*(21)$, $b^*(21)$ of the vital left central incisors. A 3D-mathematical model of this dependence of the age-related changes trichromatic coordinates of the vital erupted and the extracted impacted teeth were used to determine theoretical values of standard tooth. This model used data to determine the extrapolated values of the trichromatic coordinates for near-zero age.

For all significance tests and for construction of confidence intervals of parameter estimates and confidence intervals of prediction (Figs 2 and 3) at the significance level $\alpha = 0.05$ was used.

Figure 2 shows the age dependence of mean spectral parameters E* of vital erupted teeth 21 as well as extracted impacted teeth (IMP - immediately after extraction).

Figure 4 shows estimated models for total discolouration $CMC_{(2:1)}$ vital erupted teeth (21), extracted impacted teeth (IMP) and differential values (21-IMP) for erupted and impacted teeth after surgery as a function of age based on experimental data (Fig. 4a) and mathematical models of total, extrinsic and intrinsic discolouration derived from spectral parameters (Fig. 4b). The outer limits are 0.25% quantiles of the data, ie ± 3σ. The relationship is described by the formulas in Table 1.

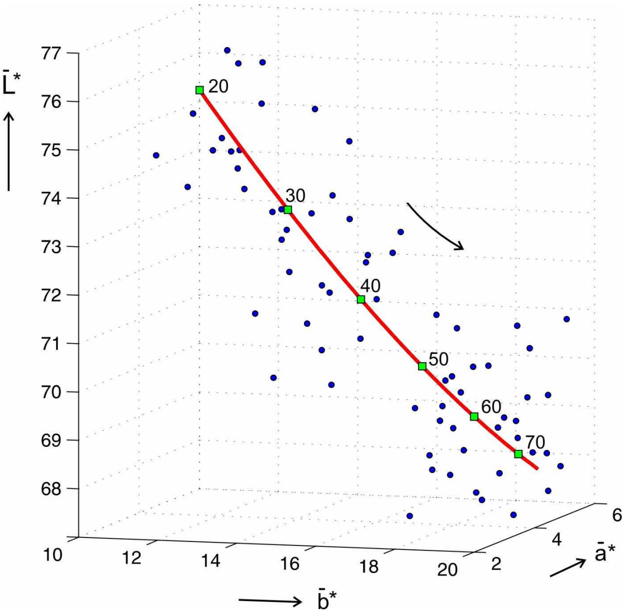

Fig. 1. The age dependence of mean trichromatic coordinates L*(21), a*(21), b*(21) of the vital left central incisors. Method CIELAB (L* - lightness, +a* - redness, +b* - yellowness). *Note: Estimates of the trichromatic model parameters are given in Table 1*

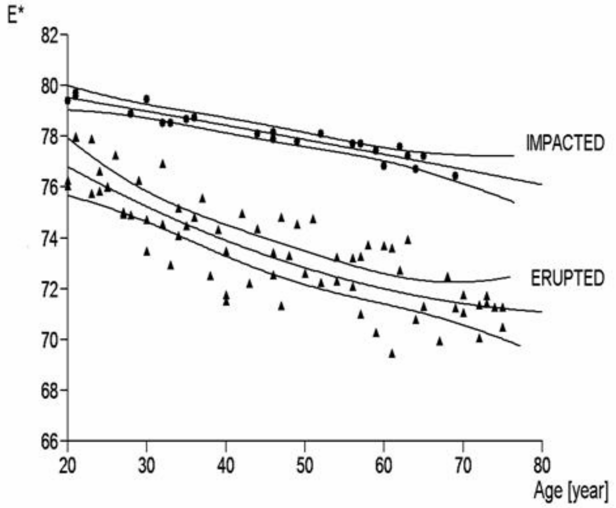

Fig. 2. Age dependence of mean spectral parameters E* of vital erupted teeth (21) as well as extracted impacted teeth (IMP) immediately after extraction

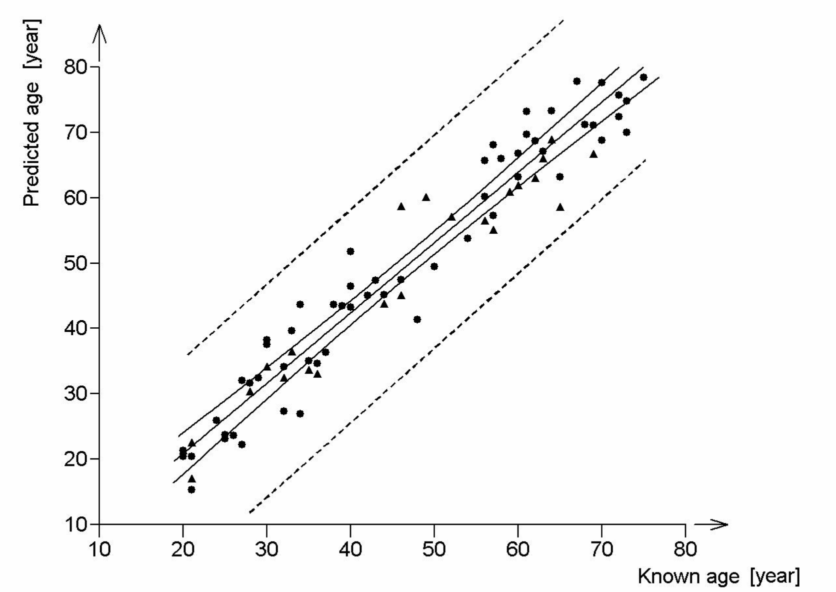

Fig. 3. Mean age estimation from inverse models of spectral parameters of the vital erupted teeth (21), use of inversion exponential function $^{Inv}CMC_{(2:1)}$ and extracted impacted teeth (IMP), use of inversion linear function of $^{Inv}CMC_{(2:1)}$

Table 1

Estimates of parameters and their confidence intervals for the models of trichromatic coordinates and spectral parameters of vital teeth (21), extracted impacted teeth (IMP) and differential values (21-IMP) for vital (21) and impacted teeth (IMP) after surgery

Spectral parameter Y	**Age t [year]**	**Number of teeth MF(M)(F)**	**Parameter estimates of the model Y = A ± C∗exp(-k∗Age) and Y = A ± C∗Age with their confidence intervals (CI)**					
			A	**CI** (A)	**C**	**CI** (C)	**k**	**CI** (k)
L*(21)	20÷75	69 (35) (34)	66.09	64.23	18.26	13.61	–0.03	–0.06
				68.44		22.78		–0.02
a*(21)	20÷75	69 (35) (34)	4.99	3.83	–4.75	–5.90	–0.04	–0.06
				6.11		–3.55		–0.02
b* (21)	20÷75	69 (35) (34)	26.32	25.00	–18.01	–21.53	–0.02	–0.03
				27.62		–14.1		–0.01
E*(21)	20÷75	69 (35) (34)	69.97	68.18	15.21	10.68	–0.04	–0.06
				71.76		19.74		–0.02
CMC(IMP)	20÷69	23 (12) (11)	0.07	0.04	0.08	0.07	0.00)^x	---
				0.09		0.09		---
CMC(21)	20÷75	69 (35) (34)	23.90	18.05	–22.22	–25.76	–0.02	–0.03
				26.39		–22.03		–0.01
CMC(21-IMP)	20÷69	46 (24) (22)	13.05	11.99	–13.02	–10.97	–0.03	–0.04
				15.22		–14.98		–0.01

Note:)^x ... Very low value of the parameter k allows the use of linear model

Figure 4 shows approximate age estimation, significant correlation with the known real age p-value < 0.001, from inverse models of spectral parameters of the vital erupted teeth

(21), use of inversion exponential function ${}^{Inv}CMC_{(2:1)}$ and extracted impacted teeth, use of inversion linear function of ${}^{Inv}CMC_{(2:1)}$.

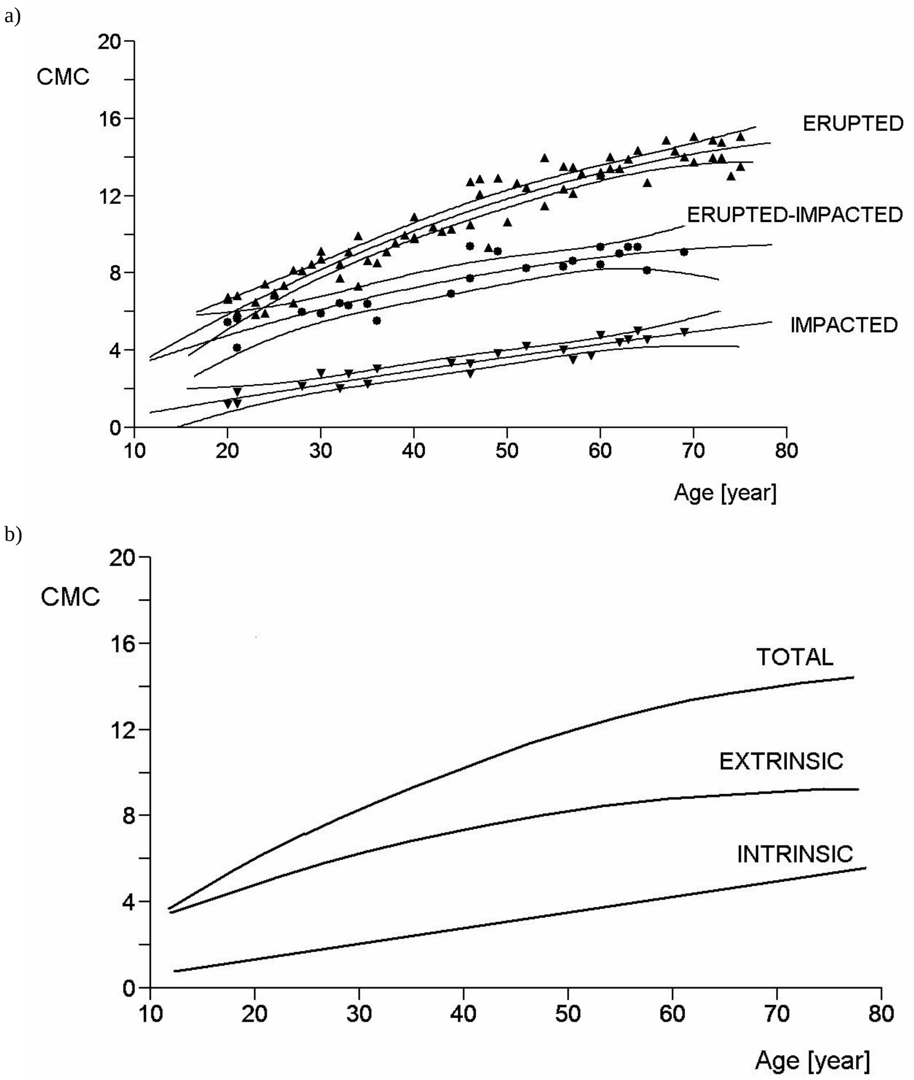

Fig. 4. Estimated models for total discolouration of vital teeth (21), extracted impacted teeth (IMP) immediately after extraction and differences values of vital erupted and extracted impacted teeth (21-IMP) from patients after surgery as a function of age based on experimental data (Fig. 4a) and mathematical models of total, extrinsic and intrinsic factors of discolouration derived from spectral parameters (Fig. 4b). *Note: Estimates of the trichromatic model parameters are given in Table 1*

Table 1 gives age dependence of the mean trichromatic coordinates $L^*(21)$, $a^*(21)$ $b^*(21)$, $E^*(21)$ of the vital left central incisors and estimates of parameters and their confidence intervals for the mathematic-statistical models of Total factors-vital erupted teeth (21), Intrinsic factors-extracted impacted teeth (IMP), immediately after extraction and of Extrinsic factors-differences values of vital erupted and extracted impacted teeth (21-IMP) of probands after surgery as a function of age based on experimental data.

Table 2 gives of the contributions of intrinsic and extrinsic factors to the total discolouration and rate of colour changes obtained from mathematical models of spectral parameters of vital and extracted impacted teeth as a function of age.

Table 2

Estimates of the contributions of intrinsic and extrinsic factors to the total discolouration and rate of colour changes obtained from mathematical models of spectral parameters of vital teeth (21), extracted impacted teeth (IMP) and differential values (21-IMP) for vital (21) and impacted teeth (IMP) after surgery

Age group	**Number of teeth**	**Discoloration**		**Discoloration rate [year^{-1}]**			
		W (Intrinsic)		**S (Intrinsic)**		**S(Extrinsic)**	
[year]	**MF**	$\frac{\mathbf{CMC(IMP)}}{\mathbf{CMC(21)}}$	**s.d.**	$\frac{\mathbf{d(CMC(21}}{\mathbf{d(Age)}}$	**s.d.**	$\frac{\mathbf{d(CMC(21\text{-}IMP))}}{\mathbf{d(Age)}}$	**s.d.**
20÷29	20	0.26	0.02	0.08	0.01	0.18	0.03
30÷39	20	0.27	0.03	0.08	0.02	0.14	0.04
40÷49	20	0.29	0,07	0.08	0.02	0.11	0.04
50÷59	20	0.31	0.08	0.07	0,02	0.08	0,06
60÷69	12	0.34 $)^{x}$	–	0.07 $)^{xx}$	–	0.06 $)^{xx}$	–

Note: $)^{x}$ extrapolated values, $)^{xx}$ calculated from extrapolated values

The statistical software package QC-Expert 3.1 (TriloByte Statistical software, CZ) was used to calculate the regression models and to plot the figures. This software is validated with US National Institute for Standards in Technology (NIST).

The vital erupted central left incisor of 69 (35 M, 34 F) probands was measured using the method CIELAB. The averaged values and standard deviations for trichromatic coordinates were L* = 71.3 (s.d 4.7), a* = 3.6 (s.d. 1.3), b* = 15.4 (s.d. 4.8). Hasegawa and Motonomi [10] published trichromatic coordinate values of L* = 73.0 (s.d. 5.0), a* = 3.5 (s.d. 1.5), b* = 16.5 (s.d. 5.0) for the upper incisors (87 humans, age 20-75). Analogous values were determined by Xiao et al [11] (405 young humans) and Tenbosch and Coops [12] (102 extracted teeth). According to these results, the developed method of tooth colour measurement is comparable with the above-mentioned authors.

The trichromatic coordinates of the vital impacted teeth, immediately after extraction 23 (12M, 11F) were measured by the same method. The average values were L* = 77.5 (s.d. 1.9), a* = 2.7 (s.d. 0.5), b* = 8.7 (s.d.1.65). It is very difficult to compare this, because it was measured for the first time. Schafer [13] measured 124 skulls post mortem (0-83 years), which was isolated from the environment too. Trichromatic coordinates values were L* = 72.5 (s.d. 8.22), a* = –7.4 (s.d. 3.22), b* = 16.4 (s.d. 5.52).

The difference between the male and female age-related colour change of the vital central incisor 21 is not conditioned sexually, this difference was not found in the same group in impacted areas (two sample t-test, p-value < 0.05). The divergence in the average

L* value for the male and female group could be merely caused just by a different level of hygiene at a younger age.

The age dependences of the trichromatic coordinates vital erupted teeth *in vivo* are usually measured for use in prosthetic stomatology Gozalo-Dias et al [14] and are expressed by a linear mathematical model. In forensic odontology linear mathematical model was used for age related colour changes of extracted teeth [15].

Experimental age related data of L*, a*, b*, E* discolouration of vital erupted teeth have decreasing rate of colour change and the spectral parameters converge to a constant. However, the total discolouration E* is not suitable for differential method of determination extrinsic factors. A modern weighting method based on $CMC_{(l:c)}$ could not be applied because the trichromatic coordinates of the standard tooth are experimentally unavailable. A 3D-mathematical models of this dependence of the age-related changes trichromatic coordinates of the vital erupted and the extracted impacted teeth was used to determine theoretical values of standard tooth. This model used data to determine the extrapolated values of the trichromatic coordinates for near-zero age.

These values L_0^*, a_0^*, b_0^* rounded to 2 decimal places were defined as the absolute standard for teeth colour. The computation of CMC was based on predefined this values of the trichromatic coordinates theoretical standard tooth and weight factors *l, c*.

The ratio of extrinsic and intrinsic factors for vital and non-erupted teeth was demonstrated by mathematic-statistical models. The curves were calculated by new weighting method to the general spectral parameter $CMC_{(2:1)}$ from the above values of the spectral parameters of vital erupted teeth 21 as well as extracted impacted teeth (immediately after extraction) and their differential determination. The extrinsic factors are more powerful in terms of the colour change over the life of the human being compared with intrinsic factors. This effect decreases with age. The extrinsic/intrinsic ratio is more than three times larger for the youngsters than for seniors. The rate of complex discolouration decreases over the life. The rate of colour change caused by intrinsic factors is nearly constant over time and the extrinsic change decreases with the age. The contributions of intrinsic and extrinsic factors of the total discolouration obtained from the differential values for vital erupted and impacted teeth (immediately after extraction). The rates of colour changes were obtained as the mean first derivatives from the curves of total, extrinsic and intrinsic environmental factors within ten-year intervals.

The inverse model age-related colour-changes $CMC_{(2:1)}$ of teeth can also be used to estimate standard deviations and confidence intervals of the estimated age. Mean errors of age determination as well as two calibration systems CIELAB and CMC system were compared. The mean error of the determined age from 46 people (23 males, 23 females) of the vital erupted teeth 21 was 11.9 years for linear regression model CIELAB and 6.2 years for nonlinear regression model $^{Inv}CMC_{(2:1).}$ In the case of 20 extracted impacted teeth (10 males, 10 females), immediately after extraction, this error for linear regression model CIELAB was 3.1 years. More convenient for approximate age estimation are the vital impacted teeth immediately after extraction (inversion linear function of $^{Inv}CMC_{(2:1)}$). There is only one possible article for comparison [13]. Correlation between the subjects age and the yellowness of b* values of skulls is ($r^2 = 0.80$). The similar correlation between the persons age and yellowness of b* values of impacted teeth ($r^2 = 0.79$) suggests a presumably similar mechanism of colour changes in bone and impacted teeth.

Conclusions

Extracted impacted teeth are relatively available biological samples and can be obtained without any difficult medical or ethical issues. These teeth may become suitable objects for research of the age-dependent and environmental changes in human organism. The new biophysics concept of custom-made instrument with fibre-optic spectrometer, new use of the CMC system for calculating colour differences and 3-D mathematical models described here is suitable even for other biological objects where the estimation of intrinsic and extrinsic environmental factors or properties reflecting biological age is necessary.

Acknowledgement

This research was supported by Ministry of Education, Youth and Sports of Czech Republic by the projects MSM 111600002, MSM 111100006 and MSM 619 895 9214.

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OCENA CZYNNIKÓW WEWNĘTRZNYCH I ZEWNĘTRZNYCH POWODUJĄCYCH ZMIANY BARWY ZĘBÓW ZWIĄZANE Z WIEKIEM

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Abstrakt: Zmiany koloru obiektów biologicznych wraz z wiekiem są wynikiem zmian w ich strukturze i właściwościach, które mogą odzwierciedlać wpływ środowiska zewnętrznego i wewnętrznego. Dotychczas nie badano wpływu środowiska jamy ustnej zębów niewyrzniętych. Opisano stanowisko do badania zarówno zębów niewyrzniętych, jak również zębów usuniętych (bezpośrednio po ekstrakcji), zbudowane z elementów dostępnych w handlu (spektrometr światłowodowy), obsługiwane sterownikami programowanymi. Metodę pomiaru całkowitej zmiany barwy zębów w systemie CIELAB 1976 oceniono jako niewystarczającą. W badaniach wykorzystano bardziej precyzyjną metodę $\Delta E_{CMC}(l{:}c)$, wprowadzając współrzędne trójbarwne standardowych zębów oraz stosunek zewnętrznych i wewnętrznych czynników istotnych dla zębów niewyrzniętych i zatrzymanych. Do opisu zmian zastosowano wielowymiarowy model regresji 3D. Szybkość przebarwienia zębów spowodowana przez wiele czynników zmniejsza się w ciągu życia człowieka. Zmiana barwy zębów spowodowana przez czynniki wewnętrzne jest prawie niezmienna przez cały okres życia. Ocena wieku zębów wyrzniętych 21 (odwrotna zależność wykładnicza funkcji $CMC_{(2:1)}$) jest tylko przybliżona (SD = 6,2 roku). Wygodniejsze dla przybliżonej oceny wieku są zęby badane natychmiast po ekstrakcji (odwrotna funkcja liniowa $CMC_{(2:1)}$), dobrze korelująca ze znanymi, prawdziwymi długościami życia $p < 0{,}001$ (SD = 3,1 roku). Korelacja między wiekiem badanych szczątków oraz zażółceniem wartości b* czaszek jest znacząca ($R^2 = 0{,}80$). Podobna zależność między wiekiem pacjentów i zażółceniem wartości *b zębów zatrzymanych ($R^2 = 0{,}79$) sugeruje podobny mechanizm zmiany koloru kości i zębów. Zęby są stosunkowo łatwo dostępną próbką biologiczną i mogą być uzyskane bez trudności natury medycznej lub etycznej.

Słowa kluczowe: zmiany barwy zębów związane z wiekiem, czynniki wewnętrzne i zewnętrzne, model regresji 3D, szacowanie rzeczywistego wieku