

CORRELATION BETWEEN ALTERED ENAMEL MINERAL CONTENT AND FINAL TOOTH SHADE

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ABSTRACT

Objectives: This study was conducted to evaluate the correlation between alteration of enamel mineral content and tooth shade.

Materials and Methods: A total of 33 sound bovine enamel blocks (5 x 5 mm, 2 mm thickness), were obtained from the middle third of the labial enamel surface. The samples were divided into three subgroups (n=11/group), where Group I received no surface treatment; Group II represents the demineralized enamel surface, Group III represents the remineralized enamel surface. Scanning Electron microscopic (SEM) examination and Elemental Analysis (EDXA) testing, as well as Color measurement using a color spectrophotometer and micro-hardness evaluation were performed for all samples.

Results: The L values revealed a significantly strong negative correlation with carbon ($r = -0.74$, $p = 0.002$), but being positively correlated for oxygen ($r = 0.59^*$, $p = 0.02215$), phosphate ($r = 0.62^*$, $p = 0.01315$) and calcium ($r = 0.76$, $p = 0.00115$). In contrast, both a and b values were only positively correlated for carbon ($r = 0.67^{**}$, $p = 0.006$; $r = 0.72^{**}$, $p = 0.003$ respectively), and negatively correlated for the rest of elements. Vickers microhardness values on the other hand were only negatively correlated with carbon ($r = -0.88^{**}$, $p = 0.000$), but positively correlated for the rest elements.

Conclusions: The change in the surface elemental content of the bovine enamel affected the lightness and the blue-yellow axis of tooth color, correlating respectively with the L* and b* values.

KEYWORDS: Enamel demineralization; Microhardness; Shade.

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INTRODUCTION

Since a beautiful healthy smile is of major importance for the self-esteem of humans, the concern about esthetics and preventive dentistry has captured not only the attention of dentists but also that of dental patients throughout the years.

Upon light illumination, the “phenomenon of the observed color” of the object results because of light scattering. Hence, irregular light paths through the structure occur prior to emerging out in the direction of the incidence surface reaching the observer’s eye.¹

Nowadays, a healthy tooth shade depends mainly on intrinsic factors (optical property, mineral content,... etc), congenital factors (heredity, gender and race), along with extrinsic factors inclusive illumination and observer’s perspective). Such factors are thought to be modified by the surface texture and surface properties of enamel, which may be affected drastically by aging together with various chemical challenges met through life.^{2,3}

Optically, the properties and characteristics of dental enamel are mainly influenced by its micro-composition. Thus, awareness and recognition of their origin is mandatory for the development of new optical methods for the measurement of enamel composition and shade.⁴

Unlike dentin, dental enamel is a mineralized uniquely arranged prismatic structure, with low organic and water content, evident translucency, and light transmission, hence produces optical phenomena. The mineral content of enamel is mainly affected by acid attacks. Such attack is executed by a low hydrogen ion concentrated environment either produced by bacterial plaque metabolism, exogenous, or endogenous erosive factors, causing either sub-surface or surface demineralization, respectively, that is clinically presented at early stages as white spot lesions.⁵

Typically, demineralized enamel defects are either evaluated directly using surface alterations, (profilometry) or indirectly, by assessing surface softening (microhardness). Although regarded as the gold standard for measuring mineral damage in dental hard tissues, Transverse microradiography (TMR) and Scanning electron microscopy (SEM) are harsh and extremely difficult to perform on the fragile surfaces of erosive lesions. However, Optical coherence tomography (OCT), Inductively coupled plasma atomic emission spectroscopy (ICP AES), Non-contact profilometry (NCP), and Confocal laser scanning microscopy (LSCM) are non-destructive techniques but are non-clinical or with limited sensitivity. Therefore, a practical technique, or a combination of techniques, is required to achieve a non-destructive assessment of both areas.⁶

Until now, non-destructive methods that can perform a clinical, qualitative, and quantitative assessment of mineral loss for enamel demineralization do not exist. This study aimed to evaluate and correlate the mineral content and enamel color change through a statistical correlation model. The null hypothesis tested denied the existence of any correlation between mineral content of the tooth enamel and its color, represented by the Commission Internationale de l’Eclairage (CIE L*a*b*) color system.

MATERIALS AND METHODS

Sample size estimation

Power analysis was performed for sample size estimation, using the color parameters (L, a and b) as a primary outcome. The effect size $f = 0.5796368$ was calculated based upon the results of Vieira-Junior et al. 2018.⁷

If the standard deviation within each group = 1.7, using alpha level of 5% and Beta level of 95%. The minimum estimated sample size was a total of 33

samples (11 samples per group). G*Power version 3.1.9.2 was used for sample size calculation.

Sample preparation

A total of 33 intact bovine incisor teeth were kept at 4°C in 0.01% thymol solution for 30 days until tested. A low-speed, water-cooled diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) was used to prepare enamel blocks (5x5 mm, 2 mm thickness) from the middle third of the labial tooth surface.

Enamel samples were imbedded in resin blocks with the labial surfaces facing upwards. Acid-resistant varnish was painted on the surface of the disc leaving an exposed area of enamel (roughly 3X3 mm) (Fig. 1). The area was measured from a photograph of the sample against a ruler to calibrate the pixel dimensions using Fiji (Image J, Ver.1.52i).⁶

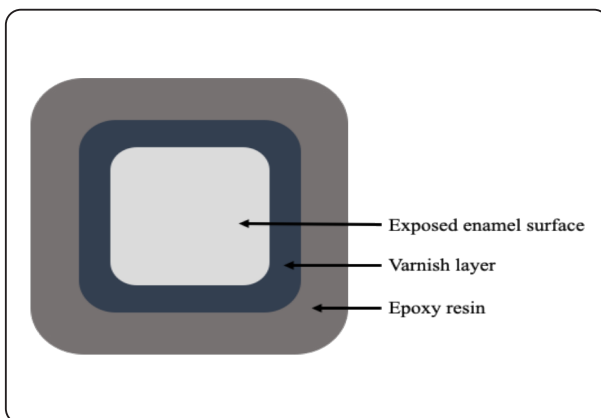


Fig. (1) Schematic presentation of single block sample preparation.

The 33 specimens were divided into three subgroups (n=11/group), where group I (Control group) received no surface treatment and were stored in distilled water at room temperature (22°C) until tested, group II (Demineralized enamel surfaces), group III (Re-mineralized enamel surfaces).

Surface demineralization:

Enamel samples of groups II and III were put separately for 72 hours into a plastic container filled with a 30 ml demineralizing solution (1.5 mM CaCl₂, 0.9 mM KH₂PO₄, 50 mM CH₃COOH, and 3mM NaN₃) at a pH 4.8, to be changed daily.

After demineralization, the samples were taken out of the solution, rinsed with running tap water for 5 min, then re-rinsed with distilled water for 30 s. Finally, these samples were dried with Oil-free air spray. After removal of the nail polish, white spot lesions (WSL) were confirmed visually on the samples, which were stored in distilled water at 4°C until treated.

Surface re-mineralization:

Demineralized enamel samples of group III were re-mineralized, using a disposable micro-applicator, to apply MI Paste Plus to the WSL, covering it. Afterwards, the applied paste was brushed manually under minimum pressure; in a brushing procedure (3 times for 3 minutes). Subsequently, the samples were kept in distilled water at room temperature (22°C) until examined.

SEM Examination and Elemental Analysis (EDXA) Testing:

Enamel surfaces for each group (control, demineralized, and re-mineralized) were characterized using a SEM (Model Quanta 250 FEG Field Emission Gun) attached to an EDX unit (Energy Dispersive X-ray Analysis), with 30 K.V accelerating voltage. Prior to characterization, all teeth were dried.

Color Measurement:

Color measurements of the 33 enamel surfaces were performed for the three groups. For evaluation of color change, an intra-oral spectrophotometer (VITA Easyshade V® Vita Zahnfabrik, Germany) was used, to determine CIE L*a*b* color parameters of differently treated enamel specimens. The data

pattern automatically used the average of the 3 measurement values.

Microhardness testing:

After color change assessment, the same samples were placed on a Vicker's micro-hardness tester (Wilson ® Hardness Tester, Model Tukon 1102, Buehler, Lake Bluff, IL, USA). Vickers hardness numbers (VHN) were recorded by forcing the indenter into the tested specimens under impact-free, smooth application of a 500-gram load (maintained in place for 15 seconds). Controlled physical quality of the indenter, along with the accuracy of applied load are of prime importance for obtaining correct results. Upon load removal, a magnifying eye piece was used to assess the indentation, measuring both impression diagonals with a digimatic screw micrometer (Mitutoyo, Kawasaki, Japan) to the nearest $0.1 \mu\text{m}$ and averaged. The VHN was

calculated ($\text{VHN} = 1854.4 L/d^2$), where load $L =$ load (in gf) and the $d =$ average diagonal (in μm).

STATISTICAL ANALYSIS

Numerical data was collected, tabulated, and examined for normality (Shapiro–Wilk test). The data was proved to be normally distributed and a parametric test, one-way analysis of variance (ANOVA), was used to ascertain the presence of statistically significant differences between the tested groups. The post-hoc test was used to exactly identify the groups differing from each other. Pearson correlation was used to detect the existence and type of correlation between enamel mineral content and tooth shade throughout the study. IBM SPSS statistics for windows, was used for statistical analysis, setting the significance level at $p \leq 0.05$.

RESULTS

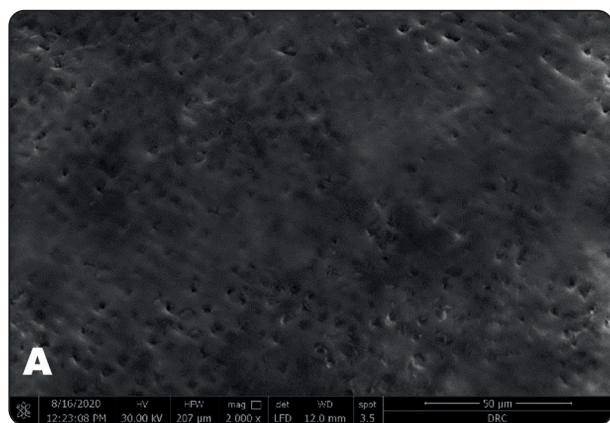


Fig. (2A) Scanning electron microscopic image of sound enamel.

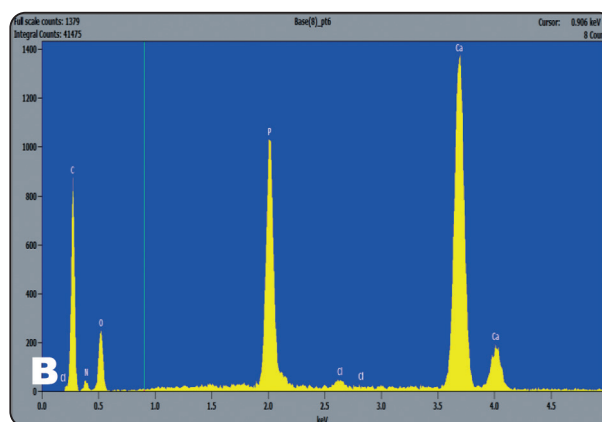


Fig. (2B) Spectrum of elemental analysis of the specimens by energy-dispersive X-ray for sound enamel.

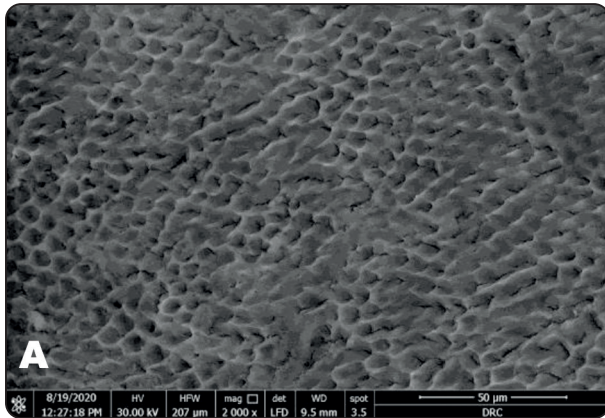


Fig. (3A) Scanning electron microscopic image of demineralized enamel.

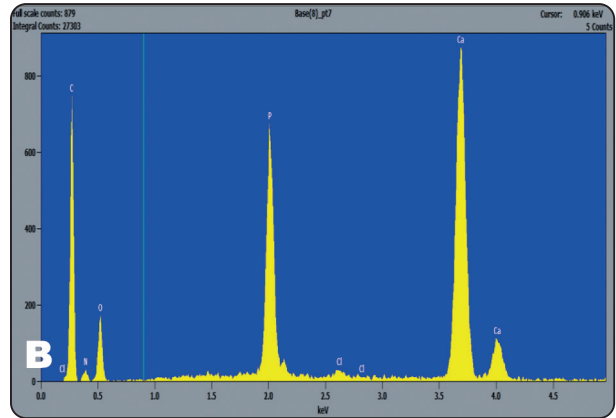


Fig. (3B) Spectrum of elemental analysis of the specimens by energy-dispersive X-ray for demineralized enamel

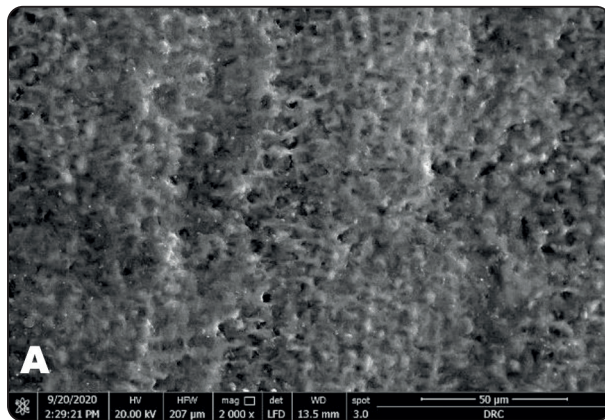


Fig. (4A) Scanning electron microscopic image of remineralized enamel.

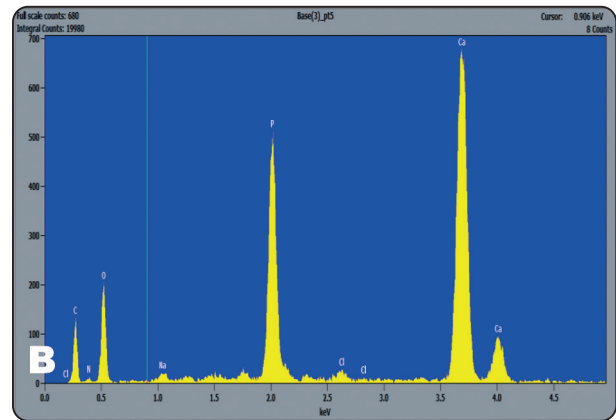


Fig. (4B) Spectrum of elemental analysis of the specimens by energy-dispersive X-ray for remineralized enamel.

TABLE (1) Means, standard deviation, and significance for EDXA analysis of the tested groups.

Groups	C	O	PO ₄	Ca
Sound	12.01±1.425 ^{xxx}	43.09±0.98 ^o	12.54±1.344 [*]	32.2±1.269 ⁱ
Demineralized	36.2±9.094 ^x	37.79±3.92 ^{oo}	6.8±2.514 ^{**}	17.37±3.546 ⁱⁱⁱ
Remineralized	19.06±0.957 ^{xx}	44.66±1.41 ^o	9.72±0.895 ^{**}	26.46±1.079 ⁱⁱ

Different superscript letters show statistically significant differences (p ≤ 0.05).

TABLE (2) Means, standard deviation, and significance in shade parameters (L, a and b) for the tested groups

Groups	L	a	b
Sound	73.19±1.879 ^b	0.53±0.613 ^b	8.72±1.859 ^a
Demineralized	68.51±0.48 ^a	1.78±0.566 ^a	13.69±1.49 ^c
Remineralized	72.61±1.785 ^b	0.59±0.293 ^a	7.15±0.732 ^b

Different superscript letters show statistically significant differences (p ≤ 0.05)

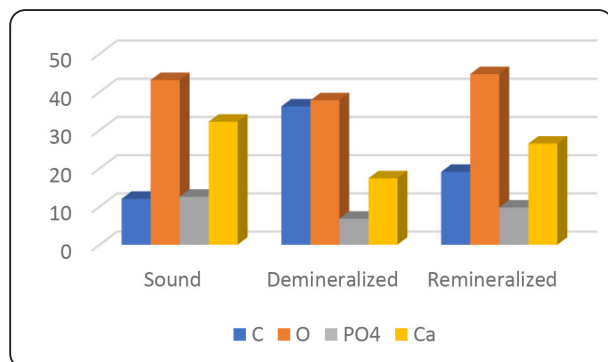


Fig. (5) Mean elements' concentrations of tested enamel surfaces.

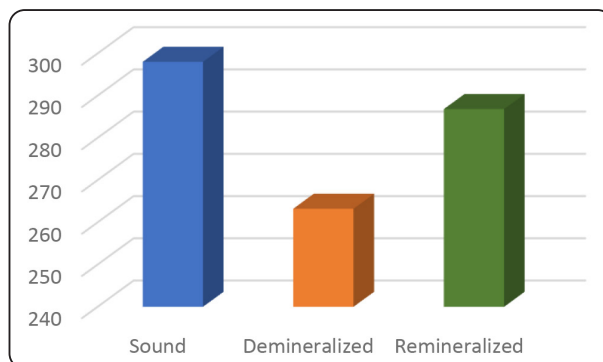


Fig. (6) Mean VHN for tested enamel surfaces.

A statistically significant correlation does not necessarily indicate a strong correlation. Coefficients with an absolute value of <math><0.40</math> are frequently indicative of weak correlations; while a coefficient value of 0.40–0.69, denotes moderate correlations; and finally, a value of ≥ 0.70 , denotes strong correlations. A significantly strong negative correlation was detected for the L value with Carbon ($r = 0.74^{**}$, $p = 0.002$), but significantly positively correlated with Calcium ($r = 0.76^{**}$, $p = 0.00115$). Thus, the increased calcium and decreased carbon enamel contents resulted in an increased L value, while oxygen and phosphates on the other hand showed moderate positive correlation with L.

All values showed a moderate correlation with all the elements tested, such that the correlation was positive for carbon and negative for oxygen, phosphates, and calcium.

The b values revealed a significantly strong negative correlation with oxygen, a relationship that was reversed for carbon.

A strong correlation was confirmed to exist between the surface hardness (VHN) of the specimens and all tested elements except oxygen. The correlation was negative with Carbon, but such correlation was reversed in the case of Calcium and phosphates with the correlation being positive in both. Thus, the increase in the calcium and/or phosphates and decrease carbon contents of the enamel samples will lead to an increase in the VHN values.

Other than this all the tested subgroups showed moderate correlations.

TABLE (3) Means, standard deviations in VHN for the tested groups.

Groups	VHN
Sound	298.02±1.815 ^a
Demineralized	263.23±1.151 ^c
Remineralized	286.83±3.862 ^b

Different superscript letters show statistically significant differences ($p \leq 0.05$).

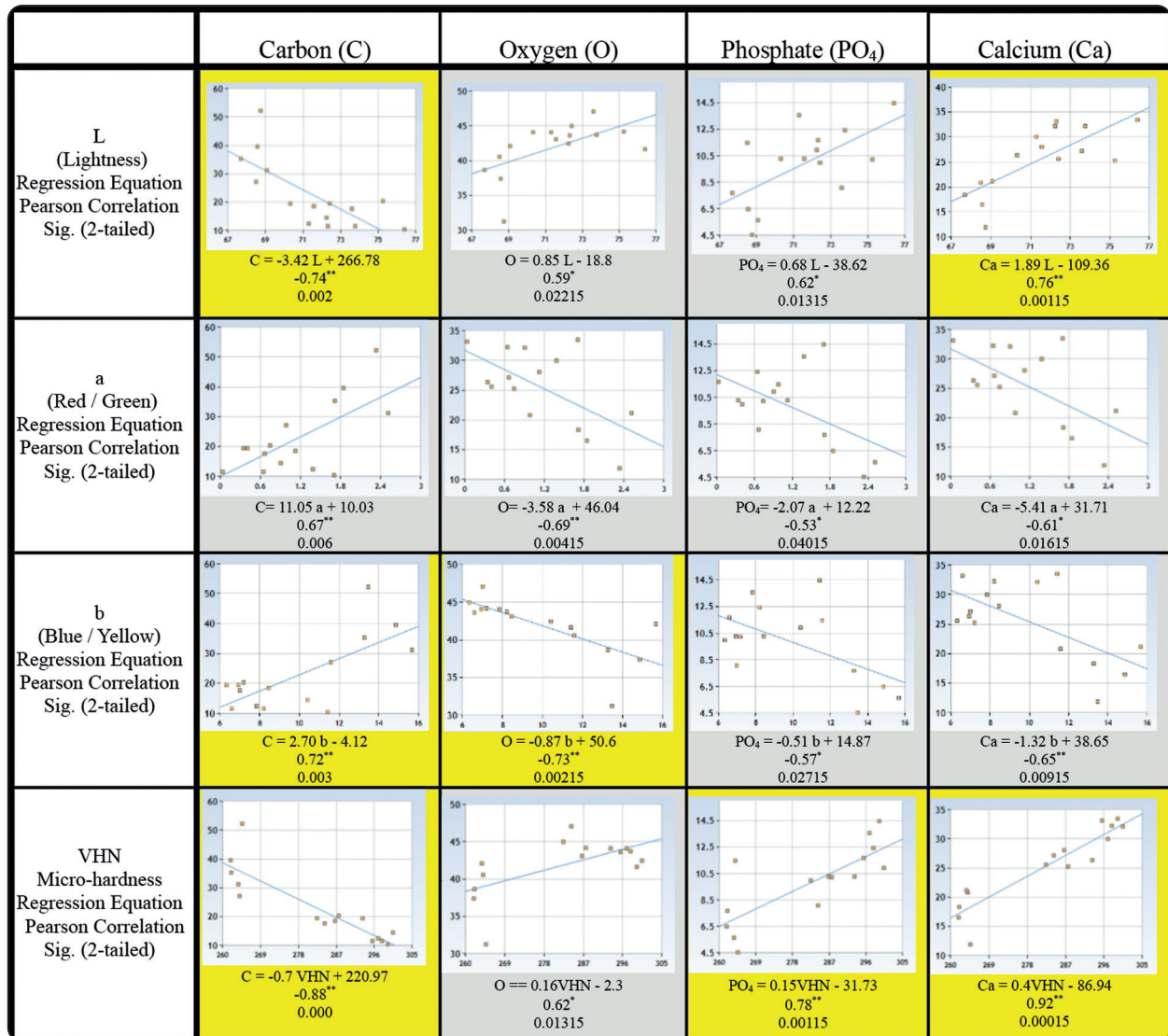


Fig. (7) Correlation [Pearson Correlation Sig. (2-tailed), Different superscript letters show statistically significant differences $p \leq 0.05$.

DISCUSSION

The null hypothesis tested was partially accepted, due to the existing correlation between the alteration of some of the enamel elements and the L*, a* and b* values of the color spectrum.

Whereas the L represents the white-black axis related to the luminosity of the teeth.

Several factors may influence humans' perception of colors, and can be modified by the light source,

the surface properties of inspected objects, as well as the inspector himself looking at the object.⁸

This study focused on the evaluation of enamel samples, represented by changes in their mineral content, determining the color under standardized illumination and measurement geometry.

Due to the difficulty of clinical determination of tooth color, different methods have been used by several authors throughout the literature to scientifically measure the color change.^{9, 10, 11, 12, 13, 14.}

Hue, chroma, and value all together, in addition to color coordinates can describe the modern approach to color determination, which explains its use in esthetic dentistry, upon dealing with non-self-luminous objects. Recent digital shade detecting protocols use CIE L*a*b* color scale for clinical shade selection.¹⁵

Where L represents the black/white scale relating to the luminosity of tooth, a represents the red/green, and b stands for the yellow/ blue scale.^{16, 17}

The current study confirmed a correlation among mineral content, L* values, b* values, and VHN. It is well known that dental enamel participates in expressing the complex phenomena of tooth color by modifying light waves reflected from the underlying dentin. and variation of tooth shade intensifies by any change in enamel shape, structure, composition or even surface properties. That led to the suggestion that hydroxyapatite crystals' size of enamel, composition and orientation play a major role in tooth-shade determination.¹⁸

Previous studies¹⁸ suggested that dentin mainly controls tooth shade; however, the current work demonstrates that changes in the elemental composition of enamel alter its scattering and reflection of light.

Even though the examination of the role of enamel elemental composition on tooth color is clinically significant, according to the results of Pearson's correlation in this study, it may play a role in study models that aim for the evaluation of these variables, thus contributing new insights.

Therefore, clinical diagnostic and follow-up techniques need to be improved and developed to support a safe treatment, specifically regarding the repair of affected enamel. This is because it is crucial to develop a clinical investigatory method that supplies data about the impact of elemental composition of tooth structure on shade change as well as its reaction to demineralization attacks and remineralization protocol.

The null hypothesis was rejected because due to the existence of a correlation between the mineral content of enamel and the color of the teeth, although not all correlations revealed the same power.

CONCLUSIONS

The alteration in the surface elemental content of bovine enamel influenced the lightness and the blue-yellow axis of tooth color, correlating in turn with the L* and b* values.

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