TOOTH ROOT COLOUR AS A MEASURE OF CHRONOLOGICAL AGE

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ABSTRACT

The purpose of this study was to assess a possible colour shift in the root surfaces of adult human teeth and if so, whether this colour change is related to chronological age.

Teeth extracted from persons of known age and gender were obtained from Ontario dental practitioners and grouped into five-year age ranges. Three experiments were undertaken: (1) to identify a possible difference in yellow colouration between the four surfaces of tooth roots (mesial, distal, lingual, and buccal), (2) to investigate the difference in yellow colouration of tooth roots between non-molar teeth and molar teeth and (3) to assess the correlation between the age of teeth and root colour saturation for yellow, magenta, cyan and black. The teeth in all investigations were scanned by a flat-bed digital colour scanner with a Kodak* colour scale control and viewed on a colour computer monitor. In the first two experiments the yellow colour saturation of the root surfaces was measured at six points on each root using Photoshop 5.0** software. A significant difference was observed in the percentage yellow colour saturation between the mesial and the other three anatomical surfaces ($p<0.01$), and between the root surfaces of non-molar and molar teeth ($p<0.01$) (ANOVA with Bonferroni post-test). The authors then randomly assigned tooth surfaces to select an equivalent number of posterior and anterior teeth in the study, assessing the relationship between age and root colouration. Four points of colour measurement on 40 teeth (sample size permitting, see Table 1) for each known age and gender were assessed for colour saturation (cyan, magenta, yellow and black). The correlation of chronological age to colour saturation was linear for all colours, with correlation coefficients ranging from $r = 0.81$ to $r = 0.94$. The high correlation values strongly support the conclusion that chronological age is related to increased root colouration. **(J Forensic Odontostomatol 2000;18:37-45)**

Keywords: forensic odontology, age determination, teeth, cementum, colouration

INTRODUCTION

Police, coroners or pathologists consult forensic odontologists when there is a need to derive forensically significant information from teeth or dental restorations.[1,2] Gustafson[1] introduced the measurement of six dental regressive changes to estimate age 50 years ago. These regressive changes in dental tissue are still commonly used to estimate the age of adults.

Gustafson's[1] method of aging teeth involved cutting them into 250 to 350 m thick slices and ranking the following characteristics from 0-3: attrition, apical migration of periodontal attachment, secondary dentine formation, cementum apposition, root resorption and root-end translucency. The characteristic ranks were added and an estimated age was extrapolated.[1] Drawbacks to Gustafson's method include: a) none of the criteria can be used alone; b) when only one tooth is available the standard deviation increases greatly; c) expert training in dental histology is required; d) root translucency assessment must be made on sections 1 µm thick; e) the standard deviation increases significantly on teeth over 50 years-old; f) anterior teeth should be used whenever possible and g) the police often require rapid results whereas sectioning requires significant time and expense.[3,4]

* Kodak colour block, Eastman Kodak Corporation, Rochester NY, USA

** Adobe Photoshop 5.00, Apple Computer Inc., USA

The age at death for sub-adults can be estimated by referring to charts indicating age of eruption of teeth. There are, however, differences between races and the genders.[5] Environmental factors and disease can also cause individual variations to be too large to allow accurate estimation of age.[6] Using charts depicting age of root crown mineralization is a good method of estimating age because development is comparatively unaffected by nutritional, endocrine and other factors which impact on the rate of dental maturity.[7]

Since Gustafson[1] introduced his method for age assessment, several similar methods have been performed.[8-15] Of the six criteria introduced by Gustafson[1], the variable with the strongest correlation to age is apical translucent dentine.[13-15] The canine teeth provide the most reliable estimate using this variable.[15]

More recently, the racemization rate of D and L enantiomers of amino acids in enamel, dentin and cementum has been explored.[16-18] The racemization reaction proceeds at a constant rate in dentin and cementum, confirming that cementum like dentin has a low metabolism and is stable throughout an individual's life.[16-18] Therefore, age estimation is possible using the racemization reaction in teeth.[16-18]

Ten Cate *et al.*[3] showed that teeth could be aged by comparing the root colour to a known standard. Their experiments further showed that untrained individuals trying to estimate age of teeth by visual comparison were not consistent but results from trained personnel were more accurate. They also measured optical density of colour using photographs and transmission densitometry. Yellow was measured using a Wratten blue 47 filter and a regression line relating age in terms of optical density was constructed, with the results being better than visual matching. However, a densitometer can only measure a small root surface at any one time, and the processing time is lengthy. Using reflective spectrophotometry, Solheim[19] reported that crown and root dentin colour was related to age. The procedures outlined by Ten Cate *et al.*[3] and Solheim[19] have advantages over Gustafson's method in that any tooth may be used, no sectioning is required, and it is relatively easy to do. Since the studies of Ten Cate *et al.*[3] and Solheim[19] colour measurement has become simplified using colour scanning and computer assisted measurement.

The purpose of this study was to assess a colour change in the root surfaces of adult human teeth and if so, whether this is related to chronological age. We hypothesized that there may be a difference in the colour between the anterior and posterior teeth as well as between the four anatomical surfaces of the roots. In addition, we hypothesize that there is a linear relationship between tooth root cyan, magenta, yellow and black colouration and chronological age.

MATERIALS AND METHODS

A sample of extracted teeth of known age and gender was obtained from Ontario dental practitioners, cleaned with pumice and stored in corked glass jars in cupboards. The teeth were pooled by gender into five-year age groups: 15-19, 20-24, 25-29, and so on until 80-84[3] and three experiments were performed.

Experiment 1: Comparison of yellow saturation of the four anatomical surfaces of the tooth roots

From each of the 20-24 and 70-74 female age groups 21 teeth were scanned by AGFA Arcus II Scanner# using Fotolook## software and then viewed on their mesial, distal, buccal and lingual sides (4 separate scans) by Adobe Photoshop 5.0.** Each scanned image of a particular tooth's root surface was measured at six points for percent yellow saturation – two points from the cervical third, two points from the middle third and two points from the apical third of the root. Areas of environmental staining were avoided. A Kodak colour block* served as a control for variations in the percent yellowness from image to image. The data from each side were entered into Instat 2.0† and a comparison of the percentage yellow between the four sides was tested by ANOVA. A Bonferroni post-test determined the percentage yellowness variation from surface to surface of a tooth's root.

Experiment 2: Comparison of yellow colour saturation of anterior vs. posterior tooth roots

Agfa-Gevaert AG, Mortsel, Belgium
Fotolook 2.0, Microtek Corporation, California, USA
† Instat 2.0, Graphpad Software, Inc., San Diego, CA, USA

Table 1: *Columns depicting how many teeth were used for each age group and both genders in Experiment 1*

AGE GROUP	Number of specimen teeth	
	Female	Male
15-19	38	22
20-24	40	40
25-29	40	40
30-34	40	40
35-39	40	36
40-44	40	40
45-49	40	40
50-54	40	33
55-59	40	40
60-64	40	33
65-69	40	26
70-74	26	10
75-79	6	8
80-84	11	10
85-89	10	

Table 2: *Summary of experiment 1 data compares the difference in tooth root yellowness between the four anatomical root surfaces of the 20-24 aged female group. Values connected by underlines are not significantly different. Values not connected by underlines are significantly different at least 0.01 according to Bonferroni post hoc test for multiple comparisons.*

Yellow value	52.82±3.8	56.32±5.72	56.56±5.49	56.57±5.86
Surface	Mesial	Distal	Lingual	Buccal

Table 3: *Summary of experiment 1's comparison of the difference in tooth root yellowness between the four anatomical root surfaces of the 70-74 aged female group. Values connected by underlines are not significantly different. Values separated by underlines are significantly different at least 0.01 according to Bonferroni post hoc test for multiple comparisons.*

Yellow value	67.81±4.63	69.18±5.31	69.63±4.40	69.90±5.55
Surface	Mesial	Distal	Lingual	Buccal

The female 20-24 years data from Experiment 1 were used, but data from both non-molar and molar (10 non-molars, 11 molars) were grouped by tooth surface only (mesial, distal, buccal, and lingual). Thus, the mean female 20-24 mesial anterior tooth yellowness was compared to the mean female 20-24 mesial posterior tooth yellowness and similarly for the other three sides. The data were entered into Instat 2.0† and the means compared by ANOVA and a Bonferroni post-test determined the percentage yellowness of root variation between anterior and posterior teeth.

†† Cricket graph 1.3.2, Computer Associates International, Islandia, NY, USA

Table 4: *Summary of Experiment 2 data compares the difference in tooth root yellowness between the four anatomical root surfaces between non-molars and molars of 20-24 aged females. Values connected by underlines are not significantly different. Values not underlined are significantly different at least 0.01 according to Bonferroni post hoc test for multiple comparisons.*

Yellow value	54.37±3.97	53.33±3.60
Surface	Mesial Anterior	Mesial Posterior
Yellow value	55.53±6.52	57.04±4.85
Surface	Distal Anterior	Distal Posterior
Yellow value	58.72±5.1	54.61±5.06
Surface	Lingual Anterior	Lingual Posterior
Yellow value	58.90±5.98	54.45±4.89
Surface	Buccal Anterior	Buccal Posterior

Experiment 3: Measuring colour change of tooth roots with age

Forty teeth (20 non-molars and 20 molars) [sample size permitting, see Table 1] from each age group and gender were placed randomly on the scanner, the images were scanned by Fotolook 2.0## and viewed by Adobe Photoshop 5.0**. Four points on each tooth's root were selected at random (two above the midline of the root and two below). Once again environmentally stained areas were avoided and Photoshop 5.0** was used to measure the percentage cyan (c), magenta (m), yellow (y), and black (k) at each of the four points. Instat 2.0† performed an analysis of the mean values and confidence intervals for each age and gender group. The mean and standard deviation with a 95% confidence level for each of the colour values, age groups and genders were determined and plotted. The mean

and 95% confidence levels of the various colour values and age groups were then graphically displayed using Cricket graph 1.3.2[††] for display.

RESULTS

Experiment 1

The one way analysis of variance with Bonferroni post-test for multiple comparisons in experiment 1 yielded unexpected results. The anatomic surfaces of the teeth for the female 20-24 year group revealed a significant difference between the percentage yellow of the mesial surface of tooth roots when compared to the other three root surfaces (Table 2). The p value was < 0.01 between the mesial and distal surfaces and < 0.001 between the mesial and both the buccal and lingual surfaces, indicating that the mesial surface of the roots were less yellow than the other three surfaces.

The female 70-74 year data (Table 3) indicated a significant difference in colour value between the mesial surface and the buccal and lingual, but failed to show a significant difference between the percentage yellow of the mesial and distal surfaces. This finding is unlike the female 20-24 year category which indicated a significant difference between the mesial and the other three surfaces. However, the measured t value ($2.18<2.65$) for the comparison between the percentage yellow of the mesial versus distal root surfaces indicated a non-significant, linear trend.

Experiment 2

The second experiment's results indicated that there was a significant difference in the percentage of yellowness between two of the four root surfaces (Table 4). The one way ANOVA with a Bonferroni post-test multiple comparison gave a $p<0.001$ for the comparison between lingual and buccal surfaces on anterior versus posterior root surfaces. The difference between the anterior and posterior root surface yellowness is therefore not due to chance. Comparing mesial and distal anterior with posterior surfaces, however, gave $p>0.05$ resulting in a failure to show a statistically significant difference between these surfaces.

Experiment 3

Figures 1 to 8 depict the relationship between the percentage cyan, magenta, yellow, black and the age of the genders. All of these figures indicate a positive increase in the percentage of the measured colour with age. A correlation value of 0.93 between the percentage cyan and age for males was calculated (Fig. 1) while values of 0.93, 0.90 and 0.94 were determined for the correlation between age and the percentage magenta, yellow, and black in males. The slope of the male data graphs ranged from 1.16 to 1.96% colour/5 years, the smallest slope being 1.16% magenta/5 years, while yellow and cyan produced slopes of 1.20 and 1.23 respectively. Figure 4 depicts the change in percentage black with age for males, which yielded the steepest slope of 1.96% black/5 years.

The linear model fitted the data well. In all the male graphs (Figures 1-4) the straight line model intersected more than half of the values within their 95% confidence intervals.

Figures 5-8 show the correlation between female age and percentage cyan, magenta, yellow and black respectively. The correlation values for the female data ranged from 0.81 to 0.93, the lowest and highest correlation values being attributed to magenta and cyan in figures 6 and 5 respectively. This was unlike the male data which indicated that the lowest and highest correlation values were from yellow and black respectively. The female slopes ranged from 0.83 to 1.30 and in agreement with male data, the females' magenta graph in Fig. 6 gave the lowest slope of 1.30, while the black gave the greatest slope of 1.30. The slopes for the cyan and yellow were 0.99 and 1.18, which were different from the male data.

Another difference between female and male graphs in Figs. 1 to 8 is that all the female graphs have a higher y-intercept values of 25.43, 23.65, 64.81 and 5.22 for percentage cyan, magenta, yellow and black respectively whereas the male y-intercepts for the graphs of cyan, magenta, yellow and black versus age were 24.26, 21.41, 54.77 and 1.63 respectively. For females the straight line model fitted the data well but there were less cases when compared to the male graphs of the line intersecting a majority of the measured values within their 95% confidence interval.

DISCUSSION

The results of experiment 1 indicate that there is an uneven colouration of the four anatomic root surfaces. The percentage yellow was similar on three of the four surfaces, and considerably less on the mesial surface of the female 20-24 year teeth. This may indicate that less cementum is deposited on the mesial surfaces which coincides with the phenomenon known as "mesial drift", defined by Jablonski[20] as the gradual movement of the teeth mesially as a consequence of natural interproximal tooth wear and which could result in cementum deposition on the other 3 surfaces. Similarly, the 70-74 year group data indicate a significant difference among three of the four surfaces. Our study was not specifically concerned with the cause of the difference in percentage yellow, but this difference will be the subject of further research.

For the purpose of the current analysis of root colouration as an indicator of chronological age, the results of experiment 1 encouraged us to expand our sample size to 40 teeth. In addition, randomly selected tooth surfaces were scanned due to the observed varying colouration between mesial and other root surfaces.

A significant difference in the degree of yellowness between buccal and lingual non-molar versus molar teeth was observed. However, no significant difference was observed among the other two surfaces and these results coincide with the observations in experiment 1 where tooth roots have an uneven distribution of cementum, among non-molar and molar teeth on certain anatomical surfaces. The cause of this variation requires further research. This data indicated that a similar number of non-molars and molars would be necessary in experiment 3 to give a meaningful colour measurement.

The data in experiment 3 confirm conclusively that there is a relationship between tooth root colour and age, in which all four colour measurements correlated well. This change in colour with age is due to continued cementum deposition through life[3,8] or perhaps due to some undefined intrinsic change in the dentine.[21] The authors have not attempted to determine this. With the lowest correlation value of 0.806 and the majority of the values above 0.9, these data clearly indicate an important and indisputable relationship between root colouration and age and from a forensic dental viewpoint this correlation could prove to be quite useful when the age of found remains needs to be estimated.

Variation between the genders was present, yet small. In all of the graphs the females had higher y-intercept values which may coincide with the observed sexual dimorphism between male and female tooth eruption times. Since female teeth, with the exception of third molars erupt earlier than in males, these higher values may be due to slightly longer cementum deposition time or development of coloured substances in the dentine.[21] This argument contrasts with Solheim's[22] finding that there is less cementum present on female teeth and further, that less cementum on female teeth may be due to their smaller tooth size or the weaker masticatory force applied. Another difference between the male and female graphs of colouration with age is that all the male graphs had greater slopes. Could it be possible that there is dimorphism in the rate of deposition of cementum or other coloured products? Once again, the biological mechanism behind the increased colouration with age needs to be explored further.

The advantage of this technique over other methods is that it does not require long and expensive laboratory time and can be performed with a minimal knowledge of dental anatomy and computer skills. The tooth is not destroyed and it is inexpensive to perform. The disadvantage and limitations of this technique however are that teeth need to be extracted, the standard regression equation may not apply to all populations, racial groups and conditions of the remains, whether a body has been burned or buried, and for how long, all may have an effect on tooth root colouration. It may however be possible to make new standard correlation equations for each of these conditions using the same technique.

Another problem with this study is that the correlation values only apply to this sample of teeth which originate from a limited geographical site and probably contain a limited number of ethnically diverse subjects. It however creates a further data base to help cover the multiplicity of geographical regions and populations that exist.

Although the reason for each tooth's extraction was not known, one could argue that these factors might alter its root's colouration but despite this the linear trend remained true in all cases for all colours.

To establish the usefulness of this technique, further testing is required. One method would be to use these data to conduct blind tests of single or groups of teeth of known age and compare the results. In addition, field-testing could be undertaken in actual forensic cases to determine this technique's potential in age determination compared with other currently used methods.

A significant difference was observed between the colouration of the four root surfaces, as well as a significant difference between non-molars and molar tooth root colouration. The regression lines for male and female teeth from 15-89 and 15-84 years respectively indicated an excellent correlation between the measured colour and age. This technique may be valuable to forensic dentists faced with the task of estimating age.

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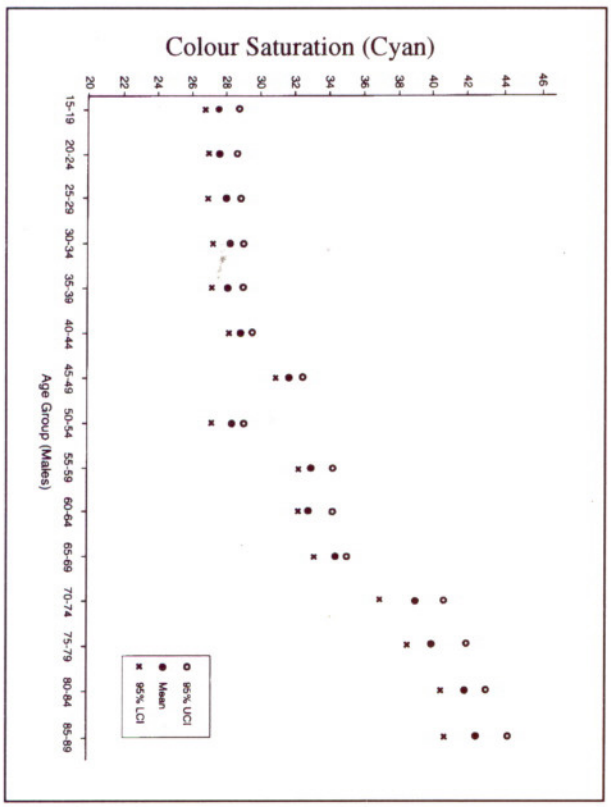

Fig.1: *The percentage of in tooth root cyan colouration as measured in teeth of male subjects displayed by age group r=0.93*

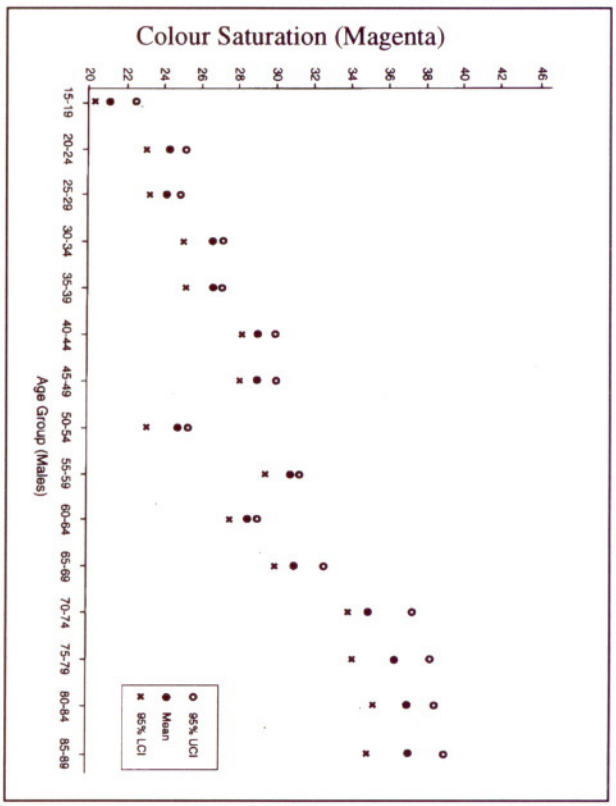

Fig.2: *The percentage of in tooth root magenta colouration as measured in teeth of male subjects displayed by age group r=0.93*

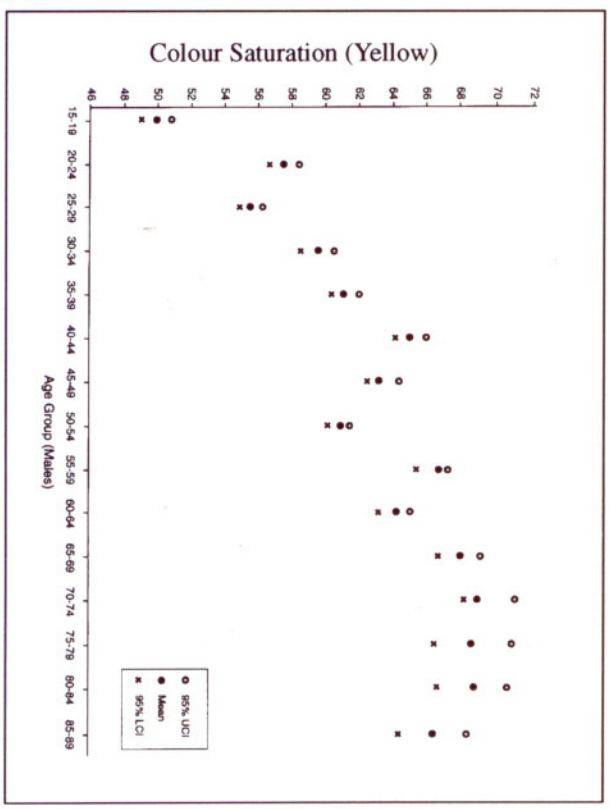

Fig.3: *The percentage of in tooth root yellow colouration as measured in teeth of male subjects displayed by age group r=0.90*

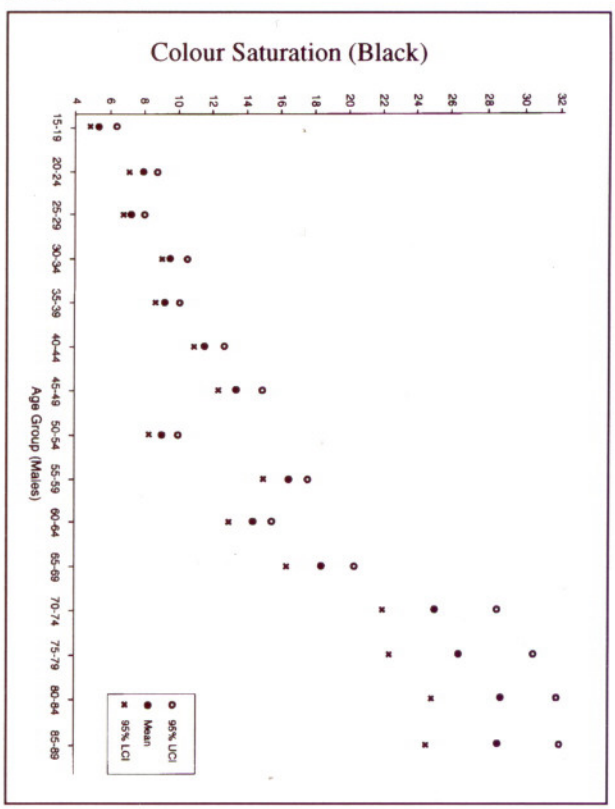

Fig.4: *The percentage of in tooth root black colouration as measured in teeth of male subjects displayed by age group r=0.94*

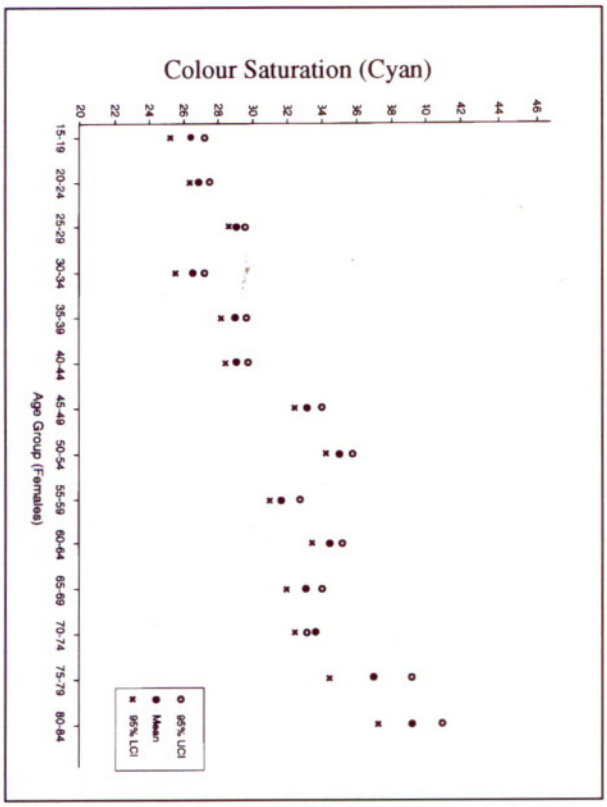

Fig.5: *The percentage of in tooth root cyan colouration as measured in teeth of female subjects displayed by age group r=0.93*

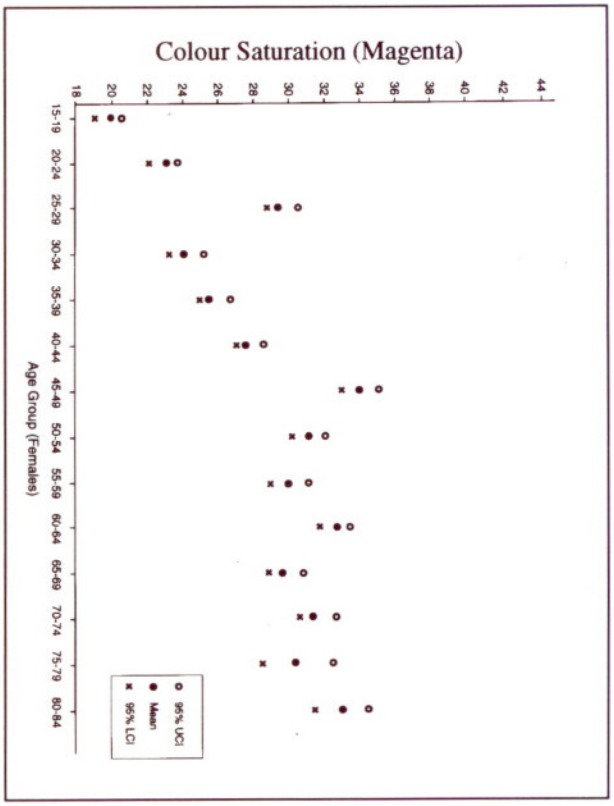

Fig.6: *The percentage of in tooth root cyan colouration as measured in teeth of female subjects displayed by age group r=0.81*

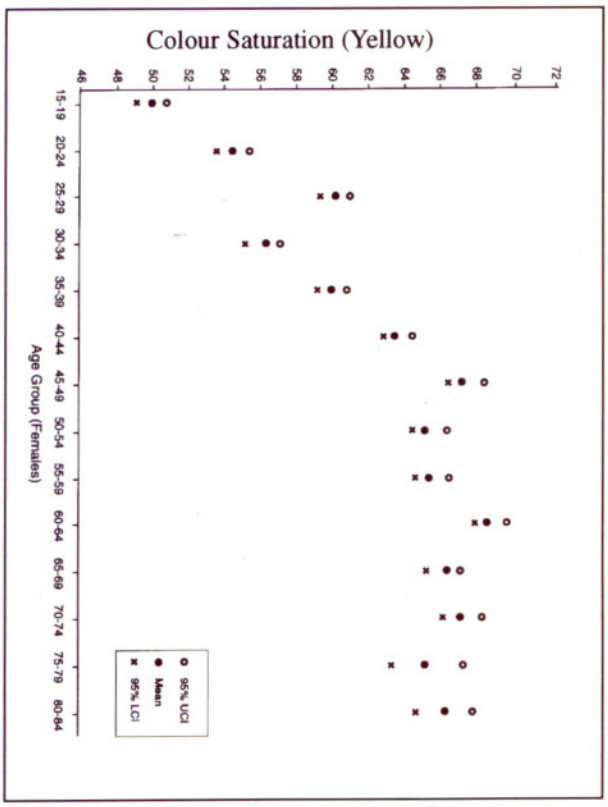

Fig.7: *The percentage of in tooth root yellow colouration as measured in teeth of female subjects displayed by age group r=0.86*

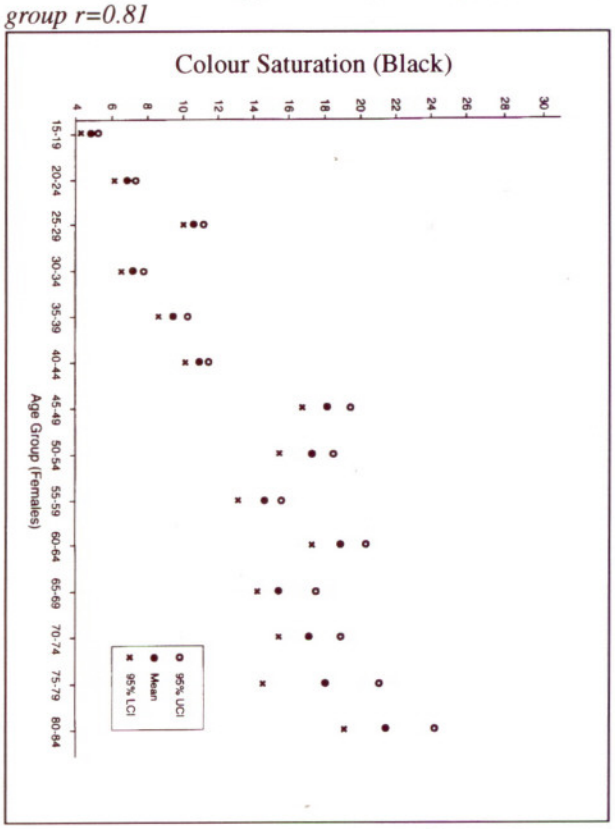

Fig.8: *The percentage of in tooth root black colouration as measured in teeth of female subjects displayed by age group r=0.92*