Changes in Color and Roughness with Laser Bleaching using different Peroxide Concentrations

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Abstract
Purpose: The use of different H2O2 concentrations for in-office bleaching has been questioned. Thus, the aim of this study was to evaluate the efficiency of laser bleaching on color changes and surface roughness of bovine enamel with different peroxide concentrations.
Materials and methods: Forty eight bovine incisors cleaned and labial surface polished up to #1200. Half of them were artificially stained with black tea and divided into three subgroups and bleached according to the concentration of H2O2 (15%, 25% and 35%) (n=8). Specimens were bleached with laser hybrid system (DMC Whitening Lase II, Sao Paulo, Brazil). CIE Lab color system was used to evaluate color using Vita Easyshade Spectrophotometer (Ivoclar Vivadent, Liechtenstien). The remaining specimens were used for surface roughness evaluation following bleaching. The enamel roughness (Ra) values were measured by Stylus Profilometer (Suf-Corder SE 1200, Tokyo, Japan) and Atomic force microscopy (AA3000,Bosten,USA) before and after bleaching. Data were analyzed with Paired sample T-test to evaluate color changes and Ra values at each concentration. ANOVA and Tukey test were used to evaluate the difference between the groups with different bleaching agent concentrations. All tests were computed at 5% significance level.
Results: Significant increases in degree of lightness (L*) values observed following bleaching at all concentrations. Different concentrations of peroxide produced significantly different Ra values. Bleaching with 35% agent produced significantly higher L* value than 15% but similar to 25%. Exposing enamel to high peroxide concentration (25% and 35%) significantly increased Ra value in comparison to unbleached enamel.
Conclusion: Color changes and surface roughness of bovine enamel were influenced by hydrogen peroxide concentrations of DMC laser bleaching system.

Key words: Tooth bleaching, Hydrogen peroxide, DMC Laser, Bovine enamel, Color changes, Surface roughness.

Introduction
Of paramount importance for human beings in relation to their social, psychological and professional needs is minimizing discoloration particularly of anterior teeth (Wetter,2004). Different options are available to treat the discolored teeth. They include bleaching, direct or indirect composite veneer and full crowns. With keeping over all philosophy of tooth restorations, conservation should be given first to the bleaching of the teeth. Bleaching has gained popularity with many techniques by utilizing different peroxide concentrations and activation sources (Watts,2001).
Donald's medical dictionary defines bleaching as "the act or process of removing stains or color by chemical means" (Haywood,1989). A number of methods have been described for the bleaching of vital teeth (Joiner, 2006). However, basically, there are three techniques: in-office, over the counter bleaching, and home bleaching (Heymann, 2005). Since its introduction by Haywood and Heymann 1989, several products have been employed for bleaching. These products are mainly available in gels containing several concentrations of hydrogen peroxide (Ziebolz et al. 2007). Although different concentrations of hydrogen peroxides may show similar teeth whiting results (Götz et al., 2007), higher concentrations of H2O2 and increased application times may cause enamel surface alterations, such as loss of mineral content and increase surface roughness (Al-Salehi et al. 2007).
One of the newest advancement in the field of in-office bleaching techniques is laser application and there are many types of lasers. The most recent laser activating system is Whitening Lase II DMC hybrid system which is a combination of infrared laser with LED in one light so is provided best result with less time and sensitivity. Whitening Lase II provided is with three different bleaching agents, based on the concentration of hydrogen peroxide ,(Lase PeroxideLite-15%, Lase Peroxide Sensy II - 25% & Lase Peroxide Sensy II - 35%). For effective stain removal, hydrogen peroxide must be able to move through tooth structure. This is possible since hydrogen peroxide has a low molecular weight that permits proteins denaturing, and will consequently increase tissue permeability and allow the movement of ions through the tooth (McEvoy,1989). The penetration of hydrogen peroxide and the possible effect of the bleaching on the enamel structure must be considered by restorative dentists because in instances where bleaching of the enamel and/or dentin has been unsuccessful or not accepted clinically, masking of the discolored crown with acid etch veneering technique has been advocated (Tavares et al. 2009).
Until recently, little attention has been given to the influence of the increase of hydrogen peroxide concentration on the enamel surface textures and color changes within the same experimental unit. Improvement of discolored teeth may be affected by various concentrations of peroxide. In addition, alteration in enamel surface roughness may follow bleaching process as well.

Hypothesis of the study:
There would be no change in enamel color and surface roughness after laser bleaching with different peroxide concentrations.

Objectives:
Evaluate bleaching efficacy of laser activating system utilizing three different concentration bleaching agents, (15%,25%&35%) on color changes and surface roughness of bovine enamel.

Materials and Methods:

The materials used for preparing the specimens and the equipment used for measuring the color changes and surface roughness are shown in Table (1).

Specimens collection and preparation:

Forty eight bovine cattle incisors were stored frozen after extraction until their use. Specimens were cleaned and polished with non-fluoridated polishing paste. Roots were cut using a diamond disc with straight-type micro motor handpiece (NSK, Tokyo,Japan). Pulpal tissue was removed by a reamer. Pulp chambers were irrigated with 5 mL of 5% sodium hypochlorite to remove any tissue remnants, then washed and dried. Labial surfaces were polished to create a smooth and flat enamel surface with ascending-grit water proof silicon carbide papers starting from #400 up to #1,200 under running water (Fig.1). The specimens were randomly assigned into two groups, two groups, half is for detecting color changes and the other half is for surface roughness.

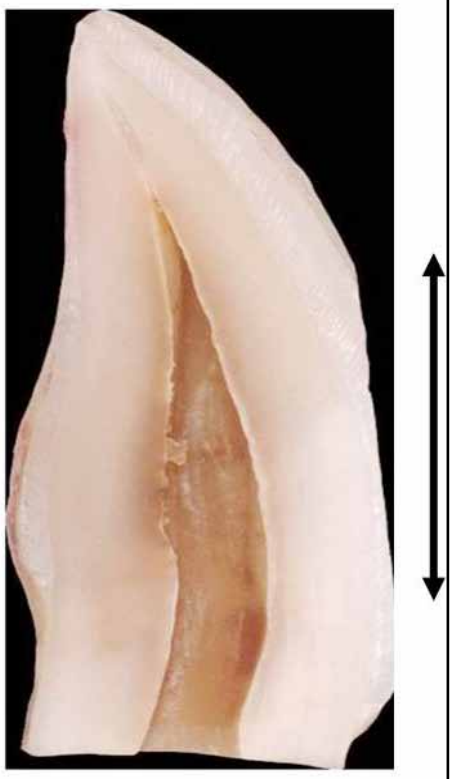

Fig 1 Crosscut surface of the prepared specimen.

Table.1. The materials used for preparing the specimens and the equipment used for measuring the color changes and surface roughness.

	Materials and Equipment	Company
1	Turbine Handpiece	W&H, Austria
2	Straight-type micro motor Handpiece	NSK, Japan
3	diamond disc	Mani, Germany
4	Handpiece bur	Mani, Germany
5	Waterproof silicon carbide paper	Mitchell Elliott, Korea
6	Acid Etch	Promedica, Germany
7	Tea	Lipton, India
8	Whitening Lase II system	DMC, Brazil
9	Lase Peroxide Lite - 15%	DMC, Brazil
10	Lase Peroxide Sensy II - 25%	DMC, Brazil
11	Lase Peroxide Sensy - 35%	DMC, Brazil
12	VITA Easyshade Spectrophotometer	Ivoclar Vivadend, Germany
13	Digital camera	Sony, Japan
14	Incubator	Jerad, Syria
15	Stylus Profilometer	Surf-Corder, Japan
16	AFM / SPM	Angstrom Advanced Inc, USA
17	Dental Cold Cure Acrylic	Ivoclar, Vivadent, Germany

Color Changes

Staining procedure:

Specimens artificially stained by immersion of two grams of black tea (Lipton, India)was immersed in 100 mL of boiled water for five minutes (Sulieman et al.,2003; Ayaka et al., 2011). Specimens were immersed in the solution and stored inside the incubator for 7 days at 37°C. Specimens before and after staining are shown in (Fig.2). Labial surfaces of stained specimen were covered with a masking tape with a 5 mm-diameter hole to fit the probe of Spectrophotometer (Fig.3).

The CIE L*a*b* color system was applied for the evaluation of color changes by using VITA Easyshade Spectrophotometer. The CIE L*a*b* values of enamel surfaces were recorded prior to bleaching and considered as a baseline data. Each specimen was measured three times, then the average value was considered for calculation. In order to decrease the variation between the specimens, only the specimens which showed an L* value between 45 and 65 were included in the research.

Fig 2 Specimen before and after staining.

Fig 3 Specimen with masking tape with a 5 mm-diameter hole.

Bleaching Procedure:
Specimens were divided into three subgroups according to concentrations (15%, 25% & 35%) (n=8). The bleaching procedure was performed using Whitening Lase II, DMC (810nm wavelength diode laser mix with LED) according to manufacture. Figure (4) shows the specimens bleached with different peroxide concentrations in comparison with stained specimen. Color values of L*a*b* were measured at both baseline and after bleaching. The difference in L*a*b* value between the baseline and after bleaching were expressed as ΔL, Δa, and Δb respectively. The color difference (ΔE) was calculated according to the following equation:

$$\Delta E=[(\Delta L)2+(\Delta a)2+(\Delta b)2]1/2$$

Surface Roughness
Surface Roughness Measurement:
Specimens following polishing procedure and after bleaching were used for evaluation of Ra. Such evaluation was conducted by two methods, one considered as contact methods via stylus profilometer (Surf-Corder SE 1200, Tokyo, Japan) and other as non-contact method using AFM(AA3000, Angstrom Advanced Inc.USA).The differences in average (Ra) value between polished and bleached value for each specimen were recorded and analyzed.

Statistical analysis
Mean for all groups before and after bleaching were recorded and the data was analyzed Table (2&3). Paired Sample T-test was used to evaluate color changes and surface roughness between stained and bleached surfaces at each concentration. T-test used to compare the difference between polished and bleached surfaces at each concentration. One way (ANOVA) was used to determine any significant difference in the mean of (L*) value & (Ra) value among the various treatment groups followed by Tukey HSD as post hoc comparisons test within various treatment groups.

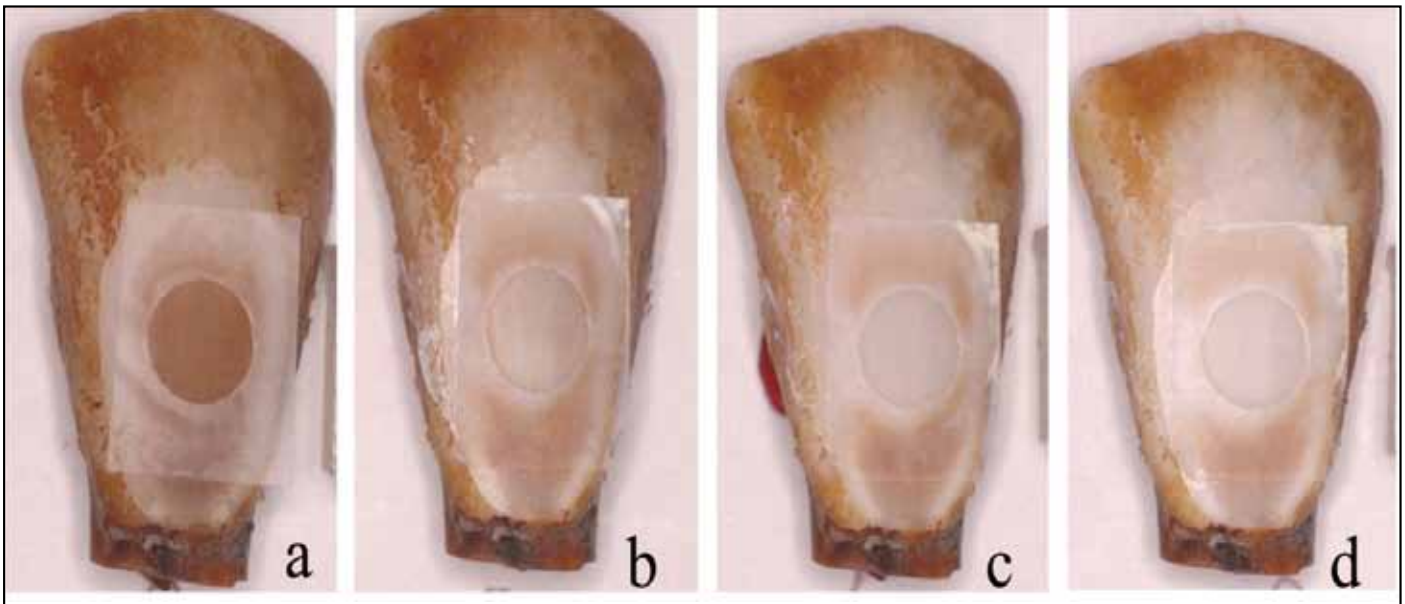

Fig 4 Color changes following bleaching with three different concentrations of H2O2. (a) Before bleaching of the experimental surface. (b) After bleaching with peroxide 15%. (c) After bleaching peroxide 25%. (d) After bleaching peroxide 35%.

Table.2. Mean (L* value) and Standard Deviation of stained and bleached Groups

H2O2%	Mean (L*) value		Std. Deviation		Std. Error Mean	
	Stained	Bleached	Stained	Bleached	Stained	Bleached
15%	52.57	65.3	4.87	5.20	1.72	1.83
25%	53.77	71.7	6.26	6.27	2.21	2.21
35%	52.07	73.3	5.67	4.97	2.00	1.76

Table.3. Mean (Ra) value and Standard Deviation of polished and bleached Groups at different concentrations

H2O2%	Mean (R_a) Nanometer		Std. Deviation		Std. Error Mean	
	Polished	Bleached	Polished	Bleached	Polished	Bleached
15%	137.5	142.5	10.4	8.9	3.7	3.2
25%	138.5	177.5	8.4	7.1	2.0	2.5
35%	137.5	210	7.1	14.2	2.5	5.0

Results

Color Changes

The result of T-test revealed that there were significant differences between stained and bleached specimens at each concentration, this differences in L* value were illustrated in fig (5). One way analysis of variance for (L*) value between various concentrations is given in table (4). The results revealed that there was a significant difference in the mean of L* value between the various treatment groups. Tukey multiple comparisons demonstrate a significant increase in L* values with an increase in peroxide concentrations. Bleaching with 35% agent produced significantly higher L value than 15% .While no difference was observed between 15% and 25% and between 25% and 35%. The results of Tukey test were explained in fig (6).

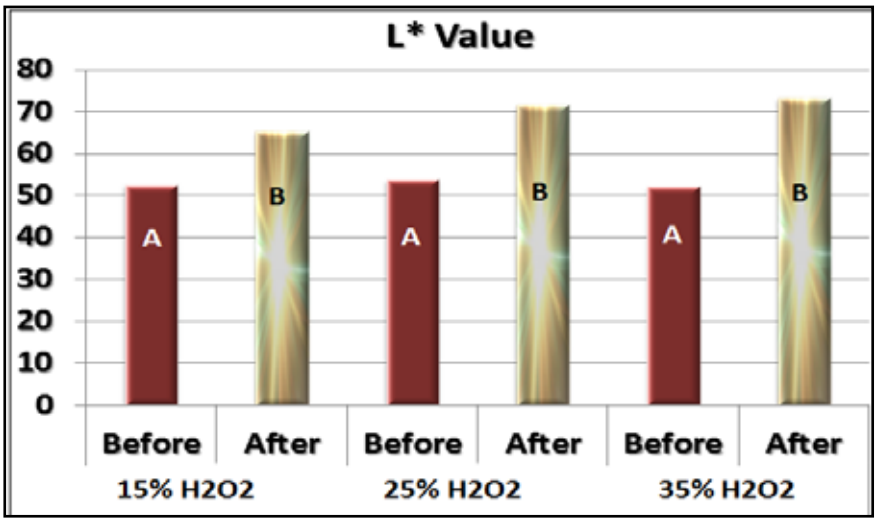

Fig 5: L* value and differences between stained and bleached specimens (different letters indicate significant difference)

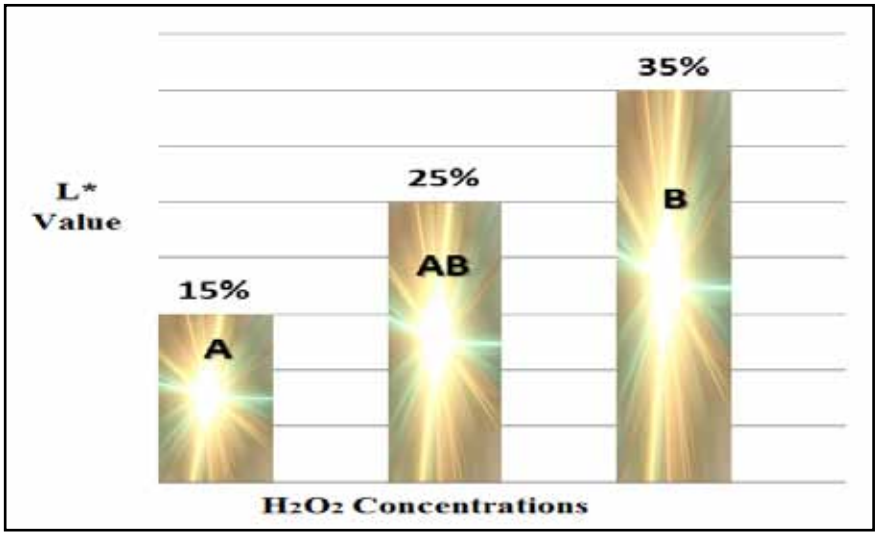

Fig 6. L* value after bleaching with different concentrations (AB) letter indicate no significant difference.

Table.4. One Way Analysis of Variance for L* value among various H2O2 concentrations

L* value	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	282.258	2	141.129	4.641	.021
Within Groups	638.559	21	30.408		
Total	920.816	23			

surface Roughness

Paired sample T-test was used to compare the difference in surface roughness between polished and bleached surfaces at each concentration. The results revealed that there were no significant differences in (Ra) value between polished and bleached groups at peroxide 15%. A significant difference in (Ra) values was observed between polished and bleached groups at peroxide 25% & 35% fig (7). One way analysis of variance for surface roughness between various concentrations is given in table (5). The results revealed that there were a significant difference in the mean of Ra value between the various treatment groups. The Tukey multiple comparisons demonstrate a significant increase in Ra values with an increase in bleaching agent concentrations. Bleaching with 35% agent produced significantly higher Ra value than 15%.While no difference between 15% and 25% and between 25% and 35% fig (8) .For AFM evaluation of surface roughness, the result shows an increase in both numbers and depth of vale of the enamel subjected to different peroxide concentrations as show in Figures (9).

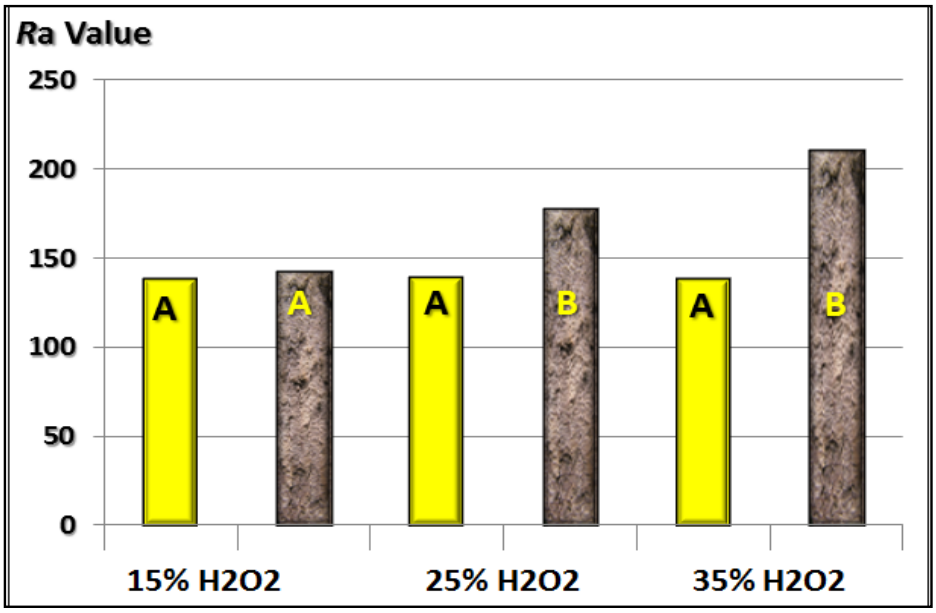

Fig.7: Ra value of polished and bleached groups at each concentration (different letters indicate significant difference)

Table.5. Analysis of Variance for the three Groups Ra value

R_a value	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	18233.333	2	9116.667	83.239	.000
Within Groups	2300.000	21	109.524		
Total	20533.333	23			

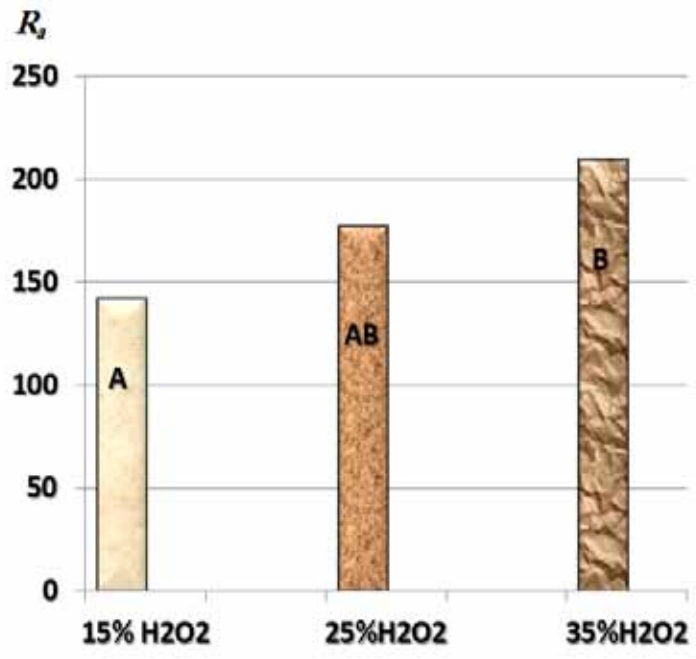

Fig 8. Ra value after bleaching with different concentrations (AB) letter indicate no significant difference

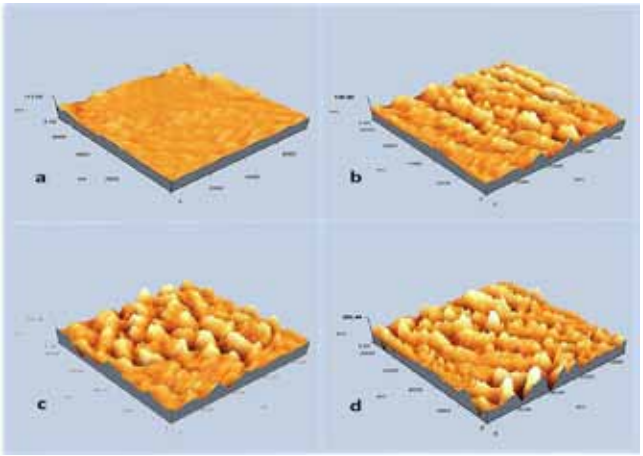

Fig 9 Three dimension (3D) Surface topography of enamel by Atomic Force Microscopy (AFM) were made with areas of 10 µm2 captured with a slow scan rate(0.1 Hz). Nano roughness (Ra, in nanometers) was measured with proprietary software (Nano scope Software, version V7). :a) Polished enamel sample Ra = 140 nm. b) Enamel sample after bleaching with 15% H2O2. Ra = 150.4 nm. c) Enamel sample after bleaching with 25% H2O2 Ra = 190.74 nm. d) Enamel sample after bleaching with 35% H2O2 Ra = 200.44 nm.

Discussion

Esthetic dentistry has become an increasingly essential requirement of modern dental practice. Laser teeth whitening is one of the newest and efficient methods available for office teeth whitening. The objective of laser bleaching is to achieve ultimate bleaching process by using the most efficient energy source while avoiding any adverse effect (Dostalova et al. 2004). On the other hand, the goal of the dental practitioner is to whiten the tooth efficiently with controlled peroxide concentration but with no morphological and chemical changes of enamel. To date, numerous in vitro models have been used to evaluate the efficacy of tooth bleaching products and methods (Suyama et al., 2009, Sulieman et al., 2003). An artificial discolored bovine tooth model with tea extract for staining the teeth was applied in this study. It's based on the discoloration model reported by (Sulieman et al., 2003,2004&2005; Davidi et al, 2008; Polydorou et al., 2009). Many researchers have preferred to use animal teeth because of the increasing difficulty in obtaining human teeth for dental research, and because of the requirements made by ethics committees about their use. Bovine teeth have been suggested as possible substitutes for human teeth because it has been reported that chemical and physical properties of bovine tooth such as composition, enamel density, heat capacity, hardness, dentin tubule density and permeability are similar to human teeth (Tagami et al., 1989, Esser et al., 1998, Fonseca et al., 2004, Reis et al., 2004).

In this study, Spectrophotometers (Vita Easyshade) were used for the evaluation of the color change. The Easyshade is an intraoral contacting spectrophotometers that can supply parametric data (L*,a* & b*) which could be easily applied for statistical analysis. The possible changes related to in-office different peroxide-containing bleaching agents are capable of causing alteration in enamel at high concentrations (for example 35%) as well as the degree of lightness. Previous studies evaluated the influence of high peroxide concentration like 35% on enamel surface roughness and color changes (Dostalova et al. 2003) it was found that high concentrations produce a great improvement in color especially in deep discoloration but such improvement will affect enamel surface roughness. The reason for these changes could be related to loss of Ca^{+2} ion by the action of hydroxyl group of peroxide gel (Berger et al., 2010). These results are in consistent with our results for color changes and surface roughness where there is a slight surface modification after laser-assisted bleaching in-office procedure especially in high concentration. In such a case, we recommend using remineralizing products to overcome such enamel destruction like Gc Tooth Mousse on bleached enamel (Baljeet et al.,2012; Vasconcelos et al.,2012). The mean (L*) value obtained after bleaching the stained teeth was greater than the stained one. This indicates that hydrogen peroxide solution was able to react and activate in the presence of laser beam. Such activation will produce active free radicals that act as a potent oxidant and subsequently react with and cleaves the bound of chromophores leading to alteration of pigments in the enamel and dentin and the lightening effect is achieved. The improvement in the degree of lightness between stained and bleached specimens at each concentration was observed in this study. These results imply that regardless of the peroxide concentration provided with Lase II system, the bleaching process was successful at the applied time protocol. Such a result confirms the company claims that even low concentrations of peroxide when activated with laser could produce a substituent difference in color toward greater lightness (Marvin et al., 2009). The analysis of variance shows a significant difference between groups (15%,25% & 35%). exposure of the teeth enamel to higher peroxide concentration (35%) produce higher lightness values (higher L*) when compared with low concentration (15%, 25%). This indicates that there is a relation between increasing peroxide concentration and color changes. This could be explained by the amount of free radicals such as oxygen (O•) and hydroxyl (OH^-). Therefore, higher concentrations will produce higher amounts of free radicals and consequently higher lightness. This coincides with the finding by Sulieman et al. (2004) who compared the in vitro tooth bleaching efficacy of gels containing 5–35% peroxide. This study found that with high peroxide concentration, the number of gel applications required to produce uniform bleaching was decreased. From the results of our study and Sulieman's study we can suggest that whitening result can be obtained in a short period of time by utilizing high peroxide concentrations. The short time of gel applications might decrease post-operative sensitivity. In the present study, the degree of lightness ΔL gradually increased in all groups by increasing peroxide concentrations, while (Δa) and (Δb) that represent the degree of redness and yellowish showed a gradual decrease. This behavior of color improvement resulted from the fact that the homogenous changes in ΔE value among different concentrations dependent on the amount of free radical release as excessive amount is expected to be released with higher peroxide concentration. Such a phenomenon was also observed by other studies (Sulieman et al., 2003,2004&2005; Davidi,2008; Polydorou et al., 2009). It should be noted that a mean for (ΔE) value for all three peroxide concentrations was differed statistically. In the present study, the mean surface roughness (Ra) value obtained after bleaching was significantly higher than those values without bleaching treatment for both 25% and 35% concentration. Although the 15% did not show significant difference in Ra value but the mean comparison revealed an increase in value. This study demonstrates that there is a relationship between the surface roughness value and an increase in peroxide concentrations, where exposure of the teeth enamel to 35% hydrogen peroxide during office bleaching produced higher roughness values when compared with 25% and 15% hydrogen peroxide. Such observation was also applicable when comparing the roughness value between 15% and 25% groups. Accordingly, low peroxide agent should be selected whenever possible to avoid the possible drawback associated with increasing the peroxide concentration. However, this might be guided clinically by the discoloration degree. Recent advances in the bleaching process always focus on the lower peroxide concentration agents even when the treatment time is prolonged (Joiner, 2006). Hydrogen peroxide at lower concentration did not have a major effect on the enamel structure and this coincides with the finding of (Oltu and Gurgan, 2005,McCracken and Haywood, 2004) who found a significant Ca^{2+} loss of enamel after treatment with different peroxide solution. The presence of hydration shell makes the enamel crystal electrically charged and can therefore attract ions. Free radicals that emit from hydrogen peroxide during bleaching are able to play a part in demineralization and cause Ca^{2+} loss from enamel crystals. Lewinstein et al, (2006) came to the conclusion, that peroxide could cause demineralization in enamel at low and high concentrations. Another study concluded that higher concentrations of H2O2 caused more Ca^{2+} loss than lower concentrations (Hüseyin Tezel et al.,2011). Atomic force microscopy revealed that exposing enamel to high peroxide concentration (25% and 35%) significantly increased (Ra) value

in comparison to unbleached enamel Fig (9). Also different concentrations of peroxide produced significantly different (Ra) value. This result was consistent with the result of profilometer analysis. Our results showed changes in superficial enamel after hydrogen peroxide treatment which affected the highly mineralized enamel even at low (15%) concentration. More detectable changes was observed with higher peroxide concentration (25% & 35%). It is recommended to perform tooth whitening by using low concentration of hydrogen and a short treatment time to reduce the possible destruction but to bring about the required change in color. This might be applied for mild discoloration to overcome such effect but this is not recommended in deep discoloration where mild color improvement will be obtained.

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Conclusions:

Color changes and surface roughness of bovine enamel were influenced by the use of DMC laser bleaching system. Higher peroxide concentration produces higher color changes and surface roughness value.

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