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Research Article

The Effect of Bleaching Agents on Micro Chemical Structure of Human Enamel by FTIR Spectroscopy Method (An *in-vitro* study)

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Abstract: Bleaching has been accepted as the most conservative treatments for discolored teeth. In vitro examination of the effects of the bleaching agents such as carbamide peroxide 10%, carbamide peroxide20%and hydrogen peroxide 40%, on micro chemical structure of human enamel by Fourier Transform Infrared (FTIR) spectroscopy, was the aim of this study. In this study, thirty healthy human teeth were used and they were divided into three groups of ten. From each tooth two enamel blocks with dimensions of 4 × 3 × 2 mm were prepared. In each group, a block was considered as a control block (immersed in distilled water) and the other block was treated in bleaching condition as follow: 1) hydrogen peroxide 40%, 2) carbamide peroxide 10%,3) carbamide peroxide 20%.During the tests, the samples were submerged(immersed)in distilled water. Then, the samples were powdered separately, and the spectra of powdered samples were taken and each spectrumwasconsistedofPO_4^{3-}, CO_4^{-2}, OH^-signals respectively. Frequency changes were evaluated and data was analyzed by variance test, ($P < 0.05$). In the results the spectra of control and bleached samples, structural change were not detected but in terms of quantity, it was found that the frequency average of bleached samples was decreased. The maximum reduction of hydrogen peroxide 40% was P = (OH^- (3313.00), $CO3^{-2}$ (1447.30),PO_4^{3-} (1031.80), and the minimum reduction in the carbamide peroxide 10% was P = (OH^- (3347.90),CO_3^{-2} (1451.20),PO_4^{3-} (1040.30),respectively. In conclusion Based on this study, bleaching agents may reduce enamel mineral content, especially when the concentration of peroxide is high. As well as, the presence of fluoride and desensitizing agents in bleaching agents, balance the reduction of enamel mineral contents.
Keywords: carbamide peroxide, hydrogen peroxide, spectroscopy

INTRODUCTION:

The increasing demand for esthetic dentistry of patients, has led to the development of various methods to improve the appearance of the teeth. One of the considered methods is (Bleaching) of the teeth that involve the whitening of the teeth color by using chemical materials in order to oxidize the organic pigments in the teeth. Today, bleaching of discolored teeth, has become more widespread and in this view, bleaching of the (Vital Teeth) because of its effectiveness, easy application and its ability to create immediate results, is very common [1].

Moreover, bleaching is the conservative approach, compared to other methods such as veneers or crowns in achieving esthetic [2 & 3].

The common assumption about bleaching is that, the free radicals attack the organic molecules in order to be stable, this leads to release of other radicals, which react with unsaturated bonds, and disrupt the electron conjugation and alter the absorption energy of enamel organic molecules. Simpler molecules are formed; they reflect less light, so that the teeth look brighter, .Regardless of the work trend, bleaching can be done with different materials such as hydrogen peroxide, carbamide peroxide, sodium perborate which can be used with different concentrations [4].

In IR (Infra Red) spectroscopy, molecular vibrational (?)Spectra are recorded and then based on the spectral data, the molecular structure is discussed. The interpretation of the spectrum is(?)That, each chemical bond in molecules has its own specific frequency (like a fingerprint), so by recording the IR spectrum, one can be detect the bond type and molecular structure ultimately. In addition, existing groups in the molecule, such as C= O, O- H, have their own related frequencies, as well. In the IR spectra, molecules of hydroxyapatite, Ca10 (Po4) 6 (OH) 2

except the frequencies of the OH group that is special, the phosphate groups constitute the main structure of the spectrum [6].

According to the daily increase in the use of different bleaching materials, we decided to examine the effect of these materials on tooth structure. Hence, the aim of this study was in vitro examination of the effects of bleaching compounds such as hydrogen peroxide 40%, carbamide peroxide 10%, andcarbamide peroxide 20%, on micro chemical structure of human enamel by spectroscopy Fourier Transform Infrared (FTIR).

In one study, carbamide peroxide effects on mechanical, chemical and morphological properties of human enamel were examined, in which, tray soft vinyl matched on the dental arches, one side was received bleaching agent and the other side was considered as the control group. The bleaching of live teeth was done three times with different concentrations, and each time new enamel parts were used, the Scanning Electronic Microscopy (SEM), Knoop Micro hardness, Energy Dispersive X-ray (EDX) tests were done. Data was analyzed with ANOVA test, results showed that there is no chemical, mechanical and morphological change on the enamel surface, resulted from using three different carbamide peroxide agent [7].

In one study, the effect of 30% neutral and acidic hydrogen peroxide on the chemical composition, mechanical properties, surface morphology and color of tooth enamel were studied and in which, distilled water was used as a control group. In this study, acidic hydrogen peroxide30%, compared with neutral type and distilled water, has led to a prominent reduction in micro hardness (Micro hardness) and morphological changes of the enamel surface [8].

In a research, the effects of different bleaching systems and thickening agents on human enamel hardness were examined invitro, and the in vitro effects of treatment by micro hardness tests which were performed before and after treatment, showed that all treatments reduced the enamel micro hardness [9].

A study which was carried out in order to evaluate the effect of adding calcium and fluoride into hydrogen peroxide gel35% on the surface and sub-surface hardness of enamel, and in which, micro hardness of enamel surface examined using Vickers microdurometer immediately after bleaching treatment showed that, bleaching treatment with hydrogen peroxide (35%) reduces the surface and sub-surface micro hardness of enamel significantly, and adding calcium and fluoride in bleaching agent increases the micro hardness of bleached enamel. [10]

In a study of bleaching effect with fluoride and fluoride treatment after bleaching on whiteness and micro hardness of bovine enamel showed that, treated parts with fluoride bleaching caused smaller reduction in micro hardness compared to those treated with agent without fluoride. As a result, fluoride bleaching agent results less demineralization in morphology and surface micro hardness of enamel and increasing the amount of added fluoride don't prevent bleaching effect [11].

METHODS AND MATERIALS:

In this experimental study, Thirty extracted healthy human premolar teeth, which were selected, based on the entry and exit criteria of persons (?) less than 20 years for the orthodontic treatments, periodontal disease , so that they had no cracks, caries, fillings, wear buccal, lingual, occlusal and any discolorations with internal or external origin, and congenital imperfection such as fluorosis in the visual examination, as well as, it is past at most six months over their teeth extracted.

Before the beginning of the experiment, any calculus and tissue debris were removed from the teeth by scaler and then by rubber cap and pumice powder without fluoride, then the teeth were cleaned and disinfected with 2% formalin. In the next step the teeth were randomly divided into 3 groups of 10 teeth. Then the enamel blocks with dimensions of (4 × 3 × 2) from the middle third of the buccal and lingual of the each tooth were prepared by high-speed hand pieces, water and air spraying and needle-shaped diamond bur, and the cut parts were polished by round fine diamond bur, in order to remove any dentin, then all debris produced from cutting were removed and the blocks were washed and cleaned with brush and distilled water.

Dimensions of enamel blocks were controlled by periodontal probe. Two blocks of each tooth in each group were named and set separately, so that a block of each dental sample was considered as the control group and other block considered as the sample under the test of target group. All prepared blocks were kept separately, in distilled water at room temperature until the start of the experiment. After preparing 2 dental blocks, one as a control sample and the other as a tested sample, 20 dental blocks were prepared in each group, that will be explained in the following:

The first group of 20 obtained blocks in this group included, 10 blocks immersed separately in distilled water and 10 blocks under hydrogen peroxide (40%) of fluoride test (Opalescence Boost / Ultra dent / USA). So in the first day, each block is submerged for 15 minutes in the containers separately, then they are cleaned by cotton, and this action is repeated twice within 15 minutes until 45 minutes. All these steps are repeated on the seventh and fourteenth days and the samples were stored in distilled water during this time.

The second group consists of 20 obtained blocks, 10 submerged blocks in distilled water

separately and10 other blocks set under carbamide peroxide 10% test(Opalescence PF / Ultra dent / USA). In this way, in the first day each blockish submerged separately in the vessels containing this material for 6 hours. This step is repeated with fixed intervals-every day for a week, at day intervals - the samples were stored in distilled water.

The third group with 20 blocks obtained by this group, 10 blocks immersed in distilled water separately, and 10 blocks exactly the same as the second group, are subjected to carbamide peroxide 20% test(Opalescence PF / Ultra dent / USA).The distilled water of control samples is changed routinely until the end of the test. Then blocks of each group are grounded separately in the chemical lab, and they change to powder. The diagram of obtained powder samples spectrum and sample changes spectrum are interpreted. The data is entered into SPSS 16 software, and the descriptive study is expressed by calculating the average, percentage and standard deviation, and in order to analyze the study assumptions, the analysis variance test with P value <0.05 is used.

RESULTS & DISCUSSION:

In Table 1, the average frequency ofPO_4^{3-}, CO_3^{-2}, OH^-signals, as well as the standard deviation of the mean frequencies in the studied groups are shown.

As it is presented in Table 2, Analysis of variance in PO_4^{3-} signals frequencies showed that there is no significant statistical difference between distilled water and peroxide 10% group (P = 0.997) and carbamide peroxide 10% with carbamide peroxide 20% group. (P = 0.062).While there is considerable statistical differences between distilled water with hydrogen peroxide 40% group and carbamide peroxide 20% , and distilled water with hydrogen peroxide 20 % and the group of hydrogen peroxide 40% with carbamide peroxide 10% and carbamide peroxide 20% (P <0.05).

According to Table 3, the variance analysis in the frequency of CO_3 signals, showed that, there is no significant statistical difference between hydrogen peroxide 40% with carbamide peroxide 10% (P = 0.206) and carbamide peroxide 20% (P = 0.327)with carbamide peroxide 10% with carbamide peroxide 20% (P = 1/000)group, while considerable statistical difference was found, between distilled water with hydrogen peroxide (40%) and carbamide peroxide 20% and carbamide peroxide 10% (P <0.05).

As it is cleared in Table 4, the variance analysis in OH^-signals frequencies showed that there was a significant statistical difference in all groups (P <0.05).

Table 1: Average frequency PO_4^{3-}, CO_3^{-2}, OH^- signals in studied groups.

The used material	The Average Frequency of PO_4^{3-} Standard Deviation	The Average Frequency of CO_3^{-2} Standard Deviation	The Average Frequency of OH^- Standard Deviation
Distilled water	1040.63 1.27	1457.80 3.80	3402.23 1.77
Hydrogen peroxide 40% fluoride	1031.80 1.61	1447.30 4.11	3313.00 1.33
Carbamide peroxide 10 % fluoride	1040.30 2.11	1451.20 3.79	3347.90 1.91
Carbamide peroxide 20 % fluoride	1038.10 1.10	1451.100 4.44	3324.10 2.46

Table 2: Comparison of the frequency average of PO_4^{3-} signals in studied groups.

Frequency	Group		The average difference	P value
PO_4^{3-}	Distilled water	Hydrogen peroxide 40% fluoride	8.83	0.0001
		Carbamide peroxide 20 % fluoride	2.53	0.0001
		Carbamide peroxide 10 % fluoride	0.33	0.997
	Hydrogen peroxide 40% fluoride	Distilled water	8.83	0.0001
		Carbamide peroxide 20 % fluoride	6.30	0.0001
		Carbamide peroxide 10 % fluoride	8.50	0.0001
	Carbamide peroxide 20 % fluoride	Distilled water	2.53	0.0001
		Hydrogen peroxide 40% fluoride	6.30	0.0001
		Carbamide peroxide 10 % fluoride	2.20	0.062
	Carbamide peroxide 10 % fluoride	Distilled water	0.33	0.997
		Hydrogen peroxide 40% fluoride	8.50	0.0001
		Carbamide peroxide 20 % fluoride	2.20	0.062

Table 3: Comparison of average frequencies of CO_3signals in studied groups.

Frequency	Group		The average difference	P value
CO_3^{-2}	Distilled water	Hydrogen peroxide 40% fluoride	10.50	0.0001
		Carbamide peroxide 20 % fluoride	6.80	0.004
		Carbamide peroxide 10 % fluoride	6.60	0.001
	Hydrogen peroxide 40% fluoride	Distilled water	10.50	0.0001
		Carbamide peroxide 20 % fluoride	3.70	0.327
		Carbamide peroxide 10 % fluoride	6.90	0.001
	Carbamide peroxide 20 % fluoride	Distilled water	6.80	0.0001
		Hydrogen peroxide 40% fluoride	3.70	0.327
		Carbamide peroxide 10 % fluoride	0.20	1.000
	Carbamide peroxide 10 % fluoride	Distilled water	6.60	0.001
		Hydrogen peroxide 40% fluoride	6.90	0.206
		Carbamide peroxide 20 % fluoride	0.20	1.000

Table 4: Comparison of average frequencies of OH^-signals in studied groups

Frequency	Group		The average difference	P value
OH^-	Distilled water	Hydrogen peroxide 40% fluoride	89.23	0.0001
		Carbamide peroxide 20 % fluoride	78.13	0.0001
		Carbamide peroxide 10 % fluoride	54.33	0.0001
	Hydrogen peroxide 40% fluoride	Distilled water	89.23	0.0001
		Carbamide peroxide 20 % fluoride	11.10	0.0001
		Carbamide peroxide 10 % fluoride	34.90	0.0001
	Carbamide peroxide 20 % fluoride	Distilled water	78.13	0.0001
		Hydrogen peroxide 40% fluoride	11.10	0.0001
		Carbamide peroxide 10 % fluoride	23.80	0.0001
	Carbamide peroxide 10 % fluoride	Distilled water	54.33	0.0001
		Hydrogen peroxide 40% fluoride	34.90	0.0001
		Carbamide peroxide 20 % fluoride	23.80	0.0001

In this study, we compare the spectrum diagrams of control groups, and there were no changes based on transformation of the spectrum diagram in the whole experimental groups, however, in terms of quality, the frequency reduction exhibited these three signals. Comparison of frequency reduction of PO_4^{3-} control group (1040.63) with HP 40% (maximum reduction1038.10) was considerable and with CP 20% (minimum reduction 1040.30)was not significant. Furthermore, comparison of HP 40% with CP 10% and CP 20% was significant while there was no difference in comparing CP 20%with CP 10%. On The other hand, the mean frequency comparison of CO3-2control groups (80/1457) with all experimental groups was significant, in a way that, maximum frequency reduction was associated with the maximum of the HP 40% (1447.30) and the minimum reduction was related to the frequency of CP 10% (1451.20) after control group.

Pair wise comparing of experimental groups showed that the CO3-2 frequency difference was not significant. The OH- average frequency difference groups were considerable. The more reduction in the spectra frequencies is, the more mineral content of the tooth will be lost.(?)The enamel (the HA) has the signals related to PO43-, CO3-2, OH-groups. The results mean that in the CP 10%, due to reduced frequency of PO43-, CO3-2, OH-, the new hydrogen bonds have beenformed. The PO43-frequency reduction was not-significant, however, a reduction has occurred in the mineral content of enamel.

In a study, fulfilled by Smidt *et al.;* carbamide peroxide 16-15% had no significant impacts on micro hardness and morphological changes of enamel mineral components (calcium, phosphate, carbonate and fluoride) which was dissimilar to our research results. The variation in results can be related to differences in the time and frequency of bleach usage, bleach and commercial combinations of bleach and different PH in these two studies. ON the other hand, in the Smidt's research, the samples were treated and bleached under In Situ condition in saliva, while the samples of our presented study were submerged in distilled water during bleaching test, under, in vitro condition. Saliva

can cause dilution and buffering of the environment and act as a source of calcium and phosphate for remineralization [7].

In a study of Shannon *et al.;* and Seghi *et al.;* it had been shown no alterations in enamel hardness after treatment with CP 10% [12&13], that their conditions were dissimilar with our study.

In the Attin *et al.;* study (?), using of Opalescence Quick (HP 38%) for two hours, indicated a significant reduction in Knoop hardness of enamel to 700 microns depth [14], which is consistent with our study.

As well as, in the Tezel *et al.;* research, the loss of calcium caused by HP 35% and HP 38% with the use of light catalyst was prominent, while it was not significant with CP 10% [5]. Which the first part of our study was consistent with our study and the second part was opposed to it, this is resulted by using brushing with fluoride toothpaste after bleaching, and being soaked in the saliva in the study of Tezel.

In the study of Cadenaro *et al.;* which was done In Vivo, bleaching material HP 38% had no significant effect on enamel surface roughness (Surface Roughness) [15]. Although their material was similar with our study (HP) and the difference was just 2%, it was contrasting with our results because of the existence and impact of saliva in the study of Cadenaro. Furthermore, Cadenaro studied the mechanical properties and surface roughness of enamel, while our work was based on the accurate investigation of the chemical components changes in Enamel.

In Suleiman *et al.;* study, using HP 35% for 30 minutes did not cause significant changes in enamel and dentine hardness values [16] which this point is inconsistent with our results, and its reason could be resulted from differences in the using time of the bleaching material in these two studies (in our study treatment was complemented during 3 sessions of 45 minutes).

Hegedus *et al.;* study showed that HP has more damaging effects on tooth enamel compared to CP [17] which was similar to our research results of comparing the composition of CP and HP.

As well as, factors such as bleach compound, the PH, frequency and duration of bleaching material usage, the effect of oxidative compounds, the amount of thickener bleach substance in order to stabilize the material, the presence of fluoride, and the variety of the morphology of enamel influence on the mineralization rate of enamel structure [15&18]. Of course, note that bleaching gels PH used in this study was 7, however, frequency reduction of sampled teeth is not related to bleaching gel PH.

CONCLUSION:

The IR investigation in this study showed that, the experimental bleaching agents did not affect the shape of the spectrum diagram, while in terms of quantity, they reduced the frequencies of PO43-, CO3-2, OH-signals (except the carbamide peroxide10% that had no impact onPO43-), in this research the maximum reduction infrequency average was related to hydrogen peroxide 40% and minimum reduction was related at the beginning to the control group and then to carbamide peroxide10% . As among the used whitening compounds, CP 10% showed the least lost of mineral content of teeth. Fluoride and the desensitizing agents of the bleaching agents, can somewhat balance the mineral content reduction of the enamel.

Further studies using bleaching ingredients, along with the environmental impact study, preferably in the oral environment are required. Another limitation of commercial bleaching products is their jelly form that may contain some other added substances such askarbamr, glycerin, fluoride, metal salts and sweeteners. It is essential that these factors be considered in future studies.

REFERENCES:

1. Heymann HD; Additional conservative esthetic procedures, In: Roberson TM .Editor: Rudolph TM. Editor: Rudolph penny. Sturdvents art and science of operative dentistry.15th ed. North Carolina: Mosby Elsevier; 2006, ch (15):625-685.
2. Blankenau R, Goldstein RF, Haywood VB; The current status of vital tooth Whitening techniques. Compend Contin Educ Dent 1999; 20:781-794.
3. Haywood VB, Leonard RH, Dickinson GL; Efficiency of six months of night guard vital bleaching of tetra cycline stained teeth. J Esthet Dent 1997; 9:13-29.
4. Haywood VB, Berry TG; Natural tooth bleaching, In: Summitt Y, Robbinson W, Hilton T, Schwartz R. Editor: Summitt Y. Fundamentals of operative dentistry .3rd ed. Texas: Quintessence publishing co, Inc; 2006, ch (15):437-459.
5. Tezel H, SogutErtas O, Ozata F, Dalgar H, OnurKorkut Z; Effect of bleaching agents on calcium loss from the enamel surface. Quintessence Int; 38:339-347.
6. Dauglas A. Skoog Waugh, James Haller, Nieman; chapter17.principles of instrumental analysis.
7. Smidt A, Feuerstein O, Topel M; Mechanical, morphologic, and chemical effects of carbamide peroxide bleaching agents on human enamel in situ. Quintessence Int 2011; 42:407-412.

8. Sun AL, Liang S, Yue Sa, Wang Z, Xiao Ma, Tao J *et al.;* Surface alteration of human tooth enamel subjected to acidic and neutral 30%hydrogen peroxide. Journal of dentistry 2011; 39:686-692.
9. Soldani P, Amaral CM, Rodrigues JA; Micro hardness evaluation of in situ vital bleaching and thickening agents on human dental enamel. Int J Periodontics Restorative Dent 2010; 30(2):203-11.
10. Borges AB, Samezima LY, Fonseca LP, Yui KC, Borges AL, Torres CR; Influence of potentially remineralizing agents on bleached enamel micro hardness. Oper Dent 2009; 34(5):593-7.
11. Chen Hp, Chang CH, Liu JK, Chuang SF, Yang JY; Effect of fluoride containing bleaching agents on enamel surface properties. J Dent 2008; 39(9):718-25.
12. Shannon H, Spencer P, Gross K, Tira D; Characterization of enamel exposed to 10% carbamide peroxide bleaching agents. Quintessence Int 1993; 24:39-44.
13. Seghi RR, Denry I; Effects of internal bleaching on indentation and abrasion characteristics of human enamel vitro. J Dent Res 1992; 71:1340-1344.
14. Attin T, Vollmer D, Wiegand A, Attin R, Betke H; Subsurface micro hardness of enamel and dentin after different external bleaching procedures. Am J Dent 2005; 18:8-12.
15. Cadenaro M, Navarra CO, Mazzoni A, Nucci C, Matis BA, Leonarda RD *et al.;* An invivo study of the effect of a 38 percent hydrogen peroxide in-office whitening agent on enamel .JADA 2010;141:440-454.
16. Suleiman M, Addy M, Macdonald E, Rese JS; A safety study in vitro for the effects of an in-office bleaching system on the integrity of enamel and dentin. J Dent 2004; 32:581-900.
17. Hegedus C, Bistey T, Flora-Nagy E, Keszthelyi G, Jenei A; Anotomic force microscopy study on the effect of bleaching agents on enamel surface. J Dent 1999;509-515.
18. Bisty T, Nagy IP, Simo A, Hegedus C; In vitro FT-IR study of the effects of hydrogen peroxide on superficial tooth enamel. J Dent 2007; 35(4):900-4.