TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

Comparison of whitening effect of hydrogen peroxide and fluoride on female human teeth.

Biology Extended Essay

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Abstract

The aim of this extended essay is to investigate and compare the whitening effect of Hydrogen Peroxide and Fluoride on human female teeth. The research question is: “Is there a significant difference between whitening agents Hydrogen Peroxide and Fluoride in terms of their effect on the colour change of human teeth ?”

It was hypothesized that there will be a significant difference in terms of their whitening effects of Fluoride and Hyrogen Peroxide on female human incisors. The teeth treated with Hydrogen Peroxide will show a greater color change than the teeth treated with Fluoride.

To investigate the whitening effect, a two-step experiment is planned. In the first step teeth were stained in solutions of coffee, tea and sour cherry juice for 7 days. The second step is the whitening step where the teeth are cleansed with the whitening agents for another 7 days. The color tones at each step are measured using a colorimeter and data analysis is done in order to decide whether there is a statistical difference between the efficiency of the whitening agents.

Resultantly, on the enamels of teeth that Hydrogen Peroxide is used, a larger color tone change is observed. The outcome of t-Test showed there is a significant mean difference between Fluoride and Hydrogen Peroxide in terms of their whitening effects on human incisors.

Word Count: 222

Table of Contents

I. Introduction

Discoloration of teeth is a common aesthetic problem. Color changes of teeth are usually classified as intrinsic, extrinsic and internalized stains. [1]

Intrinsic teeth stains result from systemic or pulpal origin within the teeth such as exposure to tetracycline and similar minerals, they are mostly formed during teeth formation. Trauma, medications, nutritional deficiencies, genetic defects, hereditary diseases, aging and taking excessive fluoride in the diet are also causes of intrinsic stains. [2]

Extrinsic factors of discoloration include staining by absorption of colouring substances that are exogenous sources such as smoking, drinking, exposure to antimicrobial agents.[3] It is also reported that production of colored components by chromogenic bacteria may contribute to the formation of extrinsic stains. Some factors such as enamel defects result from salivary dysfunction and inadequate oral hygiene are predisposition to extrinsic stains of human teeth. [4]

Internalized stains are formed when the extrinsic stains enter the dentine (appendix III) from the tooth defects such as cracks on the enamel.[5] Fissures and defects are the parts where the food, beverages and tobacco are accumulated, causing stains. Beverages such as coffee, tea, red wine, carbonated drinks etc. have varying degrees of staining on teeth according to their composition and properties. [6]

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[1] Watts A, Addy M. *"Tooth discolouration and staining: a review of the literature."* British Dental Journal. 2001 Mar 24;190(6):309-16.
[2] "Tooth Discoloration" 27 Dec 2010. <http://emedicine.medscape.com/article/1076389-overview>
[3] Omata Y, Uno S, Nakaoki Y, Tanaka T, Sano H, Yoshida S, Sidhu SK. "Staining of hybrid composites with coffee, oolong tea, or red wine." Dental Materials Journal. 2006 Mar;25(1):125-31.
[4] Hattab FN, Qudeimat MA, al-Rimawi HS. Dental discoloration: an overview. J Esthet Dent. 1999;11(6):291-310
[5] Sulieman M. "An overview of tooth discoloration: extrinsic, intrinsic and internalized stains." Dental Update. 2005 Oct;32(8):463-4, 466-8, 471.
[6] Ertaş E, Güler AU, Yücel AC, Köprülü H, Güler E. "Color stability of resin composites after immersion in different drinks." Dental Materials Journal. 2006 Jun;25(2):371-6.

Extrinsic stains or discoloration mostly occurs on teeth enamel. If the stains are mild, tooth could be turned back to normal by removing of the stain from the enamel by simple dental processes such as brushing teeth and prophylactic dental cleaning since tooth is not affected in its original structure.[7] If not managed in time, these stains can penetrate into teeth dentine and cause permanent staining which is difficult to remove.

Studies have shown that at-home teeth whitening agents are safe and effective (Demarco, 2009) The various teeth whitening products with active components of flouride, carbamide, hydrogen peroxide oother whitening agents such as pastes, gels, rinses, gums, strips are available at pharmacies or supermarkets. Products for at-home tooth whitening procedures contains generally low levels of carbamide peroxide and hydrogen peroxide (3 %) whereas products used in in-clinique procedures have higher levels of hydrogen peroxide (%35) [8]

Peroxide compounds such as carbamide and hydrogen peroxide have been in use to whiten teeth for many years and it is the most active ingredient in nearly all tooth whitening products. Hydrogen peroxide penetrates into the enamel and causes numerous reactions such as oxidation and reduction to lighten the dentinal regions.[9] Time and concentration are the main factors affecting the efficiency of the whitening of peroxide compounds. The efficacy increases with increasing concentration of peroxide but if the treatment is extended over a large time period (ie. 3 months) the low concentrations can be as effective as higher concentrations.[10] Sodium Monofluorophosphate (0.15% w/v fluoride ion) commonly called "Flouride" is also found in whitening products. There are studies on the whitening effect of fluoride showing it is an effective whitening agent. (Hu D.)

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[7] Roy, Lagnajeet.Teeth Discoloration and Teeth Whitening Solutions. 26 Nov. 2010 <http://ezinearticles.com/?Teeth-Discoloration-and-Teeth-Whitening-Solutions&id=4657936>.
[8] Wetter NU, Branco EP, Deana AM, Pelino JE. “Color differences of canines and incisors in a comparative long-term clinical trial of three bleaching systems.” Lasers Med Sci. 2009 Nov;24(6):941-7.
[9] European Commission on Consumer Products. Scientific committee on consumer products. Preliminary opinion on hydrogen peroxide in tooth whitening products. http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_cons_01_en.pdf SCCP. 2005;0844(4):1-50.
[10] Joiner A. “The bleaching of teeth: a review of the literature.” J Dent. 2006 Aug;34(7):412-9. Epub 2006 Mar 29. Review.

Many studies are available for research on hydrogen peroxide but any research on comparing the whitening effects of hydrogen peroxide and fluoride were found, which insipired me to carry out the study myself. So, this study compares the effectiveness of various home-applied whitening systems on discolourated teeth that are previously immersed in staining solutions of tea, coffee and sour cherry juice.

A debate over the whitening products whether they are effective or just claiming to be effective raised the research question in my mind; **"Is there a significant difference between whitening agents Hydrogen Peroxide and Fluoride in terms of their effect on the colour change of discolorated female human teeth ?"**

II. Hypothesis

Tooth discoloration is influenced by a combination of extrinsic and intrinsic factors. Instrinsic stains are related to enamel and dentin properties while extrinsic stains are associated to deposition of food or beverage stains on the tooth surface.

Home-applied whitening products appeared as alternatives to whiten discolorated teeth without dentist supervision.[11] The majority of these products contain hydrogen peroxide and fluoride in their ingredients. Hydrogen peroxide is a highly effective substance that forms the backbone of whitening in in-office and home-applied products while fluoride is a substance that is a substance widely used in bleaching and whitening processes. Both of these substances affect the color of the teeth by penetrating into the enamel, causing reactions.

Therefore, **it is hypothesized that there will be a significant difference in terms of their whitening effects of Fluoride and Hyrogen Peroxide on female human incisors.** It is expected that the teeth treated with Hydrogen Peroxide will show a greater color change than Fluoride.

[11] Demarco FF, Meireles SS, Masotti AS. "Over-the-counter whitening agents: a concise review." Braz Oral Res. 2009;23 Suppl 1:64-70. Review.

III. Method Development and Planning

To design an appropriate method for this study, I consulted a number of dentists and researched on scientific articles on teeth discoloration and whitening systems. To get accurate results and test my hypothesis effectively, I examined the experimental studies done on this topic.

The first problem I faced was the selection of the teeth. To select the appropriate samples for the study, the factors that affect the discoloration of the teeth are investigated. These factors are found to be smoking, age and sex. Smoking causes yellowish brown stains on tooth surface when nicotine and tar are deposited.[12] Discoloration due to ageing is another factor that 9 out of 10 people have to face since teeth develop a secondary dentin over time leading to a darker appereance.[13] Also further research revealed that discoloration is affected by the gender of the human. One study carried out in an English population showed that males tend to have more staining on tooth surface than females. (Ness)

In light of this information, the teeth were selected according to characteristics such as age and sex since these factors are effective on discoloration. Teeth used the experiment belonged to female humans between the age of 18 – 40 which is the pre-menopausal stage. The reason the age interval ends with 40 year-old teeth is that although there is hardly informative research on the effect of menopause on teeth discoloration, it is known that during the menopausal stage, the bone formation is disturbed by the hormonal changes and therefore this might affect the formation of the teeth which might lead to discoloration. Also, incisors are chosen since they are found in the front part of the mouth and exposed to outer means more than the rest of the teeth.

30 samples of female incisors that are previously extracted from patients are collected. Teeth are taken from Gazi University Faculty of Dentistry and according to the

[12] "Smoking and Health" <http://www.healthiertalk.com/smoking-and-dental-health-2473> 27 Dec. 2010
[13] "Stained-Aged Teeth and Whitening" <http://www.dentalgentlecare.com/what_ages_teeth.htm> 27 Dec. 2010

dentist's advice, teeth are kept in distilled water so as not to dry until the first measurements are taken.

Firstly, I planned to stain the teeth before the whitening process in order to clearly observe the upcoming color changes. If the teeth are immersed in staining beverages, their color tones will be relatively darker than their initial color tones and therefore when the teeth are whitened the difference between the initial and the final color tone will be larger which will be easier to measure and interpret.

The problem that came up is the decision about the types of solutions. In many studies coke, red wine, coffee, tea are used in order to stain the enamels of teeth. (Omata et al.; Topcu et al.) I decided on using coffee and tea since they are consumed everyday by millions and because of the teaflavins in tea and caffeine in coffee causes yellowish brown discoloration.[14] While choosing the third beverage I decided to use coke since it contains caffeine. Unfortunately, I found that coke results in erosion on teeth enamel which causes deep staining when exposed to a staining solution because of the corruption on the surface and therefore may result in misleading outcomes. So it was replaced with cherry juice, which is known as a natural dye and causes no erosion on enamel. Secondly, the preperation of the solutions were in ambiguity. Since the aim of the study is to compare the effects of whitening systems that can be utilized in home environment, I decided to prepare solutions as how they are prepared in daily life.

To measure the color tones I decided on using technology since the dentistry is a branch that is developing itself through the technological measurements. Color changes in dental materials may be assesed visually or by colorimetry. It is known that colorimetric measurements help to prevent the subjective interpretation of visual color comparison.[15] In light of this information using a Color Shade Scale was eliminated as an option to use in the experiment. It works according to the Visual Shade

Matching which is unable to provide accurate results and dependent upon the experimenters' interpretation. To get more reliable results, colourimeter is chosen to be

[14] Oğuz S, Mutluay MM, Doğan OM, Bek B. *"Color change evaluation of denture soft lining materials in coffee and tea."* Dental Materials Journal. 2007 Mar;26(2):209-16.
[15] Brook AH, Smith RN, Lath DJ. *"The clinical measurement of tooth colour and stain."* International Dental Journal. 2007 Oct;57(5):324-30.

used for the color measurements. The device provides accurate quantitative data of the color changes on the enamels of the samples and prevent the subjective interpretation. The colorimeter takes measurements in terms of the CIELAB Color Space (appendix I) which is a color system created by Internation Commision on Illumination to determine and evaluate the amount perceptible color changes. The device is found in the laboratories of Ankara University Faculty of Dentistry.

The main reason the teeth are immersed in solutions are to prepare the teeth for the second step of the experiment where the teeth will be whitened. With staining the teeth before the cleansing process, we will obtain a more perceptible data of the color changes before and after the whitening agents are applied.

Prior to deciding on the method for the whitening, the agents containing fluoride and hydrogen peroxide are found through consulting with dentists. According to their advice commonly used whitening products Opalescence Toothpaste containing fluoride and Pola Day Whitening System containing %7,5 Hydrogen Peroxide are used in the experiment. According to the manufacturer's instructions (appendix II) the fluoride containing toothpaste is applied by brushing the teeth and the peroxide containing gel is applied by keeping the teeth in gel in petri dishes. The application methods are adapted to the experiment such as the brushing was made twice a day for the fluoride group and the teeth are kept in peroxide containing gel for 90 minutes a day. In order to minimize the errors within the fluoride group that might arise from the brushing, the teeth are brushed according to Bass Method (appendix IV) which suggest to brush the teeth holding the toothbrush 45^{o} from the teeth and applying at least 10 strokes on every surface of teeth. In this experiment 20 strokes are applied to every sample.

The whitening is planned to continue for 7 days. In between the cleansing processes the teeth will be kept in seperate tubes filled with distilled water in order to prevent them from drying. At the end of the 7^{th} day, the color tones of the teeth will be measured and the measurements will be arranged for the data analysis. CIELAB Color Scale formula will be used in order to calculate the total color tones of the teeth. Then, the difference between the 2^{nd} and 3^{rd} measurements will be calculated and the statistical difference between the averages of the groups will be computed with t-Test: Two Sample Assuming Equal Variances.

IV. Materials

- 30 x Test Tubes (10ml)
- 30 x Teeth – Female Human Incisor
- 50 ml Graduated Cylinder
- 200 ml Distilled Water
- 3 x Petri Dish
- Gloves
- 200 ml Tea (Doğuş Çay)
- 200 ml Cofee (Nescafe Classic)
- 200 ml Sour Cherry Juice (Dimes)
- Colourimeter (Minolta CR-321, Made in Japan)
- Tooth Brush – Oral B Cross Action Vitalizer
- Pola Day SDI Advanced Tooth Whitening System %7,5 Hydrogen Peroxide
- Opalescence® Whitening Toothpaste

V. Method

A. Staining

1. 30 samples of human incisors are put into seperate test tubes in order to minimize the possibility of confusion during the experiment. Test tubes are labelled from 1 to 25. Samples 1-10 are representatives of Tea, 11-20 are representatives of Coffee, 21-30 are representatives of Sour Cherry Juice.
2. First color tone measurements are done with the colorimeter before immersing the teeth into the solutions. Before the measurements, colorimeter was calibrated with a standard white card. Measurements of a,b, and L values for each sample were repeated 3 times.
3. Teeth are then immersed into solutions of tea, coffee and cherry juice that are previously prepared. The tea solution was prepared by immersing five tea bags into 1 L of boiled water. For the coffee solution 20 g of coffee was poured into 1 L of boiled water. Both solutions were stirred every 2 minutes for 30 seconds until they cooled down to 23^{O}C which the room temperature. The other staining solution is sour cherry juice which also must be at room temperature.
4. Test tubes 1-10 are filled with tea solution, 10 ml for each tube. 11-20 are filled with Coffee, 21-30 are filled with Cherry Juice, again 10 ml for each tube.
5. Teeth are kept in solutions for 7 days under room conditions. After 7 days, the samples are taken out and the color tones are measured with the colourimeter, measurements of a,b, and L values were repeated 3 times.

B. Whitening

1. Tea, coffee and cherry juice groups are divided into subgroups labelled as “flouride” and “peroxide”. These subgroups are formed of randomly chosen 5 samples from the related group. The cleansing process will start for every group on the same day and will end after 7 days.
2. The flouride group is brushed with sodium monofluorophosphate containing Opalescence® whitening toothpaste twice a day for 7 days with the Bass Brushing

Method (appendix IV). For each teeth approximately toothpaste around a size of a bean is used. The teeth will be brushed in the morning and in the evening.

3. The peroxide group will be cleansed with Pola SDI Advanced Whitening System which does not include brushing but keeping the teeth in the tooth gel for 90 minutes a day in petri dishes. The teeth will be brushed after the application of this agent without a toothpaste.
4. For each group, between every application the teeth are put into the test tubes filled with distilled water in order not to dry.
5. The final color tones of every sample will be measured with the colorimeter.

VI. Results

Table 1 : Raw data table showing the initial color values of the samples measured with the colorimeter. a, b, L values are the different color distinctions of CIELAB Color Scale. (appendix I)

1st measurements are the initial color tones before the stainin process has begun, 2nd measurements are taken when the teeth were taken out of the staining solutions on day 7 and 3rd measurements are taken when the cleaning process is completed on day 7.

		1st Measurement			2nd Measurement			3rd Measurement		
Sample	**Trial**	**a**	**b**	**L**	**a**	**b**	**L**	**a**	**b**	**L**
	1	-4.94	7.44	92.85	-4.79	7.02	93.16	-4.76	7.08	93.01
	2	-4.98	7.45	92.70	-4.75	6.92	93.23	-4.81	7.05	93.21
1	3	-5.00	7.43	92.56	-4.78	6.84	93.34	-4.86	7.17	93.35
	1	-7.65	10.22	89.56	-5.21	0.01	80.41	-5.71	1.25	79.83
	2	-7.65	9.15	91.50	-5.17	0.86	82.38	-5.62	1.65	80.13
2	3	-7.56	7.94	93.60	-5.43	1.61	83.74	-5.58	1.98	82.4
	1	-5.23	0.50	79.51	-5.23	3.43	93.89	-5.16	0.37	90.13
	2	-5.33	0.41	80.04	-5.25	3.18	94.47	-5.14	0.11	90.26
3	3	-5.37	0.31	80.44	-5.12	2.83	94.82	-5.11	0.07	90.41
	1	-6.96	12.22	95.12	-6.08	7.77	98.47	-8.3	4.05	99.15
	2	-6.97	12.19	95.19	-6.07	7.54	98.09	-8.5	3.9	98.95
4	3	-6.93	12.08	95.30	-6.05	7.38	98.1	-8.51	3.83	99
	1	-5.87	5.46	89.43	-5.59	0.77	95.75	-0.23	0.75	88.86
	2	-6.05	5.46	89.35	-6.23	0.34	95.48	0.09	1.09	89.2
5	3	-6.17	5.29	89.36	-6.39	0.18	95.6	0.23	1.4	88.13

	1	-5.03	2.02	94.46	-5.41	7.88	82.54	-4.42	0.51	85.69
	2	-4.91	1.74	95.65	-4.36	2.22	90.1	-4.48	0.5	86.64
6	3	-4.75	1.59	96.47	-3.9	0.57	92.4	-4.53	0.5	88.67
	1	-5.17	2.19	89.98	-6.18	2.52	91.9	-7.92	0.62	95.97
	2	-4.77	2.59	89.09	-6.23	3.03	92.33	-7.81	0.84	96.07
7	3	-4.64	2.57	88.87	-6.26	4.51	93.54	-7.79	1.55	96.9
	1	-5.61	3.78	81.15	-2.99	3.69	78.71	-2.57	2.25	71.19
	2	-5.56	3.70	81.28	-3.04	4.01	79.37	-2.33	2.11	71.19
8	3	-5.60	3.63	81.39	-3.06	4.09	79.58	-2.23	2.36	70.81
	1	-7.55	5.62	83.38	-5.13	5.1	86.43	-0.27	10.89	69.5
	2	-7.62	5.63	83.57	-5.16	5.1	86.71	-0.26	10.43	69.9
9	3	-7.61	5.61	83.67	-5.16	5.11	86.82	-0.27	10.31	10.03
	1	-5.53	0.81	78.22	-6.1	10.23	85.32	-8.76	5.65	80.14
	2	-5.46	0.48	78.86	-5.86	10.96	85.54	-9.77	5.64	80.49
10	3	-5.46	0.32	78.23	-5.76	11.35	85.89	-6.31	4.51	76.33
	1	-9.06	4.38	98.36	-0.94	9.48	71.88	2.45	26.25	60.22
	2	-9.33	5.69	99.56	-1.2	8.92	72.63	2.44	25.56	60.5
11	3	-9.36	6.10	99.89	-1.25	8.61	72.93	3.98	22.9	61.95
	1	-8.96	3.84	102.75	-5.12	2.72	90.93	-7.35	1.05	97.5
	2	-8.92	3.86	103.89	-5.01	2.37	86.03	-7.6	1.15	99.57
12	3	-8.92	3.80	104.16	-4.99	2.06	86.55	-7.44	0.14	98.36
	1	-7.87	0.23	89.82	-4.13	7.39	82.84	-8.76	2.83	90.68
	2	-7.94	0.31	90.08	-4	6.76	83.55	-6.91	2.92	89.78
13	3	-7.91	0.37	90.32	-3.95	6.74	83.68	-6.91	3.02	88.85
14	1	-6.12	2.59	92.05	-3.92	4.39	91.47	-6.94	1.05	80.69

	2	-6.25	2.63	92.48	-4.06	4.44	91.81	-6.77	1.1	82.1
	3	-6.39	2.65	92.87	-4.06	4.46	91.91	-6.84	1.08	82.29
	1	-6.47	3.72	95.37	-5.61	1.47	94.42	-7.62	4.86	90.2
	2	-6.48	3.52	95.68	-5.76	1.79	95	-7.67	4.27	92.58
15	3	-6.43	3.32	95.87	-5.71	2.01	95.08	-7.68	4.37	91.71
	1	-6.05	4.29	90.67	-3.73	1.34	85.38	-8.63	3.25	98.88
	2	-6.06	4.25	90.68	-4.4	1.28	87.99	-8.65	3.55	96.35
16	3	-6.09	4.17	90.78	-4.78	1.31	90.57	-8.59	3.66	97.42
	1	-7.22	0.82	88.72	-3.42	6.37	77.53	-2.44	12.59	61.63
	2	-7.33	0.56	89.17	-3.44	6.48	76.17	-2.29	12.16	59.01
17	3	-7.38	0.50	89.36	-3.5	6.59	78.79	-2.27	12.06	62.08
	1	-3.79	5.18	89.20	-5.01	4.01	93.28	-2.7	3.4	71.35
	2	-4.30	5.01	91.01	-5.07	3.27	92.79	-2.76	3.24	71.91
18	3	-4.51	4.97	91.97	-5.07	3.16	93.86	-2.91	3.15	72.21
	1	-7.66	5.26	85.34	-7.96	1.94	87.23	-4.83	3.62	81.5
	2	-7.64	5.22	85.33	-7.53	1.89	87.62	-4.6	3.61	81.29
19	3	-7.60	5.17	85.40	-7.26	1.8	87.67	-4.37	3.61	81.09
	1	-6.96	6.60	87.81	-7.61	3.54	95.41	-9.28	2	84.17
	2	-7.00	6.61	87.77	-9.05	2.85	92.13	-9.35	1.9	83.82
20	3	-6.99	6.63	87.75	-9.42	1.65	86.14	-9.1	1.86	83.9
	1	-5.14	7.16	85.24	-3.68	1.5	84.56	-3.54	18.07	85.7
	2	-5.12	7.20	85.42	-3.6	1.46	84.53	-3.77	14.8	85.73
21	3	-5.13	7.24	85.51	-3.56	1.54	84.71	-3.76	14.75	85.75
	1	-6.61	1.63	85.48	-2.47	12.05	82.08	-5.23	17.93	88.15
22	2	-6.59	1.51	85.76	-2.33	11.89	83.11	-5.38	15.8	88.19

	3	-6.56	1.46	85.97	-2.21	12.06	83.94	-5.48	15.67	88.27
	1	-5.08	8.00	88.47	-3.29	1.9	76.46	-8.16	1.65	86.27
	2	-5.04	7.88	88.56	-3.28	1.95	83.47	-8.06	1.79	86.42
23	3	-5.05	7.86	88.60	-3.39	1.8	83.59	-9.92	2.06	86.57
	1	-6.56	5.88	92.02	-3.65	17.83	93.31	-5.34	14.41	84.25
	2	-6.95	5.91	92.43	-3.67	17.67	93.99	-5.38	14.46	84.29
24	3	-7.05	6.01	92.49	-3.84	17.41	94.96	-5.45	14.49	84.45
	1	-7.02	1.23	83.50	-5.27	5.06	87.61	-4.94	5.94	79.74
	2	-7.02	1.13	83.71	-5.49	5.33	88.4	-4.9	6.18	80.43
25	3	-7.03	1.08	83.96	-5.61	5.59	89.15	-4.92	6.3	80.9
	1	-7.89	10.91	93.87	-4.89	1.34	96.47	-2.22	2.71	84.09
	2	-7.96	10.69	93.91	-4.97	0.55	97.73	-2.28	2.71	80.3
26	3	-8.07	10.58	94.04	-5	0.13	98.16	-2.32	2.77	84.49
	1	-7.59	3.05	95.66	-6.72	5.52	104.35	-3.15	7.12	79.2
	2	-7.60	2.74	96.40	-6.86	7.25	106.83	-3.18	6.9	78.98
27	3	-7.61	2.78	96.58	-6.92	7.96	107.62	-3.17	6.83	78.95
	1	-3.61	1.92	85.33	-6.02	9.98	101.74	-0.83	10.55	81.96
	2	-3.61	1.84	85.56	-6.06	10.09	102.05	-0.74	10.49	80.47
28	3	-3.63	1.79	85.76	-5.49	15.55	104.22	-0.8	10.5	82.87
	1	-6.82	5.12	90.04	-4.95	7.12	93.13	-6.45	8.34	95.99
	2	-6.88	5.06	90.17	-4.98	7.08	93.22	-6.14	8.36	93.59
29	3	-6.87	4.99	90.28	-5.02	7.05	93.29	-5.95	8.34	97.35
	1	-4.91	9.39	96.41	-2.01	10.46	76.94	-4.36	10.17	87.82
	2	-4.92	9.38	96.52	-2.08	10.49	76.97	-4.17	9.97	89.98
30	3	-4.92	9.36	96.60	-2.15	10.54	77.15	-4.03	9.82	87.73

Table 2: Mean values of a,b and L for each sample, total color change (ΔE) values showing the difference between the 2nd and 3rd measurements.

	1st Measurement			2nd Measurement			3rd Measurement			ΔE (Between 2nd and 3rd Measurements)
Sample	**a**	**b**	**L**	**a**	**b**	**L**	**a**	**b**	**L**	
1	-4.97	7.44	92.70	-4.77	6.93	93.16	-4.81	7.10	93.19	0.18
2	-7.62	9.10	91.55	-5.27	4.59	93.23	-5.64	5.16	88.80	4.48
3	-5.31	0.41	80.00	-5.20	2.57	93.34	-5.14	3.36	84.44	8.94
4	-6.95	12.16	95.20	-6.07	0.83	80.41	-8.44	1.63	80.79	2.53
5	-6.03	5.40	89.38	-6.07	1.97	82.38	0.03	1.33	84.22	6.40
6	-4.89	1.78	95.53	-4.56	2.74	83.74	-1.37	0.82	87.60	5.36
7	-4.86	2.45	89.31	-6.22	3.15	93.89	-2.89	0.18	90.27	5.75
8	-5.59	3.70	81.27	-3.03	4.59	94.47	-4.48	1.41	93.27	3.70
9	-7.59	5.62	83.54	-5.15	6.05	94.82	-5.64	2.67	96.17	3.67
10	-5.48	0.54	78.77	-5.91	7.56	98.17	-6.75	3.93	99.03	3.83
11	-9.25	5.39	99.27	-1.13	5.23	98.09	-7.84	2.83	95.60	7.55
12	-9.93	3.83	103.6	-5.04	2.83	98.10	-6.06	1.89	92.35	5.91
13	-7.92	0.30	90.07	-4.03	0.43	95.75	-4.23	1.08	88.73	7.05
14	-6.25	2.62	92.47	-4.01	2.80	95.48	-2.38	1.00	87.67	8.18
15	-6.46	3.52	95.65	-5.69	3.43	95.60	-1.61	0.80	86.82	10.03
16	-6.06	4.24	90.71	-4.30	3.56	82.54	-0.92	0.50	87.00	6.38
17	-7.31	0.63	89.08	-3.45	1.77	90.10	-0.27	0.54	90.43	3.43
18	-4.20	5.05	90.72	-6.03	2.04	92.40	-3.10	0.65	93.57	3.45
19	-7.63	5.22	85.36	-7.58	3.35	91.90	-6.27	1.00	96.31	5.17
20	-6.98	6.61	87.78	-8.69	3.74	92.33	-8.28	1.55	88.05	4.83
21	-5.12	7.20	85.39	-3.61	4.07	93.54	-4.54	1.97	79.76	13.97
22	-6.59	1.54	85.74	-2.34	3.93	78.71	-0.47	2.24	71.06	8.05
23	-5.06	7.91	88.54	-3.32	4.40	79.37	2.96	5.12	70.50	10.89
24	-6.85	5.93	92.31	-3.72	4.76	79.58	-0.31	7.89	70.07	10.58
25	-7.02	1.15	83.72	-5.46	5.10	86.43	-3.66	10.54	49.81	37.07
26	-7.97	10.73	93.94	-4.95	6.81	86.71	-7.46	8.80	53.36	33.51
27	-7.60	2.86	96.21	-6.83	8.77	86.82	-7.93	7.20	56.89	29.99
28	-3.61	1.85	85.55	-5.86	10.85	85.32	-7.70	5.27	78.99	8.64
29	-6.86	5.06	90.16	-4.98	10.60	85.54	-7.53	12.13	72.35	13.52
30	-4.92	9.38	96.51	-2.08	9.92	85.89	-6.92	18.77	65.68	22.59

The ΔE values which represent the total color changes are calculated using the data in **Table-2** with the following formula by the CIELAB System(appendix I);

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta b)^2 + (\Delta a)^2}$$ #

Here is an example calculation for sample 1;

$\Delta L = L_2 - L_1 = 93{,}19 - 93{,}16 = 0{,}03$

$\Delta b = a_2 - a_1 = -4{,}81 + 4{,}77 = -0{,}04$

$\Delta a = b_2 - b_1 = 7{,}10 - 7{,}08 = 0{,}2$

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta b)^2 + (\Delta a)^2} = \sqrt{(-0{,}03)^2 + (-0{,}04)^2 + (0{,}2)^2} = 0{,}18$$

VII. Data Anlaysis

Table 3 : Processed data table showing the mean color tone change on the tooth surface due to the effect of the whitening agents, standard error, standard deviation and confidence interval of the fluoride and peroxide groups calculated using Microsoft Excel 2007 Programme.

Whitening Agent	Average Change in Color Tone (ΔE)	Standard Error	Standard Deviation	Confidence Level (95,0%)
Opalescence (Fluoride)	5.571	0.6714	2.6004	1.4401
Pola Day (Hydrogen Peroxide)	14.138	2.904	11.2470	6.2284

- The *average* values are calculated with the following formula;

$$\bar{X} = \frac{\Sigma x}{n}$$

where n is total trial number and x are the color tone changes for every trial. Here is an example calculation for the sample group Fluoride;

$$\bar{X} = \frac{0.18+4.48+3.94+2.53+6.4+5.36+5.75+3.7+3.67+3.83+7.55+5.91+7.05+8.18+10.03}{15}$$

$$= 5.57$$

- The *standard deviation* values are calculated with the following formula;

$$SD = \sqrt{\frac{\Sigma(X-\bar{X})^2}{n-1}}$$

where n is total trial number, X are the color tone changes for every trial, $\bar{X}$ is the mean value.

Here is an example calculation for the sample group Flouride;

$$SD = \sqrt{\frac{94.64}{15-1}} = 2.60$$

- The *standard error* values are calculated with the following formula;

$SE = \frac{SD}{\sqrt{n}}$ where SD is the standard deviation and n is total trial number.

Here is an example calculation for the sample group Fluoride;

$SE = \frac{2.60}{\sqrt{15}} = 0.671$

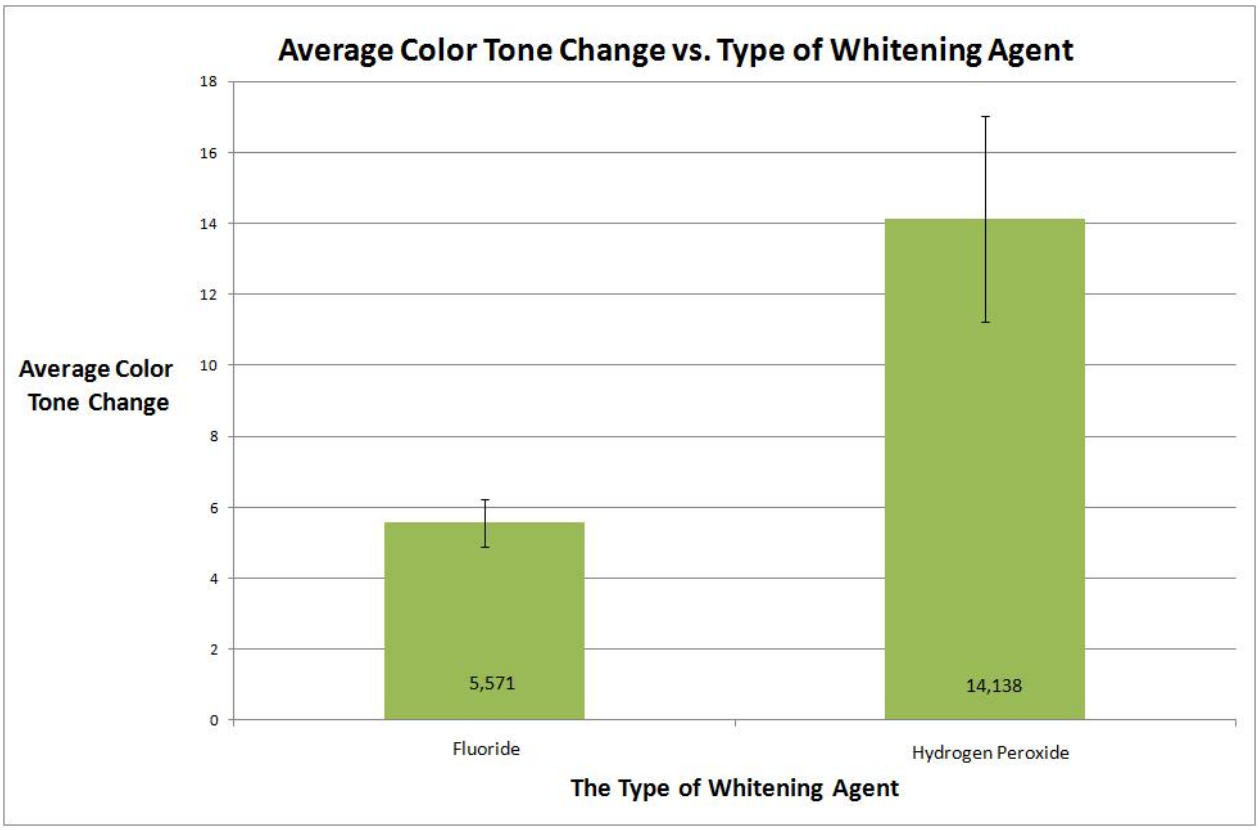
- The *confidence interval* values are calculated with the following formula;

CI = SE x t where SE is the standard error and t = 2.14479

Here is an example calculation for the sample group Fluoride;

CI= 0.671 x 2.14479 = 1.44

Graph 1: Bar graph showing the comparison of average color tone changes due to the affect of whitening agents. The error bars are drawn according to the standard error values given in Table 3.



Ho : There is <u>not</u> a significant difference between the means of color tone changes due to the effect of the whitening agents.

H_A : There is a significant difference between the means means of color tone changes due to the effect of the whitening agents.

To decide whether the alternative hypothesis or the null hypothesis is supported an anova test is made. The results are shown in the table below.

Table 4 : The results of the t-Test test made with the Microsoft 2007 Excel Programme.

t-Test: Two-Sample Assuming Equal Variances		
	Fluoride	*Hydrogen Peroxide*
Mean	5.570666666	14.138
Variance	6.762049523	126.4949314
Observations	15	15
Pooled Variance	66.62849048	
Hypothesized Mean Difference	0	
df	28	
t Stat	-2.874394077	
P(T<=t) one-tail	0.003822883	
t Critical one-tail	1.701130908	
P(T<=t) two-tail	0.007645765	
t Critical two-tail	2.048407115	

T-test results revealed that p one-tail (0.003822883) is smaller than alpha value (0.05). The difference between the color tone change of the female incisors cleansed with different whitening agents is too large to be explained by chance only. This shows there is a statistically significant mean difference. So Ho is rejected and H_A is accepted.

VIII.Conclusion and Evaluation

The intent of this experiment was to decide whether there is a significant difference between the whitening agents hydrogen peroxide and fluoride in terms of their whitening effects of human incisors. It was hypothesized that Hydrogen Peroxide would be more effective than fluoride in removing stains and therefore would produce a more perceptible color change on teeth.

In this experiment human incisors taken from pre-menopausal females were firstly coloured in staining solutions and then whitened using two whitening agents that contained hydrogen peroxide and fluoride. For 7 days, the teeth were kept in coffee, tea and sour cherry juice which are widely used in staining processes. The initial and final color tones of the teeth were measured with a colorimeter which measures the color tone in terms of white-black, red-green and yellow-blue tones. The total color changes were calculated using the formula from CIELAB Color Scale;

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta b)^2 + (\Delta a)^2}$$

After, the staining process is completed teeth are then divided into two groups of 15 teeth, randomly chosen 5 sample from each staining solution. One of the groups was cleansed using “Pola Day SDI Advanced Whitening System” containing hydrogen peroxide and the other using “Opalescense Toothpaste” contaning fluoride.

It was seen in the experiment that hydrogen peroxide has shown a greater effect on changing the color tones of the teeth than Fluoride. The mean colour change values are 5.571 for Fluoride and 14.138 for Hydrogen Peroxide. These values are the mean values of the total colour changes calculated from the 2nd and the 3rd measurements. The main reason for taking the last two measurements for calculations is that the whitening effect of the agents is observed in the second stage of the experiment. It can be clearly observed that mean colour change is greater for hydrogen peroxide. To decide whether there is a statistical difference between these mean values a t-Test is made.

The null hypothesis formed for the t-Test stated there is no significant mean difference between fluoride and hydrogen peroxide in terms of their effect on tooth color. The test revealed that the p value is 0.0038, which is smaller than the alpha value (0.05). Therefore, the null hypothesis is rejected and the alternative hypothesis, stating there is a significant mean difference between the groups in terms of their whitening effect is accepted. With the result of the statistical test, main hypothesis stating “teeth treated with Hydrogen Peroxide will show a greater colour change than the teeth cleansed with Fluoride” is supported.

The standard deviation values for hydrogen peroxide and fluoride are 2.60 and 11.25 respectively. Since the standard deviation indicates the reliability of the distribution of the data within a group, the value for Hydrogen Peroxide is quite high when we compare it to the average value, which mean that the data are not thoroughly consistent.

Graph-1 shows the teeth were whitened to different degrees with different types of agents. Since the independent variable does not show a continuous trend, a bar graph was found more appropriate for the data. The error bars were drawn according to the standard deviation values reflecting the data interval.

Factors such as gender, age, hereditary characteristics and external factors such as cracks on tooth enamel all affect teeth discoloration. To set up a reliable experiment the effect of these factors need to be minimized. As stated in introduction, the women’s teeth have more tendency to undergo discoloration than the male teeth, thus female teeth were chosen in order to observe the discoloration better. The teeth were taken from females aged 18 – 40 which is the pre-menopausal stage. Although the teeth belong to different females, it is impossible prevent the differences caused from the initial tones of the teeth and the staining rates. However, it’s possible to minimize the effects of these differences; 30 samples were collected and each measurement was repeated 3 times reducing the effect of random variation. Having many samples allowed us to have a variety of initial color tones, so the average values gave out a far more reliable data. Measurements were repeated 3 times to minimize the errors caused by the faulty measurement of the colorimeter.

Although the factors mentioned above were considered while the experimental steps were being planned, there were several systematic errors made in conducting the experiment, leading to more inaccurate results. These errors and possible improvements can be discussed as the following;

The main systematic error arose from the differences between the application of the agents. The fluoride group was brushed while the hydrogen peroxide group was just kept in the gel. Since the aim of the essay is to investigate the home-applied product efficiency it was planned to use the substances in the way the manufacturers instructed but it is clear that this decreases the reliability of the results. The experiment can be repeated with agents with the same application methods.

Secondly, in the staining step the pH of the solutions used were not taken into consideration. Research has shown that at a pH of 6 the staining of the enamel is at a maximum, and decreases with the decreasing pH.[16] If the pH level of the staining solutions were different the discoloration is clearly affected. To overcome this problem, we must measure the pH of the solutions prepared to stain the tooth enamel with a pH meter.

Although female incisors within a certain age limit were chosen, various hereditary characteristics cannot be controlled which perform as a random error since it is effective on the rate of discoloration. For example if certain samples were stained more than the others, the time it takes to establish the whiteness increases and since the experiment is carried out in limited time the results are affected.

As mentioned in introduction, some studies on the effect of time on whitening have found that if applications are spread over an extended time period like 6 months, are more effective on whitening. Although these studies are mostly on bleaching the whitening by gels and toothpastes have the same characteristics, thus time is a limiting factor in this

[16] R. Nakamura et al. "*Influence of pH on discoloration of bovine enamel" 3 Dec 2010.* <http://iadr.confex.com/iadr/japan07/preliminaryprogram/abstract_99424.htm>

experiment. If the time period can be enlarged in a future repetition the study may provide more accurate results.

The brands of the agents are also limiting factors since only one brand from each agent is used, it cannot be said that they reflect all the properties in their class and therefore this prevents to make generalized statements about the whitening effect of the agents. If the study can be revised with different brands the results may be different since the whitening is affected by all of the ingredients which is different for different brands.

The reason I chose to do my extended essay on this topic is the debate whether the dental whitening products are effective or not. In the experiment the aim was to observe only the whitening effect but since the experiment is carried out with human teeth many factors affected the results. It can therefore be said that the scope of the study is too wide for my capabilities, only professional dentists can carry out such a study efficiently. Although there are a large number of studies on teeth whitening systems, I couldn't find a study on the comparison between the whitening effect of the home-applied products containing hydrogen peroxide and fluoride.

At the end of this study my research question; "Is there a significant difference between whitening agents Hydrogen Peroxide and Fluoride in terms of their effect on the colour change of human teeth ?" is answered in favor which means there is a statistical difference between the effects of these agents.

In conclusion, the whiteness of human teeth has always been a common aesthetic problem and many whitening products have been developed in order to solve this problem. There are many whitening agents available at markets with different ingredients, all of them stating they are the most effective. At the end of this study, another question that is open for further research arises; how does the over-use of the whitening products affect the external structure of the human teeth?

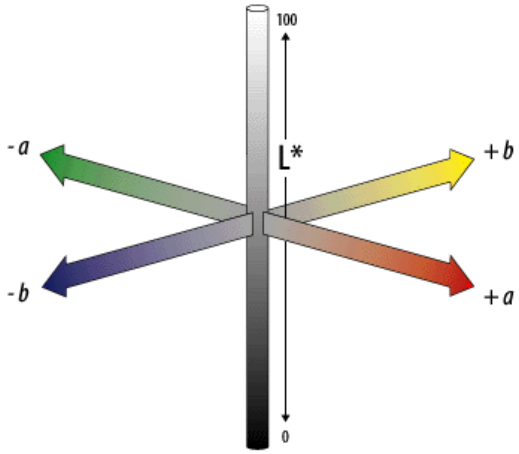
IX. Appendices

a. Appendix I

Below is information about CIELAB Color Scale that is used in data analysis.

CIELAB Color Scale[17]

CIELAB Color Scale is a uniform color scale created for the easy comparison of color values from every . CIELAB is an opponent colour system based on the system of Richard Hunter called L, a, b. It has a cubical form;



In 1960s it was discovered that the retinal colour stimuli are translated into distinctions between light and dark, red and green, and blue and yellow. CIELAB shows these distinctions with 3 axes: L*, a*, and b*. The vertical axis (L*) represents lightness, which run from 0 (black) to 100 (white). The colour axes are based on the fact that a colour can't be red and green, or blue and yellow at the same time since they are opposing to each other. This is why on each axis the values run from positive to negative. The (a*) value shows green-red and (b*) value shows blue-yellow.

[17] Cielab Color Space. 1 Dec 2010.
< www.hunterlab.com/appnotes/an07_96a.pdf>

b. Appendix II

Below is the information about the Manufacturer's Instructions of the Pola Advanced Whitening Sytem. Since the product is expected to be used by costumers the instructions are adapted to the experiment to be applied to extracted teeth.

Manufacturer's Instructions[18]

Pola Day SDI Advanced Whitening System

1. Brush and floss teeth. Take one syringe out of the kit and insert a dispensing tip by twisting it securely onto the syringe.
2. Place a small drop of gel into every compartment of the tray for the teeth undergoing treatment.
3. Seat the tray with the gel around your teeth.

   Wear the trays in your mouth for the time recommended;

10%	**16%**	**22%**
2 hrs to overnight	90 min to overnight	1 x 45 min/day

4. After treatment, remove tray and brush teeth.

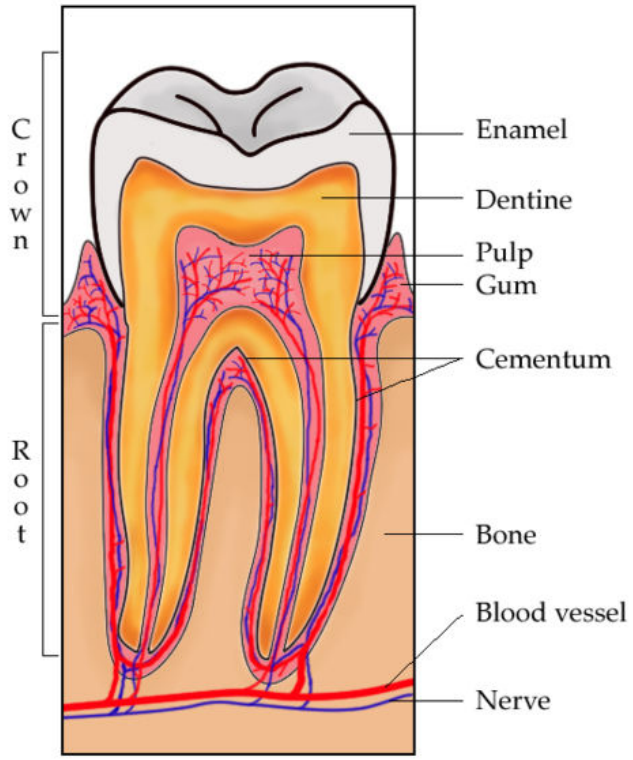
[18] Pola Day Instructions. 28 Dec. 2010 <http://www.sdi.com.au/images/stories/instructions/instructions_pdf/pola_D_N/in_pola_D_N_en.pdf>

c. Appendix III

Dental Structure

Below is a figure showing the dental structure of human. Enamel, dentine which are used throughout the essay is clearly shown. The image is taken from;

"http://www.professionalteethwhitening.org"



d. Appendix IV

Below is information about how the teeth in Fluoride group were brushed in the whitening step. The information is taken from **"www.oralcareshop.com/bass-method.htm"**

Bass Tooth Brushing Technique

Strokes

Press the tootbrush onto the surface of the teeth lightly without bending the filaments.

Vibrate the toothbrush back and forth, apply short strokes. Count at least 10 vibrations.

Apply the brush to the next group of two or three teeth.

Repeat the entire stroke at each position around the upper jaw and lower jaw arches, both for facial and lingual surfaces.

Position the Toothbrush

Direct the nylon filaments upwards for maxillary, down for mandibular teeth at a 45-degree angle. It is safer to place the brush parallel to the axis of the tooth.

Use a short (about the width of half a tooth), gentle back-and-forth motion to clean the outer surfaces of your teeth. Focus on just one or two teeth at a time.

Use this same stroke on the inside surfaces of all the teeth, except the front ones.

Scrub the chewing surfaces of your back teeth with the brush held flat in the same back-and-forth motion.

Tilt the brush vertically, and use the front part of the brush in short up-and-down strokes to clean the inside surfaces of front teeth.

e. Appendix V

Below are the photographs of the experimental setup and the whitening processes.

Figure 1 : Staining. Teeth placed in test tubes, the tubes filled with sour cherry juice, coffee and tea respectively.

Figure 2: Whitening the teeth with hydrogen peroxide. Teeth are left to rest in petri dishes filled with Pola Day whitening gel.

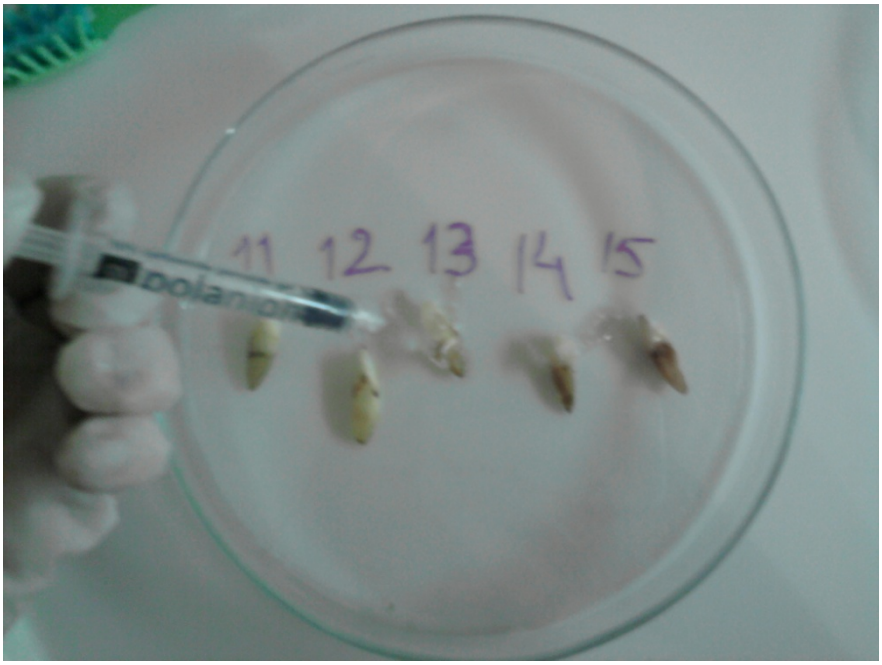
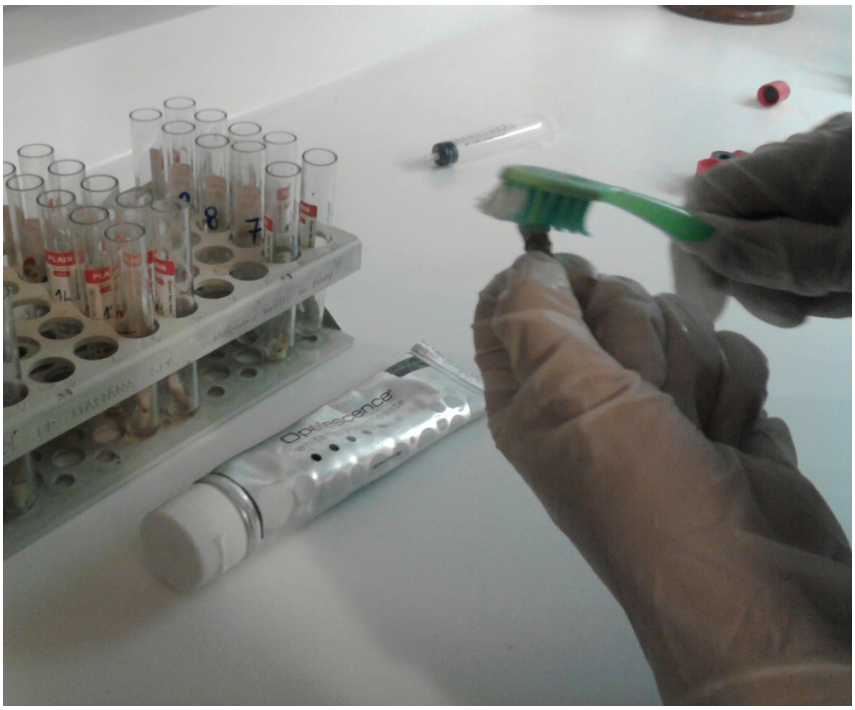


Figure 3 and 4 : Whitening the teeth with fluoride. Teeth are being brushed with Opalescence toothpaste.



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