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The Comparison of the Effects of Fruits and Whitening Toothpaste Using Scanning Electron Microscopy and Eelectromagnetic wave (X-ray) Photoelectron Spectrometer

Seoul-Hee Nam[1], Man-Seok Han[2]*, and Bo-Kyoung Song[3]*

[1]*Dept. of Dental Hygiene, Kangwon National University, Samcheok, Republic of Korea*
[2]*Dept. of Radiological Science, Kangwon National University, Samcheok, Republic of Korea*
[3]*Department of Occupational Therapy, Kangwon National University, Samcheok-si 245710, Repubilic of Korea*

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The purpose of this study is to investigate the stability of natural teeth whitening by analyzing the change on the tooth enamel surface and major mineral components, as well as tooth whitening using a natural material, such as fruit juice. Only the buccal surfaces of 20 premolar teeth extracted were assigned to 5 groups (n=4). For Group 1, normal saline was applied; for Group 2, strawberry and baking soda were mixed at a ratio of 3:1; for Group 3, lemon and baking soda were mixed at a ratio of 3:1; and for Group 4, baking soda and water were mixed at a ratio of 3:1. About 1 ml of each material was applied on the enamel surface using a toothbrush. For Group 5, 5 mm whitening toothpaste was applied using a toothbrush. Color changes using the CIELAB, pH measurement using a pH meter, surface morphology using scanning electron microscopy (SEM), and mineral content using electromagnetic wave (X-ray) photoelectron spectrometer (XPS) were measured. The whitening effect was highest in the teeth applied with lemon compared to the whitening toothpaste. The pH value was lowest with the lemon application, which showed a distinctly rough surface and porosity. The Ca and P values were lowest in the tooth surface whitened with lemon. It was confirmed that tooth whitening with natural fruit juice increased the roughness of the tooth surface due to the acidic low pH. It also caused enamel demineralization and posed harm to the teeth.

Keywords : teeth whitening, fruits, scanning electron microscopy (SEM), electromagnetic wave (X-ray) photoelectron spectrometer (XPS)

1. Introduction

As quality of life improves, modern people develop a desire for beautiful teeth and beautiful smiles that go beyond mere treatment of diseases. For this reason, patients have shown great interest in aesthetic treatment as well as functional recovery [1]. With this new demand, dentists are performing a considerable number of procedures to meet such need. Of these, a relatively easy and inexpensive procedure that is frequently performed is teeth whitening, which is an important procedure indispensable to aesthetic treatment [2].

Teeth discoloration is classified into extrinsic discoloration and intrinsic discoloration. When discoloration occurs by polysaccharide or protein adhering to the tooth surface, it is called extrinsic discoloration, while intrinsic discoloration occurs due to the internal change in the tooth by systemic or local causes [3]. A typical example of the natural factor of tooth discoloration is tooth discoloration due to aging. Pathological factors include the changes limited to the enamel such as discoloration into brown or black due to ingestion of many beverages containing pigment, such as coffee or cola, or nicotine. Excessive drug ingestion during tooth formation or excessive fluoride ingestion during enamel formation also results in tooth discoloration [4]. However, tooth discoloration, by itself, has considerable effect on the patient's smile and causes mental or social disability [5].

The main ingredient is hydrogen peroxide (HP), currently used in clinical practice, or carbamide peroxide (CP), for which hydrogen peroxide can be indirectly used. The whitening agent that is actually used has 30 % to 50 % high concentration hydrogen peroxide or 35 % to 40 % CP [6]. A whitening agent can easily be bought from

*Co-Corresponding author: Tel: +82-33-540-3383
Fax: +82-33-540-3399, e-mail: angio7896@naver.com
Tel: +82-33-540-3483, Fax: +82-33-540-3489,
e-mail: bksong@kangwon.ac.kr

shops or pharmacies in response to the change in the demand for tooth whitening, and most of the whitening agents sold on the market contain 10 % CP at a low concentration and includes 3.6 % HP. Although it contains low concentration HP, it has been reported to pose harm to the hard and soft tissues in the oral cavity due to HP [7]. As such, active oxygen generated in the process of decomposing hydrogen peroxide, which is widely used as a tooth whitening agent, penetrates into the surrounding periodontal tissues through the dentinal tubules. This results in cervical absorption, enamel shape and hardness change, hypersensitivity, and pulp injury [8]. HP also destroys the structure of organic compounds in the tooth enamel, reduces the hardness of teeth and causes corrosion [9]. In addition, tooth whitening agents containing 5 % or more HP can cause corrosion when they are in contact with the gums, skin, and mucous membranes. When tooth whitening is performed for a long time with a high concentration of hydrogen peroxide, minerals such as calcium and phosphorus are released from the teeth, weakening the teeth [10].

Today, most people want to be able to whiten their teeth easily with everyday brushing, so whitening toothpastes, which have been commercialized by reflecting the demand, have become popular. These whitening toothpastes contain various chemicals such as enzyme, detergent, and oxygen supply agent [11]. Whitening toothpastes currently available in the market have been known to have adequate abrasion power and function of removing pigmentation by chemical substances [12]. The function of whitening toothpaste is excellent because whitening can be done for a long period of time through the action of chemical substances contained in the toothpaste, and teeth whitening can be easily performed as an ordinary activity [13]. It has been reported that the whitening effect is improved when the concentration of HP in the preparation of whitening dentifrice is higher [14].

However, in recent years, there has been a growing interest in alternative whitening using natural whitening agents such as vegetables or fruits due to the various side effects of tooth whitening as well as safety concerns. Consequently, research on whitening using natural materials is increasing. This study sought to investigate the whitening effect of natural materials and the stability of natural whitening through analysis of the change on the tooth enamel surface and the major components of teeth.

2. Materials and Methods

2.1. Tooth preparation

The teeth used in this study were carefully selected by observation with a stereomicroscope (SZ-CTV, Olympus, Tokyo, Japan), and only the premolar teeth extracted from humans without morphological abnormalities were used. The 20 teeth selected were cut into buccal and lingual surfaces with a low-speed handpiece and a cutting disk. Only the buccal surfaces were used, and the manicure was applied on the cut surface to prevent the material from penetrating the tooth.

2.2. Tooth bleaching procedure

Twenty buccal surfaces of premolar teeth were randomly selected and divided into five groups of four teeth for tooth whitening. Table 1 shows the application of whitening to each group. For Group 1, a control group, 1 ml of normal saline was applied on the enamel surface. For Group 2, 1 ml of a mixture of 15 ml strawberry and 5 ml baking soda at a 3:1 ratio was applied on the enamel surface using a toothbrush. For Group 3, 1 ml of a mixture of 15 ml lemon and 5 ml baking soda at a 3:1 ratio was applied on the enamel surface using a toothbrush. For Group 4, 1 ml of a mixture of 15 ml baking soda and 5 ml water at a ratio of 3:1 was applied on the enamel surface using a toothbrush. For Group 5, 5 mm of 15ml whitening toothpaste was applied on the enamel surface using a toothbrush. After applying on the teeth three times a day (morning, lunch time, and evening) for three minutes, the teeth were washed with sterile distilled water so that no substance remained on the surface. Then they were immersed in normal saline for storage. The normal saline was replaced three times a day, and this procedure was repeated for a total of four weeks.

Table 1. Materials used in this study.

Experimental group	Manufacturer
Control (PBS): 1 day 3 min 3 times/4 weeks	Klenzo (JW Pharmaceutical Corp, Seoul, South Korea)
Strawberry: 1 day 3 min 3 times/4 weeks	Sancheong strawberry (Well-being Green Tree Farm, Sancheong, South Korea)
Lemon: 1 day 3 min 3 times/4 weeks	Sunkist lemon (Sunkist Growers, Oxnard, CA, USA)
Baking soda: 1 day 3 min 3 times/4 weeks	Sodium bicarbonate (Samchun Chemical Co., Ltd, Pyeongtaek, South Korea)
Whitening toothpaste: 1 day 3 min 3 times/4 weeks	3D WHITE (Crest, Toronto, ON, Canada)

2.3. Analysis of bleaching efficacy

The teeth were photographed before treatment and after four weeks of treatment for tooth color change. The images were stored in a personal computer with the Image-Pro Plus 5.1 software (Media Cybernetics Inc., Washington, DC, USA). The overall colour changes (ΔE) were assessed on the basis of the Commission Internationale de L'Eclairage (CIE, 1979) Lab Colour System [15]. The L* value represents the degree of lightness within a sample and ranges from 0 (black) to 100 (white). The a* value represents the degree of greenness (negative a*) or redness (positive a*), whilst the b* value represents the degree of blueness (negative b*) or yellowness (positive b*) of the sample. The differences in the values of L*, a*, and b* in each group were measured using Adobe Photoshop CS2 (Adobe Systems, San Jose, CA, USA), and ΔE was calculated for each tooth by using the CIELAB equation:

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

2.4. pH measurement

The pH of each mixed material used in each group was measured using a pH meter (Water quality pH meter, LAQUA, HORIBA, Japan). After measuring the pH value of one group, the glass electrode was washed with sterile distilled water, and then the pH value of the next mixture was measured.

2.5. SEM observation

In order to observe the change on the surface due to tooth whitening, scanning electron microscopy (SEM; S-4300, Hitachi Co., Japan) was performed in the central laboratory of Kangwon National University. The teeth were dried and coated with platinum and observed at a magnification of 2000X with 15 kV acceleration voltage.

2.6. Electromagnetic wave (X-ray) photoelectron spectrometer analysis

To quantitatively analyze the changes in the major components of the enamel surface, calcium (Ca) and phosphorous (P) were measured using an electromagnetic wave (X-ray) photoelectron spectrometer (XPS; Thermo, K-alpha+, Thermo Scientific Ltd, East Grinstead, UK) in the central laboratory of Kangwon National University. The spot size on the enamel surface was detected at 400 μm, acceleration voltage of 0.1 eV, and binding energy (BE) of 50 eV, and the peak BE on the enamel surface was measured at 3 points per specimen.

The energy of X-ray photon is related to the radiation frequency by

$E = h\nu$

h = Planck's constant (6.626×10^{-34} J/s or 4.135×10^{-15} eV/s)

v = frequency

The wavelength λ is related to the photon energy by

$\lambda = 1.240 \times 10^{-6} / E$

2.7. Statistical analysis

The SPSS program (Version 20.0, SPSS, Chicago, IL, USA) was used for statistical analysis. Changes in ΔE, pH and the main contents (Ca and P) among the groups were submitted to a one-way analysis of variance (ANOVA), complemented by Tukey's test. All analyses were performed with a $p < 0.05$ for significant differences.

3. Results

3.1. Teeth color change

Table 2 shows the mean values and standard deviations of ΔE of the teeth after whitening for four weeks. The result of observation on the change in color after four weeks showed that there was a difference between the groups ($P < 0.05$), and the color change was highest in the teeth applied with lemon.

3.2. Change of pH

The pH values of all groups were found to significantly

Table 2. The mean ΔE ± SD and P-values obtained in 4 weeks of tooth bleaching.

Experimental group	Mean ΔE ± SD	P-values
Control (PBS)	2.71 ± 0.89^a	
Strawberry	$33.84 \pm 4.17^{b,c}$	
Lemon	37.27 ± 6.54^c	0.021^*
Baking soda	23.89 ± 3.27^b	
Whitening toothpaste	$28.97 \pm 2.17^{b,c}$	

*p-values are determined by one-way ANOVA with post-hoc Tukey HSD ($p < 0.05$).

Table 3. The mean pH ± SD and P-values of all groups used for tooth bleaching.

Experimental group	Mean pH ± SD	P-values
Control (PBS)	7.38 ± 0.03^d	
Strawberry	3.84 ± 0.04^b	
Lemon	2.21 ± 0.03^a	0.000^*
Baking soda	7.58 ± 0.02^e	
Whitening toothpaste	6.98 ± 0.06^c	

*p-values are determined by one-way ANOVA with post-hoc Tukey HSD ($p < 0.05$).

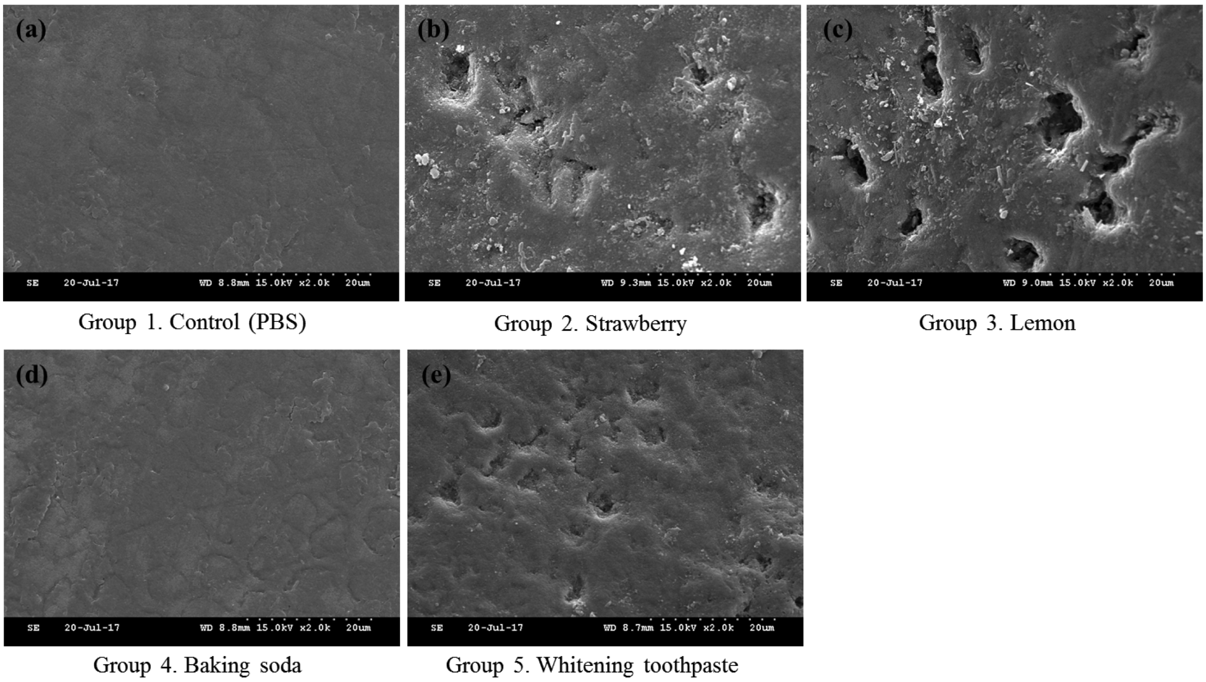

Fig. 1. Representative SEM photomicrographs (× 200). Change in enamel surface after 4 weeks of tooth bleaching.

differ between the groups (P < 0.05), and a low pH, which is acidic, manifested in the group that used lemon (Table 3).

3.3. Surface morphology changes

After applying tooth whitening for four weeks, the change on the tooth surface was observed through SEM, and the results are as shown in Fig. 1. Compared to the enamel surface of the control group, the surface of the lemon-applied tooth was distinctly rough, and the surfaces of the teeth whitened with strawberry and whitening toothpaste were slightly rough.

3.4. Mineral content using electromagnetic wave (X-ray) photoelectron spectrometer

Table 4 shows the peak BE difference in Ca and P in the enamel after bleaching with the use of XPS. The results of the composition analysis of Ca and P four weeks after whitening revealed a significant difference between the groups (P < 0.05). In terms of the changes in the Ca and P levels, the Ca value was lowest in the lemon-whitened tooth surface compared to the control group, while the P value was also lowest in the lemon-whitened tooth surface, indicating demineralization of the main mineral components of the tooth.

4. Discussion

As people want to look more beautiful due to the influence of the media, more and more people tend to want whiter teeth. Economical and simple tooth whitening is preferred, which can minimize the amount of tooth removal, rather than aesthetic prosthodontics that require teeth removal [16].

The oxidizing agent used for tooth whitening changes the organic substrate of the tooth tissue by chemical oxidation-reduction reaction and reaches the discoloration pigment in the tooth, thereby exhibiting the whitening

Table 4. The mean peak BE ± SD and P-values of the Ca and P values of enamel in all groups using electromagnetic wave (X-ray) photoelectron spectrometer.

Experimental group	Mean peak BE ± SD					P-values
	Control (PBS)	Strawberry	Lemon	Baking soda	Whitening toothpaste	
Ca	347.18 ± 0.04^{a}	346.98 ± 0.06^{b}	346.29 ± 0.04^{c}	347.04 ± 0.03^{b}	347.03 ± 0.03^{b}	0.000*
P	133.22 ± 0.03^{a}	132.96 ± 0.04^{c}	132.31 ± 0.05^{d}	133.06 ± 0.04^{b}	133.03 ± 0.03^{b,c}	

*p-values are determined by one-way ANOVA with post-hoc Tukey HSD (p<0.05).

effect [17]. This whitening process has the effect of brightening the teeth, but also leaves many side effects on the teeth and surrounding supporting tissues [18]. The side effects of tooth whitening are directly related to the HP concentration used. The use of low concentrations of HP is relatively safe, but repeated use will cause the same side effects as when high doses are used for a short period [14].

As the types and functions of toothpaste have been diversified recently, whitening toothpastes made of various chemicals have also become popular [11]. Research and development has been conducted on whitening toothpaste formulations made by mixing low-concentration HP with toothpaste ingredients. It has been reported that the whitening action of whitening dentifrices is made by a hydroxide preparation, and whitening is done by decomposing and removing the organic matters adhering to the tooth by oxidation [19]. However, studies on the side effects of tooth whitening and changes in the teeth have been lacking. Due to the side effects of HP, the interest in selecting a biocompatible whitening agent, for which the increased tooth whitening effect and stability are guaranteed, tends to rise. Therefore, this study was conducted to identify the usability of strawberry, lemon, and baking soda for natural whitening.

After tooth whitening for four weeks, the color change was observed in the following order: 37.27 ± 6.54 in lemon, 33.84 ± 4.17 in strawberry, and 28.97 ± 2.17 in whitening toothpaste. Consequently, it was found that the whitening effect of the fruit juices was 1.29 and 1.17 times higher than that of whitening toothpaste when lemon and strawberry were applied, respectively. The change on the tooth surface after whitening is dependent on the pH value. An acidic environment below pH 5.5 accelerates the demineralization of minerals, and frequent contact with corrosive chemicals causes demineralization from the surface of the hard tissue of the tooth [9]. The results of this study showed that the lowest pH value was 2.21 ± 0.03 in lemon followed by 3.84 ± 0.04 in strawberry, indicating that the environment is acidic. Also, the pH value of whitening toothpaste was 6.98 ± 0.06, indicating that it did not reach the neutral. The same results are also found in the observation of the surface change using SEM. Distinctly rough surface and porosity were shown on the surfaces of the teeth applied with lemon, while the surfaces of teeth whitened with strawberry and whitening toothpaste were also rough compared to the control group. It has been reported that the surface is roughened as minerals exposed by whitening flow out [20]. Based on these previous studies, the morphological changes shown in the present study can be attributed to the widened size of micropores as the inorganic crystal structure decomposes. McCraken and Haywood's study [21] reported a relative decrease in the concentration of Ca and P in the exposed tooth structure after whitening and deformation of the crystal structure of the enamel surface. In this study, the Ca and P values were found to be the lowest on the surfaces of lemon-whitened teeth, followed by strawberry-whitened teeth, showing that Ca and P components of the teeth in these groups were decomposed. This implies that tooth whitening with lemon is detrimental to teeth because the major minerals are demineralized. These results indicate whitened enamel, which can be attributed to the loss of mineral content due to demineralization [22].

The above results show that the whitening effect of the natural materials is higher than that of the whitening toothpaste, but since the surface of the tooth is acidified due to its low pH, mineral components such as calcium and phosphorus are dissolved, micropores are generated, and the surface becomes rough. It can be confirmed that the acid destroys the main components and causes tooth demineralization. There is a significant contradiction between the effect of actual tooth whitening and the structural change in teeth. Therefore, serious damage may accelerate due to the weakened tooth structure after whitening, so it is necessary to ensure stability when fruit juice is used as a natural whitening agent.

5. Conclusions

The purpose of this study was to identify the applicability of natural materials for natural teeth whitening to achieve superior whitening efficacy and safe whitening. In comparison with the whitening toothpaste, lemon and strawberry exhibited the whitening effect, but were found to be harmful to teeth because their acidic low pH cause demineralization of tooth enamel. Therefore, application as a natural whitening agent would be inadequate, and further research on biocompatible and safe tooth whitening is needed.

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