

A Study on the Whitening Effect of Fruit Extracts and the Changes in the Components of the Teeth

Jung-Hyun Lee and Sung-Suk Bae*

Department Dental Hygiene/ Hanseo University, Korea;
911105jh@naver.com, ssbae@hanseo.ac.kr

Abstract

Background/Objectives: In this regard, this study aimed to examine the teeth-whitening effect of undiluted and diluted extracts of Korean natural citron, grapefruit, and plum and analyze the components of teeth. **Methods/Statistical Analysis:** In this study, 55 premolars in the upper and lower jaw extracted in recent few months were used as test pieces. Extracts of citron, grapefruit, and plum were used as solutions. Each of the test pieces was deposited into undiluted solution and diluted solution (one to one ratio of undiluted solution in distilled water) for 3, 6, and 9 hours, respectively. The color difference was examined using a Spectrophotometer (CM-3500d, Minolta, Japan). Changes in components of the teeth after they were deposited into the extracts of citron, grapefruit, and plum were compared to their initial state. SPSS ver. 20.0 was used for statistical analysis. Mann-Whitney test was performed to compare the pre-post difference within and between test pieces. Kruskal-Wallis test was implemented to compare the averaged values of E* (color difference) by time. Tukey's test was carried out with 95% confidence interval for the post test. **Findings:** The whitening effect of citron extract was increased as time went by. The diluted solution with one to one ratio of the citron extract in distilled water showed the highest whitening effect after depositing for 3 hours. The whitening effect of grapefruit extract was increased as time went by. The diluted solution with one to one ratio of the grapefruit extract in distilled water showed the highest whitening effect after depositing for 6 hours. Plum extract had the highest whitening effect after depositing for 6 hours. The diluted solution with one to one ratio of the plum extract in distilled water showed the highest whitening effect after depositing for 3 hours. Components of P and Ca were irregularly increased or decreased in test pieces both before and after the depositions of teeth into fruit extracts. **Improvements/Applications:** In this study, the colors of teeth in extracts of citron, grapefruit, and plum turned brighter than the initial color as time went by. Follow-up studies are needed.

Keywords: Citron, Grapefruit, Natural Liquid Fruit, Teeth-whitening, Teeth Whitening Effect

1. Introduction

Dental bleachers currently used in the dental clinics contain carbamided peroxide, sodium perborate, and hydrogen peroxide that might cause side effects such as root resorption, dentin sensitivity, and stomatitis.¹ Tooth enamel is composed of minerals (96%) and organic

matters and water (4%). Mineral crystals are opaque with organic proteins present between them. Consequently, pigments may soak into this space.² Tooth whitening has been consistently implemented since its introduction. Various kinds of dental bleachers have been developed, produced, and used in many countries. In South Korea, various kinds of dental bleachers are being imported in

*Author for correspondence

various ways such as expert bleaching and home bleaching³. In addition, recently, Over-The Counter (OTC) bleachers have been popularized. They can be used without prescriptions from experts. As representative OTC bleachers, whitening toothpaste and patches containing hydroxyapatite and peroxide compounds are commonly used⁴. However, chemically synthesized dental bleachers might have side effects if are wrongfully used. Under these circumstances, more emphasis has been recently put on the assurance of their stability for the human body. There has been mounting interest in alternative whitening using vegetables and fruits as sources of natural dental bleachers. As a result, the assurance of their stability for the human body has recently become important and more interest arises in the alternative whitening using vegetables or fruits as natural dental bleachers. Studies on whitening with natural materials are on the rise. The whitening effect of citron has been identified in previous studies. It is currently used for skin care.⁵ The extract of grapefruit has been found to have antimicrobial effect.^{6,7} The extract from plum has been reported to have function of depressing the growth of melanin.⁸ In addition, its antimicrobial effect is increased as time goes by and when its concentration is increased.⁹ Citron, grapefruit, and plum extracts are Koreans' favorite fruit extracts. They are consumed in various ways. In this regard, this study aimed to provide basic data for developing natural dental bleachers. To meet this purpose, this study deposited extracted teeth pieces into Korean natural citron, grapefruit, and plum extracts to determine their whitening effects. In addition, the components of the teeth before and after bleaching by the three fruit extracts were analyzed.

Recent research on whitening has been performed in various ways, including expert dental bleaching and components of bleachers.^{1-4,10-23} As the result of analysis of color difference in this study, it was found that the values of E^* of tooth piece was increased after it was deposited in the extracts of citron, grapefruit, and plum compared to the initial value of the test piece, demonstrating that these fruit extracts have a statistically significant whitening effect ($P < 0.05$). Jung et al.²⁴ have also examined the teeth-whitening effect of several species of medicinal herbs. They deposited carious teeth in their extracts for 16 hours and verified their whitening effects based on the fact that test pieces turned brighter compared to their initial color. Meanwhile, Kim et al.¹² have used chemically synthesized whitening tooth paste for 3 weeks and determined its tooth-whitening effect compared to a general

toothpaste. They found significant difference at 4 weeks after using the tooth-whitening toothpaste with significant color change. Lee²¹ has reported that carbamide peroxide and hydrogen peroxide are not carcinogenic. However, they might stimulate chemical carcinogenicity. Ham¹ and Gang¹⁰ have reported that the use of highly concentrated carbamide peroxide, one of the main components in dental bleachers, might cause dentin hypersensitivity and changes in enamel and gingiva as well as excessive saliva. In other words, the whitening effect of safe and natural dental bleachers (i.e., the extracts from citron, grapefruit, and plum) can significantly increase the values of E^* . There is a need to perform further experimental research to find natural materials for the development of natural dental bleachers.

As the result of analysis of the enamel surface of the deposited test pieces before and after they were deposited in fruit extracts, it was found that the ratio of the values of P and Ca were decreased compared to those before the deposition, while the opposite was true for grapefruit extract. Kim²⁵ has performed component analysis using a scanning electron microscope and reported that the values of P and Ca of the teeth are decreased as the acidity of the drink deposited for 15, 30, 60, and 120 minutes is intensified with the passage of time. Lee²⁶ has found that enamel erosion has occurred in the anterior teeth deposited in orange juice with pH level of 4 for 10 minutes. In addition, the micro-hardness value was decreased, eventually causing an erosion on the enamel surface. Other research findings²⁵ has implied that soft drinks if frequently consumed can also influence the calcium and phosphorus contents of the enamel. Tooth deposition analysis of teeth deposited in natural fruit extracts also provided the same implication. Meanwhile, Kim et al.²⁷ have found that if the teeth are deposited in a drink with Ca added, the damage to the surface of enamel will be reduced. In this regard, a careful examination of the decrease in P and Ca contents is needed based on the results of previous studies. Additional studies are needed to verify the results of this study.

2. Proposed Work

Step 1: Producing test pieces

Teeth pieces for the experiment were premolars of the upper and lower jaws extracted due to orthodontic procedures or periodontitis. They were healthy without history of enamel hypoplasia, dentin exposure, or cavity treatment. This study prepared a total of 55 devitalized

teeth pieces, including 1 untreated tooth and 54 pieces assigned to citron, grapefruit, and plum extract (18 pieces per extract). Tissues, residues, tartars, and exogenous paint attached to the prepared teeth were removed with an ultrasound scaler and a curette to scrap off periodontal tissues. These teeth were polished with pumice containing glycerin, deposited in Perasafe® solution for 30 minutes for sterilization, washed with an ultrasound washer, and immersed in sterile distilled water. A total of 55 moulds were produced to facilitate forming the molds of the test pieces by cutting a round plastic pipe (diameter: 38 mm; height: 12 mm) with a high speed cutter. Resin-clay which was easy to form molds without the need of high pressure was used to fill the moulds and fix the extracted teeth.

Step 2: Solutions

Extracts of citron, grapefruit, and plum grown in South Korea and their diluted extracts (with one to one ratio of extracts vs. distilled water) were prepared as shown in Table 1. Each extract was put into a 30 ml experimental plastic beaker for 3, 6, and 9 hours. Meanwhile, diluted extracts were produced by mixing the extracts (15 ml) with distilled water (15 ml) at one to one ratio. Afterwards, each dilution was put into an experimental plastic beaker for 3, 6, and 9 hours. The extracted teeth (n = 18) were put into the prepared beakers (3 in each beaker) and deposited in the extract or diluted extract. The teeth pieces were stored at temperature of 37°C for 3, 6, and 9 hours and dried in a Drying Oven (C-DO, Korea) to simulate oral condition after the deposition.

Table 1. Bleaching solution used in this study

Product	Brand name	Manufacturer
citron concentrate	ES Food	Young Woo Food, Korea
Grapefruit concentrate	ES Food	ES OEM Production, Korea
Chinese plum concentrate	ES Food	Samjin Food, Korea

Step 3: Whitening measurement

To measure whitening, a spectrophotometer (CM-3500d, Minolta, Japan) was used to measure the initial colors of the test pieces and the final colors after being deposited in fruit extracts. The spectrophotometer

measurement values were obtaining for the values of E^* after identifying the values of L^* , a^* , and b^* , using Spectra magic program of the spectrophotometer connected to a computer. The program could mathematically transform the tristimulus color. Each of the test pieces was measured from the center twice. The formula of the values of E^* was expressed as follows.

$$E^* = \{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\}^{1/2}$$

where, E^* was the value of color change of the test pieces, L^* was the value of the brightness of the test pieces - the brightness of the object, 0 (black) to 100 (white), a^* was the chroma of the test piece - the value of the degree from red to green color (the closer to 0, the closer to white color), b^* was the chroma of the test piece - the values of the degree from yellow to blue color (the closer to 0, the closer to white color).

Step 4: Observation through a scanning electron microscope

Among test pieces deposited in the extracts of citron, grapefruit, and plum that were examined by a spectrophotometer, those with averaged values of L^* and E^* being the highest were chosen for scanning electron microscope (SEM) analysis. Using a scanning electron microscope (JEOL JSM-5600, Japan), the surface of the enamel of the test piece was examined by a factor of 16. Their components were analyzed to identify the difference from their initial status.

Step 5: Statistical analysis

SPSS ver. 20.0 (SPSS Inc. Chicago) was used to analyze whitening effect and changes in components. Samples used in the experiment were utilized in a non-parametric test. Mann-Whitney test was performed to effectively verify the pre-post difference within and between test pieces. Kruskal-Wallis test was performed to compare the averaged values of E^* by deposition period. Tukey's test was implemented with a 95% confidence interval for the post test to identify the averaged difference in the values of E^* of each group. Statistical significance level was set at 0.05.

Step 6: Ethical consideration

This study was an experimental study on extracted teeth using human materials. It was performed after receiving the approval from the IRB of the National Bioethics Committee regarding the research ethics. The study was exempted from deliberation (Project Management No. : P01-201511 -31-002).

3. Results

Step 1: Color difference of the test pieces deposited in fruit extracts

Results of test pieces deposited in each fruit extract and examined with the spectrophotometer are summarized in Table 2. The values of E^* of test pieces deposited in citron and grapefruit extracts were found to have significantly higher whitening effect as time went by. The highest whitening effect was found in plum extract after depositing for 6 hours followed by deposition in plum extract for 9 and 3 hours ($P < 0.05$). For the diluted extract of citron (one to one ratio of distilled water and citron extract), the higher whitening effect was obtained for test piece deposited in the diluted extract for 3 hours, followed by that after deposition for 9 and 6 hours. In the diluted extract of grapefruit extract (one to one ratio of distilled water and grapefruit extract), the highest whitening effect was found for test piece deposited in the diluted extract for 6 hours, followed by that after deposition for 9 and 3

hours. In the diluted extract of plum extract (one to one dilution of plum extract in distilled water), the highest whitening effect was found for test piece deposited in the diluted extract for 3 hours followed by that after deposition for 6 and 9 hours ($P < 0.05$).

Step 2: Changes in components before and after deposition

A scanning electron microscope (JEOL JSM-5600) was used to examine the changes in components of teeth before and after the test pieces were deposited in fruit extracts for 6 hours to determine the difference before and after the deposition. Results of the measurement are shown in Table 3.

The ratios of P and Ca of the test pieces before their deposition were 10.85 and 20.43, respectively. After deposition in the citron extract for 6 hours, the ratio of P (9.64) and Ca (17.15) were both reduced. The same was true for test pieces deposited in diluted citron extract, with the ratios of P and Ca of 10.42 and Ca 20.30, respectively. However, after

Table 2. Color change of L^* and E^* values after settling in extract over time

Division	Time	N	L^*		E^*	P
			Before	After		
citron an undiluted solution	3h	3	69.10	71.30	6.15 ^a	.000
	6h	3	69.81	73.00	8.17 ^b	
	9h	3	68.53	71.68	10.03 ^b	
Distilled water diluent(1:1)	3h	3	67.38	70.65	7.35 ^b	.000
	6h	3	68.68	71.60	6.30 ^a	
	9h	3	67.46	70.83	7.11 ^b	
Grapefruit an undiluted solution	3h	3	65.74	66.93	1.86 ^a	.000
	6h	3	66.69	68.10	2.02 ^a	
	9h	3	65.42	66.60	2.91 ^b	
Distilled water diluent(1:1)	3h	3	68.18	70.29	2.49 ^a	.000
	6h	3	69.02	70.14	4.12 ^b	
	9h	3	71.02	72.58	3.62 ^a	
Chinese plum an undiluted solution	3h	3	63.92	65.15	1.56 ^a	.000
	6h	3	66.55	67.17	2.52 ^b	
	9h	3	69.03	70.48	2.17 ^b	
Distilled water diluent(1:1)	3h	3	68.36	71.13	5.52 ^a	.000
	6h	3	67.35	70.69	7.45 ^b	
	9h	3	64.45	66.67	5.32 ^a	

Values are reported as the mean

P-values were determined by Kruskal-Wallis test

a.b. means followed by different letters are significantly different

deposition in grapefruit extract, the ratios of P and Ca were increased to 13.27 and 24.90, respectively. After deposition in the diluted grapefruit extract, the ratio of P was increased to 12.10 but the ratio of Ca was decreased to 18.99. After deposition in the plum extract, the ratios of P and Ca were decreased to 10.31 and 18.31, respectively. However, after deposition in the diluted plum extract, the ratios of P and Ca were increased to 11.88 and 21.56, respectively.

4. Conclusion

This study identified a meaningful difference in the whitening effect of fruit extracts and component changes of the teeth after deposition in fruit extracts of citron, plum, and grapefruit. A total of 55 extracted teeth were used to produce the test pieces to examine their color changes depending on the duration of their deposition in natural fruit extracts produced in South Korea. The results of this study are as follows:

1. Based on the values of E^* of test pieces, citron extract was found to have significant whitening effect as time went by. In the diluted solution at one to one ratio of the extract with distilled water, the whitening effect of the dilution extract was found to be the highest after 3 hours of deposition.
2. Based on the values of E^* , grapefruit extract had significant whitening effect as time went by. In diluted solution of grapefruit extract, the whitening effect was the highest ($P < 0.05$) after 6 hours of deposition.
3. The whitening effect of the plum extract was the highest after deposition of test piece for 6 hours, followed by that for 9 and 3 hours of deposition. In diluted plum extract, the whitening effect was the greatest ($P < 0.05$) after 3 hours of deposition.
4. Component analysis of the three fruit extracts before and after deposition showed that the ratios of P (9.64) and Ca (17.15) of test pieces deposited in citron extract were decreased compared to their initial values. The same was found for the ratios of P (10.31) and Ca (18.31) of test pieces deposited in plum extract. However, for test pieces deposited in grapefruit extract, the ratios of P (13.27) and Ca (24.90) were increased compared to their initial values.

This study found that natural fruit extracts of citron, grapefruit, and plum had teeth-whitening effect. This may have important implication for the development of natural dental bleachers. However, changes in components of teeth such as P and Ca need to be closely examined. The limitations of this study are as follows. First, this

Table 3. Results of elements analyses (Normalized)

Division	C	O	Na	Al	Si	P	Cl	Ca	Sn	Sb	I	W	%
Incipient (Untreated)	33.72	28.24	0.17	0.11	0.34	10.85	0.34	20.43	0.77	3.11	1.09	0.84	100
citron an undiluted solution	39.51	26.20	0.18	0.11	1.30	9.64	0.32	17.15	0.74	3.10	0.97	0.75	100
Distilled water diluent(1:1)	34.47	27.05	0.17	0.12	1.05	10.42	0.38	20.30	0.78	3.33	1.12	0.79	100
Grapefruit an undiluted solution	19.43	35.01	0.26	.	0.45	13.27	0.53	24.90	0.86	3.22	1.15	0.94	100
Distilled water diluent(1:1)	18.53	44.04	0.35	.	0.40	12.10	0.38	18.99	0.63	2.80	0.93	0.86	100
Chinese plum an undiluted solution	34.31	28.10	0.21	0.11	0.92	10.31	0.30	18.31	1.01	4.42	1.36	0.64	100
Distilled water diluent(1:1)	25.73	31.99	0.24	0.10	0.57	11.88	0.39	21.56	1.04	4.48	1.31	0.73	100

All results in weight %

study used and examined devitalized teeth as study materials. Additional research is needed using vitalized teeth. Second, the standards for mechanical treatment of the equipment for analyzing components were set up as a certain ratio, which might actually prevents it from accurately measuring the increase or decrease of inherent components. Nonetheless, this study provides basic data for the development of natural dental bleachers in follow-up studies in the future.

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