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Component Analysis of Tooth Surface Caused by Fruit Juice using Energy Dispersive X-ray Spectroscopy

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Dental erosion is the irreversible loss of dental hard tissue by acids not by bacteria. The prevalence rate of dental erosion is on the rise these days, and the consumption of acidic beverages is recognized as one of the main risk factors. Therefore, in this study, pH of certain commercial fruit juices was measured to determine whether they cause enamel corrosion of teeth, and the changes in calcium (Ca) and phosphorous (P) were evaluated by quantitative analysis using energy dispersive X-ray spectroscopy (EDS). As the pH decreased, the solubility of Ca increased, and as the exposure time increased, the loss of Ca was greater. Ingestion of fruit juice with a low pH affects the risk of dental erosion, and the retention time of the juice in oral cavity should be shortened as long-term contact with the juice may cause the tooth damage.

Keywords : tooth erosion, fruit juice, energy dispersive X-ray spectroscopy, electromagnetic radiation (X-ray), magnetic field

1. Introduction

Recently, as interest in health has increased, the demand for beverages that can be consumed instead of water is increasing [1]. Beverages not only satisfy the basic human desire to quench thirst, but also satisfy various needs according to preferences and lifestyles. Recently, in the domestic beverage market, the number of people who consume functional beverages is steadily increasing [2]. According to statistics from the National Health and Nutrition Examination Survey, top 30 of average intake by food included the single serving of liquid foods such as milk, beer, soju, cola, fruit juice, and makgeolli [3].

Dental erosion is the gradual absorption of tooth tissues which is caused by chemical irritation rather than bacteria [4]. The main cause of dental erosion is known to be frequent consumption of acidic foods or acidic beverages [5]. Recently, the prevalence of dental caries has declined rapidly from 20 or 30 years ago in Western industrial countries, but that of dental erosion is on the rise worldwide [6]. It shows that the damage to dental hard tissues that are not naturally regenerated tends to accumulate as age increases [7].

Zero reported that the concentration of hydrogen ion of the part that interacts with the tooth surface is important in relation to enamel dissolution [8]. The low pH and the organic acids affect dental erosion, which is the damage to dental hard tissues. According to Beverages Safety Survey conducted by the Ministry of Health and Welfare in 2000, the pH of 42 commercially available beverages was between 2.4-6.2, and 90.5 % of the beverages surveyed showed a pH of less than 5.5. The average pH of the beverages was 3.5, which is low, so it was reported that there was a concern for the tooth damage by intaking them [9]. The critical acidity at which the enamel dissolution occurs is pH 5.5, and acidic foods with a pH lower than 4.0 have a high risk of dental erosion [10]. As such, dental erosion is damage to the dentin that occurs only through direct contact with acid in the oral cavity [11].

Energy dispersive X-ray spectroscopy (EDS) is a representative surface analysis method that can be used in a variety of equipment such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and X-ray spectrometer. EDS is a non-destructive analysis, which forms an image by detecting a secondary electron when the electron generated from an electron beam reaches the surface of the sample, so that not only confirms

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physical information such as microstructure, shape, and size of the surface, but also acquires chemical information by observing the components of the surface [12, 13]. In general, the EDS detection method can analyze various types of samples, such as biological samples, minerals, particles, and metals. Furthermore, the pretreatment time is short, the operation is easy, and elemental analysis is possible in a short time. Therefore, EDS is used for shape and composition analysis in various fields [14-16].

In this study, the pH of a few commercial fruit juices were measured, and to measure the change of calcium (Ca) and phosphorous (P) on the surface of the enamel according to the frequency of drinking, EDS analysis was performed to confirm the basis of dental erosion.

2. Materials and Methods

2.1. Study materials

Three kinds of fruit juice (pomegranate juice, prune juice, and white grape juice) were selected for this experiment. The characteristics of the beverages are shown in Table 1.

2.2. Tooth preparation

The premolar teeth extracted from human without morphological abnormalities were carefully selected by observation with a stereomicroscope (SZ-CTV, Olympus, Tokyo, Japan). The enamel part of each tooth was obtained using a hard-tissue cutter (Struers Minitom, Struers, Denmark) equipped with a low-speed cutting disk. Enamel were made with a $3 \times 3 \times 2$ mm block. All surfaces, except for the exposed enamel surface were coated with nail varnish. The specimens were prepared and sequentially polished with 400-, 600-, 1200-, and 1800-grit abrasive paper in order to form a parallel and uniform surface. The enamel specimens were divided into 4 groups. The control groups were immersed in phosphate-buffered saline (PBS), prune juice, white grape juice, and pomegranate juice. The experimental groups were immersed in 10 ml of each test juice for 1, 3, 5, 15, 30, 60, and 120 minutes to observe the surface changes over time.

2.3. pH measurement

Fig. 1. (Color online) Energy dispersive X-ray spectroscopy used in this study.

The pH was measured using a pH meter (Water quality pH meter, LAQUA, HORIBA, Japan) after calibration with a standard buffer solution. After measuring the pH value of the beverage, the glass electrode was washed with sterile distilled water, and then the pH value of the next one was measured. The values were measured three times respectively, and the meaningful value among them was selected.

2.4. Energy dispersive X-ray spectroscopy analysis

The major components of the enamel surface were examined using energy-dispersive X-ray spectroscopy analysis (S-4300, Hitachi Co., Japan) (Fig. 1). The enamel specimens were completely dried in a room temperature

Table 1. Test groups used in this study.

Brand name	Content (%)	Manufacturer
Taylor prune	100 % California prune juice	Taylor Farms
If white grape	15 % Mediterranean White Grape	General Beverage Co. Ltd
Pomegranate	9.36 % Pomegranate Concentrated Juice	Seoul F&B Co., Ltd
Control (PBS)	-	-

and fixed on the specimen stand with adhesive tapes after coated with platinum. The spot size on the enamel surface was detected at 400 μm, and acceleration voltage of 0.1 eV under 15 kV. The quantitative analysis of Ca and P was expressed as the average weight % per each enamel surface.

The energy of the X-ray photon is related to the radiation frequency as shown in the equation below.

$E = hv$

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 as shown in the equation below.

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

2.5. Statistical analysis

The SPSS program (Version 24.0, SPSS, Chicago, IL, USA) was used for statistical analysis. Changes in pH, Ca, and P among the groups were submitted to a one-way analysis of variance (ANOVA), and Tukey HSD test was used as a post hoc test. The statistical significance was set at 0.05.

3. Results and Discussion

Entering the modern society, economic growth and improved income levels have brought changes in food culture, and consumers are looking for drinks that are good for their health while meeting their preferences even in their daily consumption of beverages [17].

However, it has been reported that the risk factor associated with dental erosion is the consumption of acidic foods [18], and the dental erosion occurs on the surface of the enamel [19]. Attin *et al.* [20] and Sánchez *et al.* [21] cited the risk of dental erosion when drinking beverages with low pH. Sáchez *et al.* [21] also reported that the more beverages with low pH were consumed, the greater the likelihood of dental erosion, while Lussi *et al.* [22] reported that beverages containing fruit acids had a greater effect on the erosion. In addition, it was also reported that the habit of keeping the drink in the mouth for a long time or ingesting it for a long time causes a decrease in pH [23].

Considering that the residence time of beverages in the oral cavity is less than 1 minute at a time due to the characteristics of them [24], to investigate the effect of the three beverages used in this study on the degree of erosion of enamel, the minimum time for the specimen to be exposed to the beverage was set to 1 minute. So, the total exposure time was set to 1, 3, 5, 15, 30, 60, and 120 minutes in this study. Therefore, this study analyzed the basis of erosion of enamel affected by commercial fruit juice such as prune juice, white grape juice, pomegranate juice, and observed changes of enamel according to exposure time.

Table 2. Recorded pH values of the commercial fruit juice.

Brand name	Mean pH ± SD	P-values
Pomegranate	3.56 ± 0.01^a	
Taylor prune	3.75 ± 0.001^b	0.000^*
If white grape	4.00 ± 0.02^c	
Control (PBS)	7.40 ± 0.00^d	

*The P-values were determined by one-way ANOVA with post-hoc test ($P < 0.05$).
Different letters (a, b, c, and d) refer to the presented statistically significant result of the post-hoc Tukey HSD test.

pH is known to play a major role in predicting the erosion ability to teeth [25]. When the pH of the beverage is above 4.0, the effect on dental erosion is not significant. But at pH 3.0-3.9, dental erosion is induced, and the severe dental erosion has been reported when the pH is lower than 3.0 [26]. In this study, the pH values of the commercial beverages that were used in this study are shown in Table 2. Pomegranate juice had the lowest pH (3.56 ± 0.01), followed by prune juice (3.75 ± 0.01), followed by white grape juice (4.00 ± 0.02), followed by PBS (7.40 ± 0.00). The pH values were found to be significantly different among the groups ($P < 0.05$), while the tested pomegranate juice has the lowest pH which means the most acidic. Pomegranate juice selected as an experimental drink in this study was pH 3.56 ± 0.01 and prune juice was 3.75 ± 0.01. Both were between pH 3.0-3.9 which predicts the possibility of erosion on the enamel surface. The result said that pomegranate juice had the lowest pH, followed by prune juice and followed by white grape juice, which is thought to have erosion ability compared to PBS. This result is consistent with a study [27] which showed that the acid of most fruits has a pH value of 4.5 or less and it can cause erosion. The result of this study also consistent with the research of Lussi and Carvalho [28] who reported that if the pH value is less than about 3.9, there is a high possibility of dissolving minerals in teeth regardless of the concentration of major mineral components.

About 97 % of the enamel surrounding the outermost tooth is composed of minerals of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), and Ca and P are the main components of it that make up the hard tissues [29]. As Ca and Pare minerals that make up most of the teeth, their concentration acts as an important factor in dental erosion [30].

Table 3. The concentration levels of Ca and P in experimental groups.

Experimental group		Mean ± SD				*P*-value
		Pomegranate	Taylor prune	If white grape	Control (PBS)	
Ca	1 minute	64.28 ± 0.25[a]	64.78 ± 0.07[a,b]	64.75 ± 0.71[a,b]	65.21 ± 0.12[b]	0.053
	3 minutes	64.00 ± 0.17[a]	64.54 ± 0.58[a,b]	64.45 ± 0.40[a,b]	65.21 ± 0.12[b]	0.009*
	5 minutes	63.97 ± 0.31[a]	64.39 ± 0.41[a]	64.43 ± 0.09[a]	65.21 ± 0.12[b]	0.001*
	15 minutes	63.82 ± 0.67[a]	64.30 ± 0.43[a,b]	64.43 ± 0.22[a,b]	65.20 ± 0.11[b]	0.008*
	30 minutes	63.39 ± 0.23[a]	63.61 ± 0.37[a]	63.87 ± 0.24[a]	65.20 ± 0.11[b]	0.000*
	60 minutes	63.51 ± 0.40[a]	63.52 ± 0.08[a]	63.76 ± 0.24[a]	65.19 ± 0.11[b]	0.000*
	120 minutes	63.30 ± 0.37[a]	63.41 ± 0.21[a]	63.64 ± 0.50[a]	65.19 ± 0.12[b]	0.000*
P	1 minute	35.72 ± 0.25[a]	35.22 ± 0.07[b,c]	35.58 ± 0.26[a,b]	34.79 ± 0.12[c]	0.000*
	3 minutes	36.00 ± 0.17[a]	35.46 ± 0.58[a,b]	35.55 ± 0.40[a,b]	34.79 ± 0.12[b]	0.009*
	5 minutes	36.02 ± 0.31[a]	35.28 ± 0.52[b,c]	35.57 ± 0.09[a,b]	34.79 ± 0.11[c]	0.003*
	15 minutes	36.51 ± 1.24[a]	35.70 ± 0.43[a,b]	35.57 ± 0.22[a,b]	34.80 ± 0.12[b]	0.039*
	30 minutes	36.61 ± 0.23[a]	36.39 ± 0.37[a]	36.13 ± 0.24[a]	34.80 ± 0.11[b]	0.000*
	60 minutes	36.49 ± 0.40[a]	36.48 ± 0.08[a]	36.24 ± 0.24[a]	34.80 ± 0.11[b]	0.000*
	120 minutes	36.70 ± 0.37[a]	36.59 ± 0.21[a]	36.36 ± 1.15[a]	34.780 ± 0.12[b]	0.005*

*The P-values were determined by one-way ANOVA with post-hoc test (P <0 .05).
Different letters (a, b, and c) refer to the presented statistically significant result of the post-hoc Tukey HSD test.

Hong *et al.* [31] conducted to evaluate the possibility of dental erosion according to some alcoholic beverages using EDS. It is used to evaluate the demineralization of major mineral components in teeth. In this study, EDS was used to measure changes in Ca and P, the main minerals of enamel. This component analysis equipment is used by being attached to the electron microscope series. When electrons generated in the target of the electron microscope collide with the material, various kinds of electrons, ions, and characteristic X-rays with the properties of the material are emitted from the surface of the material. Among them, only the emitted characteristic X-rays are separately detected and displayed by energy spectrum of the beam, and quantitative values are measured according to the strength of them [32].

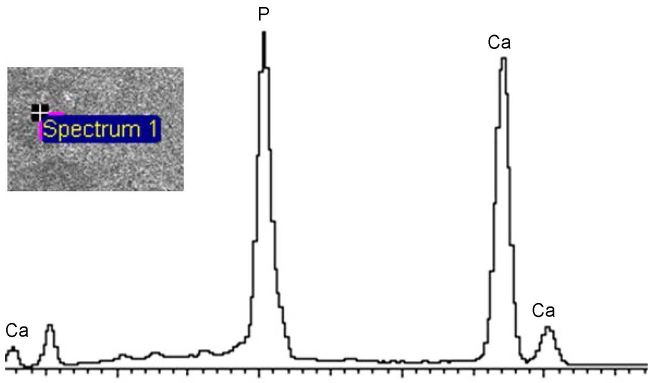

Fig. 2. (Color online) Spectrum processing and analyzed scale using EDS.

Table 3 shows the differences of Ca and P by One-way ANOVA and Fig. 2 shows the detection of Ca and P on the enamel surface using EDS. The component analysis of Ca and P showed the significant difference in Ca among the groups after 3, 5, 15, 30, 60, and 120-minutes of application and in P among the groups after 1, 3, 5, 15, 30, 60, and 120-minutes of application (P < 0.05).

After applying each time to the juice used in the experiment, the level of Ca was decreased on the enamel surface of teeth. The level of Ca was lowest from the enamel surface immersed in pomegranate juice for 15 minutes compared to the control group (P < 0.05). The greatest decrease of the Ca level on the enamel surface was pomegranate juice, followed by prune juice and followed by white grape juice. When the juice stayed in the oral cavity for 15 minutes, the pH value of pomegranate juice was the lowest and the Ca level decreased the most. After 30 minutes of application in pomegranate juice, prune juice, and white grape juice, Ca levels showed the same decrease in all three fruit juices compared to the control group. (P < 0.05). On the other hand, the level of P showed a tendency to increase as that of Ca decreased. After 15 minutes of application in pomegranate juice, the relative value of P increased. The P levels after 30 minutes of application in pomegranate juice, prune juice, and white grape juice were different from those of the PBS as control groups (P < 0.05). When the juice remained in the oral cavity for more than 30 minutes, a change in composition was caused in all three kinds of fruit juices used in the experiment. Through this, it was confirmed that the level of Ca is affected by the pH level of the beverage. In addition, it was confirmed that dental erosion is affected by the time of exposure.

As a result of observing enamel by applying it to pomegranate juice, prune juice, and white grape juice for 30 minutes, both Ca and P levels were changed compared to the control group. It is thought that the action of various organic acids added to the beverage affects the major components of teeth. When juice remained in the oral cavity for more than 30 minutes and contacted with teeth, tooth demineralization was caused regardless of the pH value of the juice. Since Ca and P appear as proportional values, a decrease in Ca value appears as a relative increase in P value. However, the decrease in Ca, which is a major component of teeth, indicates that the demineralization of the main mineral components of tooth has occurred. Based on the results of this study, the fact that the juice remained in the oral cavity for 1 minute did not affect dental erosion. It was found that the juice remaining in the oral cavity for more than 1 minute affects the dental erosion when drinking the juice with low pH level. Therefore, the risk of dental erosion can be reduced by exposing the juice to the teeth for less than 1 minute. It was confirmed that the solubility of Ca increased as the pH decreased, and it appeared to have had more impact as time passes. As a result of the above results, it was shown that even in a relatively short period of time, the consumption of acidic fruit juice can reduce the Ca components of enamel, so it is necessary to reduce the intake time. Common consumers often lack an understanding of the harmful effects of beverages on oral health. As interest in health has grown recently, fruit juice is believed to be more beneficial to health than soda or coffee in general. However, fruit juice with a low pH has the potential to cause dental erosion and severe enamel erosion can be occurred by ingesting fruit juice with exposed enamel.

As a result, fruit juice with a low pH has a high possibility of causing erosion of the tooth surface. So, it is recommended to reduce the number of intakes or shorten the drinking time when drinking fruit juice considering the possibility of dental erosion.

4. Conclusion

Through this study, the risk of dental erosion by ingestion of fruit juice is presented and the basis for having a correct perception of the possible risks to oral health has been prepared. As the exposure time to fruit juice increased, the degree of change in Ca and P was greater. Therefore, to prevent dental erosion, it is necessary to reduce retention time of the beverage in the oral cavity.

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