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The Effects of Commercial Alcoholic Drinks Determined Using Scanning Electron Microscopy and Energy-dispersive Electromagnetic Wave (X-Ray) Spectroscopy

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In modern society, drinking is becoming a culture while alcohol consumption continues to increase. Studies show that alcohol consumption in the oral cavity averages 2 hours, and alcohol consumption seems to have a high effect on tooth damage. Therefore, this study was conducted to evaluate the possibility of dental erosion due to intake of some alcoholic beverages in the market. Six types of alcoholic beverages were immersed in 1 ml of each alcohol for 10 min, 60 min, and 120 min to obtain only enamel of the tooth and observe changes over time. The crystal structure of the enamel surface was observed by scanning electron microscopy (SEM), and the changes in calcium and phosphorus, the major constituents of teeth, were analyzed by energy dispersive electromagnetic wave (X-ray) spectroscopy (EDS). Based on the results, the tooth showed a destructive pattern while and loss of calcium (Ca) and phosphorous (P) increased significantly as exposure time to the low pH alcoholic drinks increased. Since this causes the demineralization of inorganic components and greatly affects the risk of tooth erosion, long contact with alcohol should be avoided.

Keywords : enamel surfaces, commercial alcoholic drinks, scanning electron microscopy (SEM), energy- dispersive electromagnetic wave (X-ray) spectroscopy (EDS)

1. Introduction

Alcohol consumption is part of the culture of the modern South Korean society, and South Korea's per-capita alcohol consumption is the highest among Asian countries [1]. Women's alcohol consumption has been increasing of late due to the availability of various kinds of alcoholic drinks in the market, while the mental tension from social problems is also increasing the consumption of alcohol [2]. As of 2009, the average alcohol consumption time in South Korea for a single adult 19 years old or older is 2 hours, which is relatively long. This is considered not only to have a large effect on the systemic health but also to cause dental hard-tissue damage [3].

Excessive alcohol consumption affects the gingival, tongue, and oral tissue, causing dental caries, periodontal disease, oral cancer, and neglect of oral cleanliness [4]. Dental caries, a representative oral disease, is the dissolution of the enamel crystals composed of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) by the acid produced by the oral bacteria [5]. As a disease occurring in the oral cavity, dental erosion means the irreversible pathological and physiological loss of dental hard tissue due to the chemical action on the tooth surface by acid, without bacterial action. If attrition or abrasion occurs with dental erosion, serious tooth structure damage may occur [6]. In particular, dental erosion has been reported to be caused by frequently drinking acid-containing beverages with a low pH [7]. The tooth enamel is known to erode at pH 5.5 or lower [8]. As such, dental erosion occurs due to high calcium and phosphorus concentrations and a high pH in the vicinity of the dental surface in the oral cavity [8].

It has been reported recently that dental erosion frequently occurs among modern people due to lifestyle changes [9]. Among the exogenous factors of dental erosion are beverages and foods like wine, acidic fruit juices, carbonated drinks, and fruit [10]. Increasing acidic beverage consumption increased the incidence of dental erosion, among other factors [11]. The main cause of dental erosion is reportedly the consumption of acidic beverages with a low pH [12]. In addition, the ingestion

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of commercially available fruits and carbonated drinks has been reported to reduce the pH in the oral cavity for a long time, thereby causing dental erosion [13]. Thus, most of the previous studies on dental erosion that have been conducted to date have reported that low-pH beverages cause dental erosion. Although there have been reports on the pH measurement of alcoholic drinks [14], there is a dearth of research on the relationship between dental erosion and the different kinds of alcoholic drinks. Moreover, studies on the effects of the different kinds of commercial alcoholic drinks on the dental hard tissue are currently insufficient, and considering the increasing trend of alcoholic drink consumption, there is an urgent need to study the possibility that alcoholic drinks cause dental hard-tissue damage.

Therefore, this study was conducted to measure the pH values of alcoholic drinks and to evaluate the degree of dental erosion that occurs thereafter based on the microscopic change of the enamel surface and the teeth composition change, to determine the possibility of inducing dental erosion due to some commercial alcoholic drinks.

2. Materials and Methods

2.1. Study materials

Among the commercial distilled liquors, two kinds of soju (Chamisul Fresh, Soon Hari Soju [Citron flavor]), one kind of beer (Cass Fresh), and two kinds of raw rice wine (Kook Soon Dang Makgeolli, Raspberry Makgeolli), and among fruit wines, one kind of wine (Yellow Tail Cabernet Sauvignon red wine), were selected for this study. The characteristics of the various alcoholic drinks that were used in the experiment are shown in Table 1.

2.2. Dental specimen fabrication

The extracted healthy human premolar enamel surfaces without dental caries, pigmentation, and cracking were observed with a stereoscopic microscope (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 diamond saw (Struers Minitom, Struers, Denmark). The enamel specimens were divided into the control group, where the specimens were immersed in phosphate-buffered saline (PBS), and into experimental groups, where the specimens were immersed in six different kinds of alcoholic drink (1 ml) for 10, 60, and 120 minutes to observe the change over time. A total of 57 specimens, three for each of the 19 groups, were used.

2.3. pH measurement

After calibration with a standard buffer solution to adjust the pH measurement, the pH values of the alcoholic drinks that were used for each experiment were measured with a pH meter (water quality pH meter, LAQUA, HORIBA, Japan).

2.4. Scanning electron microscope observation

To observe the crystal structure and microscopic changes of the enamel surfaces, the enamel specimens were dried and coated with platinum and were fixed on the specimen mount. The enamel surface changes were observed at 5,000x magnification at 15 KV using a scanning electron microscope (SEM; S-4300, Hitachi Co., Japan).

2.5. Energy-dispersive electromagnetic wave (X-Ray) spectroscopy analysis

To quantitatively analyze the changes in calcium (Ca) and phosphorous (P), the major components of the enamel surface, they were measured using energy-dispersive electromagnetic wave (X-ray) spectroscopy (EDS; S-4300, Hitachi Co., Japan). They were expressed as the average weight % at 3 points on 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

Table 1. Test groups used in the experiment.

Brand name	Alcohol (%)	Manufacturer
Control (PBS)	-	-
Chamisul Fresh	17.2 %	The hitejiro
Cass Fresh	4.5 %	Oriental Brewery Company
Raspberry Makgeolli (raw rice wine)	6 %	Dongjinwine
Kook Soon Dang Makgeolli (raw rice wine)	6 %	Kooksoondang Brewery Co., Ltd.
Red wine (Yellow Tail Cabernet Sauvignon)	13.5 %	Australia, New South Wales
Soon Hari Soju (Citron flavor)	14 %	Lotte Chilsung Beverage Co., Ltd.

The wavelength λ is related to the photon energy as shown in the equation below.

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

2.6. Statistical analysis

Statistical analysis of each result was performed through the SPSS program (Version 20.0, SPSS, Chicago, IL, USA). The changes in pH and in calcium (Ca) and phosphorus (P) in all the groups were subjected to a one-way analysis of variance (ANOVA) complemented by Tukey's test ($p < 0.05$).

3. Results

3.1. Testing the pH values of alcoholic drinks

The pH values of the alcoholic drinks used in this study are shown in Table 2. Soon Hari Soju (Citron flavor) had the lowest pH (2.591±0.007), followed by red wine (3.636±0.004), Kook Soon Dang Makgeolli (3.879±0.008), Raspberry Makgeolli (4.030±0.003), Cass Fresh (4.095±0.007), PBS (7.402±0.001), and Chamisul Fresh (8.978±0.009). There were significant differences among the groups ($P < 0.05$), and the lowest pH was found in Soon Hari Soju (Citron flavor) (Table 2).

3.2. Surface morphology changes

Change in surface morphology was largest in the tooth surface applied with Soon Hari Soju (Citron flavor), followed by those applied with red wine, Kook Soon Dang Makgeolli, Raspberry Makgeolli, Cass Fresh, and Chamisul Fresh, showing enamel surface erosion due to enamel destruction and cracking (Fig. 1).

3.3. Analysis of mineral contents

Table 3 shows the mean±SD difference in Ca and P in the enamel, determined via XPS. The changes in the Ca and P levels were significantly different among the groups after 10-, 60-, and 120-minute application of each of the six alcoholic drinks ($P < 0.05$). The change in the Ca level was largest in the Soon Hari Soju (Citron flavor) group (10-, 60-, and 120-minute application). On the other hand, the change in the P value was significantly different in the groups to which Kook Soon Dang Makgeolli, red wine, and Soon Hari Soju (Citron flavor) had been applied for 10 minutes, and a large change into the lowest value was shown in the Soon Hari Soju (Citron flavor) group after a 60-minute application. The P values after 120-minute application of Kook Soon Dang Makgeolli, red wine, and Soon Hari Soju (Citron flavor) were different from those of the control group, where the specimens had been immersed in PBS. The lowest value was shown, however, in the tooth surface to which Soon Hari Soju (Citron flavor) had been applied.

Table 2. Mean pH±SD values of the commercial alcoholic drinks used in this study.

Brand name	Mean pH±SD	P-values
Control (PBS)	7.402 ± 0.001^{b}	
Chamisul Fresh	8.978 ± 0.009^{a}	
Cass Fresh	4.095 ± 0.007^{c}	
Raspberry Makgeolli (raw rice wine)	4.030 ± 0.003^{d}	0.000*
Kook Soon Dang Makgeolli (raw rice wine)	3.879 ± 0.008^{e}	
Red wine (Yellow tail Cabernet Sauvignon)	3.636 ± 0.004^{f}	
Soon Hari Soju (Citron flavor)	2.591 ± 0.007^{g}	

*The P-values were determined via one-way ANOVA with post-hoc Scheffe ($P < 0.05$).
Different letters (a, b, c, d, e, f, and g) refer to the statistically significant result presented by the post-hoc Scheffe.

4. Discussion

It is known that oral health is essential for maintaining systemic health, and that keeping the teeth healthy for a long time is important for having a happy life [15].

According to the Korea National Health and Nutrition Examination Survey (KNHANES), the intake of beverages in South Korea increased from 66.88 g in 2008 to 185.35 g in 2016. In the average intake by food, many one-time intakes of liquid foods like milk, beer, soju, cola, fruit drink, and raw rice wine were included in the top 30 [16]. The consumption of imported liquors like foreign wines and beer has also been increasing of late [17]. The average consumption time of commercial alcoholic drinks of a single adult 19 years or older has been reported to be 2 hours, which is a relatively long [3].

Dental erosion is defined as irreversible damage to the dental hard tissue due to the chemical action of acid, regardless of the action of bacteria [18]. The main cause of tooth loss at all ages has been found to be dental erosion [19] since the dental structures demineralized by dental erosion are more susceptible to abrasion, such as through brushing, and can be destroyed faster [20]. It has been reported that the no. 1 cause of dental erosion is the frequent drinking of acid-containing beverages with a low pH [7]. In addition, the factors causing a decrease in pH include food staying for a long duration in the mouth or long-time food or beverage intake duration [21].

Tooth damage can be generally neutralized by the saliva's buffering action when acid is produced in the oral cavity, but when the teeth are exposed to too much acid or

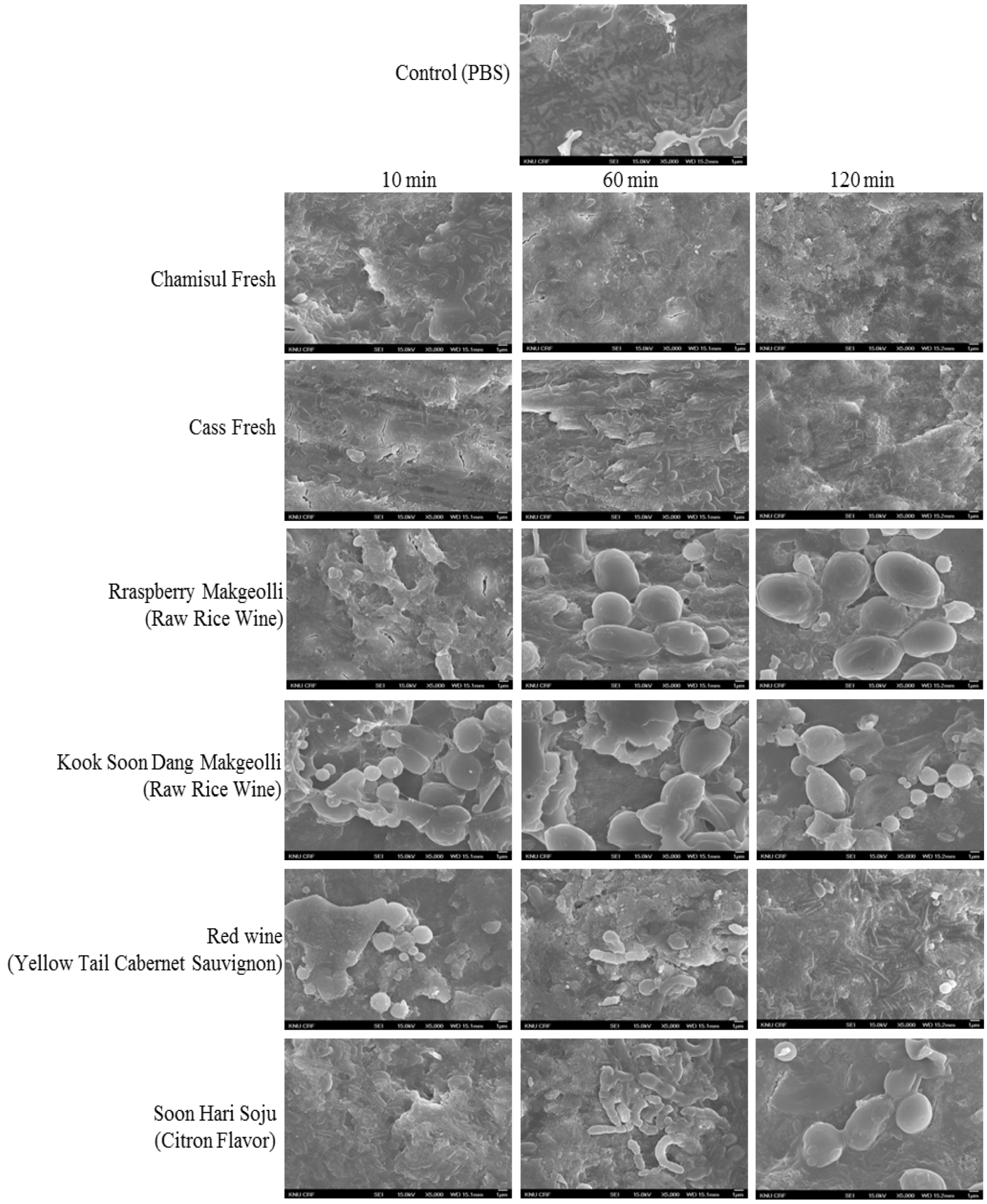

Fig. 1. Change in microscopic changes of the enamel surfaces by commercial alcoholic drinks.

exposed too frequently, the bond between Ca and P is broken and dissolved, forming holes on the enamel surface [22]. In particular, various disorders are caused in the structure or components of the developing tooth, and when osteoporosis is induced due to calcium deficiency, the developmental disorder of the tooth is further intensified [23]. This is because Ca and P, the major mineral components related to the hard tissue of the teeth, are essential for the development and maintenance of calcified tissues such as the teeth and alveolar bone.

Scanning electron microscopy is a method of observing and studying the changes in the tooth surface. SEM is used to shape the microstructure of a specimen at a high resolution, and to obtain surface topography information through the electron beam [24]. In addition, the magnification can be adjusted by changing the current flowing in the coil, yielding the advantage of more convenient surface observation [25]. Most studies have used SEM for surface measurement to determine the degree of enamel erosion [26]. In addition, EDS for component analysis of the tooth is used by attaching an energy-dispersive electromagnetic wave (X-ray) spectroscope to the electron microscope series (TEM, SEM). When the electrons generated by electron microscope targeting collide with the material,

Table 3. Ca and P concentration levels and P values on enamel by alcoholic drink.

Experimental group		Mean±SD							P-value
		Control (PBS)	Chamisul Fresh	Cass Fresh	Raspberry Makgeolli (raw rice wine)	Kook Soon Dang Makgeolli (raw rice wine)	Red wine (Yellow tail Cabernet Sauvignon)	Soon Hari Soju (Citron flavor)	
Ca	10 min	33.10 ± 0.83^{a}	$31.51\pm1.77^{a,b}$	$29.69\pm0.63^{b,c}$	$28.07\pm0.82^{c,d}$	$27.40\pm0.66^{c,d}$	$27.07\pm0.84^{c,d}$	26.64 ± 0.25^{d}	0.000^{*}
	60 min	32.97 ± 0.05^{a}	$31.41\pm1.52^{a,b}$	$28.66\pm1.64^{b,c}$	27.31 ± 1.14^{c}	26.38 ± 1.19^{c}	25.81 ± 0.91^{c}	3.30 ± 0.95^{d}	0.000^{*}
	120 min	32.96 ± 0.08^{a}	31.33 ± 0.72^{a}	28.50 ± 1.06^{b}	25.40 ± 0.75^{c}	24.82 ± 0.95^{c}	24.33 ± 0.55^{c}	1.18 ± 0.75^{d}	0.000^{*}
P	10 min	19.71 ± 0.77^{a}	$18.33\pm0.91^{a,b}$	$18.24\pm0.49^{a,b}$	$17.86\pm0.41^{a,b}$	17.37 ± 0.22^{b}	17.32 ± 0.64^{b}	17.26 ± 1.08^{b}	0.002^{*}
	60 min	19.48 ± 0.66^{a}	$18.08\pm1.19^{a,b}$	$17.30\pm1.74^{a,b,c}$	$16.33\pm0.62^{a,b,c}$	$15.19\pm0.98^{b,c}$	13.92 ± 1.51^{c}	3.68 ± 0.94^{d}	0.000^{*}
	120 min	19.71 ± 0.77^{a}	$18.04\pm0.85^{a,b}$	$16.89\pm0.52^{b,c}$	$14.83\pm0.80^{c,d}$	$14.36\pm1.16^{d,e}$	12.30 ± 0.55^{e}	$1.77\pm0.93^{e,f}$	0.000^{*}

*The p-values were determined via one-way ANOVA with post-hoc Scheffe ($P<0.05$).
Different letters (a, b, c, d, e, and f) refer to the statistically significant result as presented by the post-hoc Scheffe.

various kinds of electrons, ions, and characteristic X-rays with the properties of the material are emitted from the material surface. Among these, EDS detects only the characteristic X-rays emitted, and displays them on the screen for each energy band of the beam. In the spectrum displayed on the screen, the X-axis represents the energy of the beam, and the Y-axis represents the intensity of the beam, which is characterized by inferring quantitative values based on the intensity [27]. This study measured the pH and tooth surface changes as well as the mineral change of enamel for six kinds of alcoholic drinks with dental erosion potential. For the results of this study, the lowest pH value was shown in the Soon Hari Soju (Citron flavor) group, followed by the red wine, Kook Soon Dang Makgeolli, Raspberry Makgeolli, Cass Fresh, PBS, and Chamisul Fresh groups. After treatment of the teeth with the liquor having such pH value, the enamel surface was observed via SEM. The results showed the most destructive pattern in the Soon Hari Soju (Citron flavor) group, with the red wine group also showing a large destruction pattern. In the case of Kook Soon Dang Makgeolli and Raspberry Makgeolli, fermented bacteria were attached to them, which seemed to have caused the destruction of and changes in the enamel surface. These results are consistent with what was reported by Hughes *et al.*, that along with a low pH value, dental erosion is caused by the organic acids in wine and raw rice wine [28]. The Cass Fresh (beer) group showed some rough and small cracks while the soju group showed smooth and seamless patterns, the same as those in the control group (distilled water). These results were mostly in agreement with the pH results. In addition, it was confirmed that the surface became rougher over time. The results of the tooth composition analysis showed that the change in the Ca level was largest in the Soon Hari Soju (Citron flavor) group (10-, 60-, and 120-minute application). The P value changed greatly into a lower value in the Kook Soon Dang Makgeolli, red wine, and Soon Hari Soju (Citron flavor) groups, but the loss of P was largest on the tooth surface to which Soon Hari Soju (Citron flavor) had been applied, showing a low value. The above results indicate a greater influence over time. As the pH of the liquor decreased, the loss of Ca and P hydroxyapatite crystals increased. This will lead to the demineralization of the tooth's major mineral components, which will increase the enamel hazard because of the increased oral health risk due to dental erosion. Therefore, increasing tooth contact time especially with alcohols having low pH should be avoided [29].

5. Conclusion

The average Korean adult takes 2 hours to drink alcohol, which is a relatively long time. This is considered not only to have a detrimental effect on the systemic health but also to cause dental hard-tissue damage. Based on the results of this study, it should be noted that the long-time and frequent intake of alcohols with a low pH level is likely to cause dental erosion because a low pH level means that the liquor contains a large amount of organic acid. Thus, when drinking alcohol with a low pH level, it is better not to hold it in the mouth for a long time. Better yet, the alcohol consumption time should be made shorter to limit the time that teeth surfaces' come into contact with alcohol.

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