Comparison between carbonated and fruit-based soft drinks effect on calcium release from enamel surface of extracted permanent teeth

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ABSTRACT

Introduction: People widely consume soft drinks due to their easy accessibility and delightful taste, without realising the impact on the tooth surface. Enamel dissolves easily due to acidic products that contact acid, a chemical demineralisation process (dental erosion). This study aimed to analyse the comparison between carbonated and fruit-based soft drinks effect on calcium release from enamel surface of extracted permanent teeth. **Methods:** *In-vitro* study with a time-series design was conducted on the population of extracted premolars taken from a private dental clinic and orthodontics clinic of the Faculty of Dentistry Universitas Sumatera Utara, Medan. The sample was obtained using the pairwise difference hypothesis test formula. The samples were 24 pieces divided into two treatment groups by immersing in carbonated drinks and fruit-based soft drinks for 5 to 60 minutes. The statistical test used was the independent t-test and generalised linear model-repeated measures (GLM-RM). **Results:** The calcium level after immersion in the carbonated drink at the fifth minute was 0.476±0.397 mg/L, then increased significantly at the sixtieth minute to 3.058±0.811 mg/L (p=0.001). In the fruit-based soft drinks immersion group, the dissolved calcium at the fifth minute was 0.671±0.208 mg/L, then increased significantly to 2.258±1.351 mg/L (p-value=0.028). Neither carbonated drinks nor fruit-based soft drinks showed a significant effect on the levels of dissolved calcium (p-value=0.135). **Conclusion:** In the fifth minutes of immersion, fruit-based soft drinks caused higher calcium release level compared to carbonated soft drinks. In contrast, in the sixtieth-minutes of immersion, the calcium release is found to be higher in the carbonated soft drinks group.

Keywords: carbonated drinks; fruit-based soft drinks; calcium enamel surface; tooth erosion; acids

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INTRODUCTION

Dental health is a condition that is free from all oral diseases supported by healthy gingival conditions.[1] The condition of the oral cavity that is not clean and healthy can cause problems in everyday life.[2] Basic Health Research Data in 2018 in Indonesia suggested that the prevalence of oral problems is 57.6%.[3] Enamel is a white coating on the outer surface that protects the crown of teeth and is the hardest substance in the body. The mineral content is 95% calcium hydroxyapatite ($Ca_{10}(PO_4)_6(OH)$), and the remaining substance is 5% water and an enamel matrix. Although considered the hardest substance in the body, the enamel is susceptible to acid attack either directly from food or due to bacterial metabolism, which ferments carbohydrates into acids which will cause damage.[4]

The damage to dental hard tissue consists of both carious and non-carious lesions. The non-carious lesions include abrasion, attrition, abfraction and erosion. Tooth erosion can be defined as the progressive loss of hard tooth tissue due to a chemical process without involving the action of bacteria. The solubility of minerals in the tooth structure can occur due to contact with acids that enter the oral cavity, both from intrinsic acid sources and extrinsic acid, fruit juices and soft drinks.[5] Demineralisation of enamel occurs through a diffusion process that moves in the movement of water-soluble molecules or ions or from enamel to saliva due to differences in the concentration of acids on the surface and in the enamel.[6,7]

Calcium, as the main constituent of hydroxyapatite, is also supported by environmental conditions and can be dissolved by the acidic environment of saliva. This solubility process significantly affects the balance of the inorganic composition of the enamel so that it can be an indication of enamel demineralisation, and then it can cause tooth erosion. If calcium solubility occurs continuously, it will cause a partial loss of the enamel prism and porosity, which causes the hardness of the enamel surface to decrease.[4,8,9]

Enamel demineralisation depends on the activity of calcium ions (Ca^{2+}) and phosphate ions (PO_4^{3-}) in enamel, saliva or plaque. The presence of bacteria and food scraps adhering to the tooth surface is the initial trigger for demineralisation.

The bacteria will use lactic acid, pyruvic acid, and acetic acid to process food residue sticks to teeth. This acid will lower the pH of the enamel and diffuse into the teeth. Calcium and phosphate on the teeth will be released, where the pH will drop to 4.0-4.5, and the tooth's hydroxyapatite will break down.[10] People widely consume soft drinks due to their easy accessibility and delightful taste. According to World Wide Food, in 2014, consumption of soft drinks in Indonesia increased by 48.57% each year. Indonesia is the fifth largest country globally that consumes soft drinks as a substitute for mineral water and is often consumed by adolescents aged 15-20 years.[11] Research by Barac et al.[12] regarding the effects of erosion on various soft drinks (Coca-Cola™, orange juice, Cedevita™, Guarana™, and strawberry yoghurt) on the hardness of the enamel surface *in-vitro* showed that all types of soft drinks were erosive except yoghurt.

Erosion of the enamel surface exposed to Coca-Cola™, orange juice, Cedevita™, and Guarana™ was directly proportional to the exposure time. A systematic review on the effect of fruit juice on tooth erosion and dental caries suggested many research has been conducted on several RCT studies that have contributed to the incidence of tooth erosion in adulthood.[13]

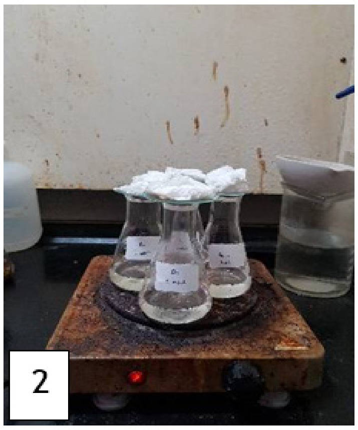
Research by Tadakamadla et al.[8] which evaluating the pH, titratable acidity and *in-vitro* enamel solubility potential of different commercially available soft drinks and fruit juices in Saudi Arabia discovered that carbonated drinks have more enamel solubility potential than fruit juices commonly found in Saudi Arabia. Eating and drinking activities affect the demineralisation and remineralisation processes of enamel. Demineralisation occurs due to exposure to acids from food and beverages for a long time, causing changes in the pH of the oral cavity so that the surface of the teeth becomes acidic, i.e. with a pH below 5.5. Currently, many soft drinks consumed by the public have a pH below 5.5, such as carbonated soft drinks with a pH between 2.3-3.4 and isotonic drinks with a pH between 2.4-4.5.4.

Erviana et al.[9] examined the differences in calcium solubility in deciduous and permanent teeth when immersed in fruit-flavoured carbonated drinks. The results showed a significant difference in the mean solubility of calcium in deciduous and permanent teeth when immersed in carbonated drinks at various times. The minerals in tooth enamel can dissolve if the teeth are often in contact with soft drinks that contain acids. Therefore, this study aimed to analyse the comparison between carbonated and fruit-based soft drinks effect on calcium release from enamel surface of extracted permanent teeth.

METHODS

An *in-vitro* study with a time-series design was conducted at the Faculty of Pharmacy Laboratory of Universitas Sumatera Utara. The statistical test used was the independent t-test and generalised linear model-repeated measures (GLM-RM). The population of this study were extracted premolars from a private dentist clinic, orthodontics clinic of the Faculty of Dentistry Universitas Sumatera Utara, Medan.

The sample was obtained using the pairwise difference hypothesis test formula. The sample used was 24 pieces divided into two different treatment groups. The first group was treated by immersion in a carbonated drink (Coca Cola™), and the second group was treated by immersion in a fruit juice drink (Buavita™). Each group was observed until the fifth and sixtieth minutes to determine the levels of dissolved calcium. Then, the dissolved calcium levels were calculated using an Atomic Absorption Spectrophotometer (AAS).



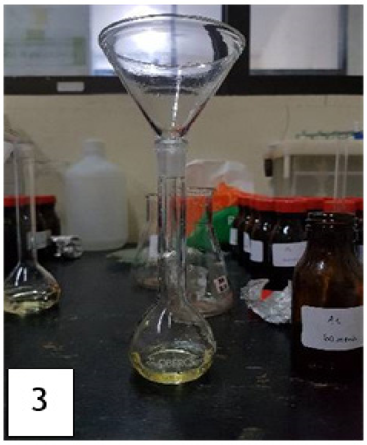


Figure 1. Tooth samples tied with floss and immersed in soft drinks
Figure 2. The formed solution was heated on a hotplate
Figure 3. The formed solution was transferred into prepared flasks

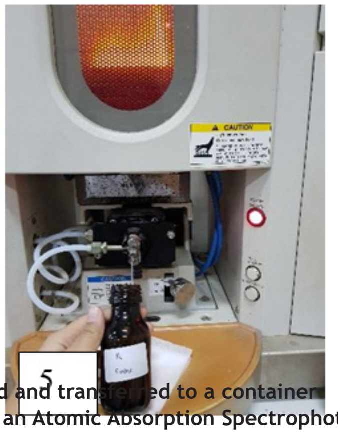


Figure 4. The solution was filtered and transferred to a container
Figure 5. The solution was calculated using an Atomic Absorption Spectrophotometer

The test was initiated by tying the tooth samples with floss and immersing them in soft drinks (Figure 1). Afterwards, the solution was heated on a hotplate (Figure 2); the sample groups were tied with floss, then immersed in soft drinks (Figure 3). Finally, each group was observed until the fifth and sixtieth minutes to see the levels of dissolved calcium (Figure 4). The dissolved calcium levels were calculated using an Atomic Absorption Spectrophotometer (AAS) (Figure 5).

RESULTS

The concentration of calcium ions in carbonated and isotonic drinks can be seen in Table 2 (initial minutes). The calcium ion of carbonated drinks in the initial minutes was 1.37 mg/L, while the mean solubility of calcium ions in the fifth minute was 1.85±0.4 mg/L and at 60-minutes immersion was 4.43±0.81 mg/L. The calcium ion of the juice drink at the initial minute was 5.98 mg/L, while the mean solubility of calcium ion in the fifth minute was 6.65±0.21 mg/L, and at the sixtieth-minute immersion was 8.24±1.35 mg/L. Table 1 presents an increase in calcium concentration in the immersion of carbonated drinks and fruit juices in the first, fifth, and sixtieth minutes (Table 1).

Table 1. Calcium levels at the first, fifth and sixtieth minutes of the carbonated and fruit juice drinks groups

Variable	Calcium Level (mg/L)		
	Initial	5 minute	60 minute
		Mean±SD	Mean±SD
Carbonated drink	1.37	1.85±0.40	4.43±0.81
Fruit uice drink	5.98	6.65±0.21	8.24±1.35

The average difference in solubility of calcium ions at five minutes of immersion in carbonated drinks was 0.476±0.397 mg/L, and an increase occurred at sixty minutes of immersion, namely 3.058±0.811 mg/L. The statistical tests showed a significant difference between the 5-minute and 60-minute immersion in carbonated drinks (p=0.001). At the immersion in juice drinks, the mean difference in solubility of calcium in the fifth minute was 0.671±0.208 mg/L, and an increase in the sixtieth minute was 2.258±1.351 mg/L. The statistical tests showed a significant difference between the fifth and sixtieth-minute immersion in fruit-based soft drinks (p=0.028) (Table 2).

Table 2. Differences in mean calcium levels at the fifth and sixtieth minutes

Variable	Calcium Level (mg/L)		
	5 Minute	60 Minute	p-value
	Mean±SD	Mean±SD	
Carbonated drink	0.48±0.39	3.06±0.81	0.001*
Fruit juice drink	0.67±0.21	2.26±1.35	0.028*

***significant**

The mean difference in solubility of calcium ions at 5 minutes of immersion in carbonated drinks was 0.48±0.39 mg/L and in juice drinks was 0.67±0.21 mg/L. The statistical tests showed no significant difference between carbonated drinks and fruit-based soft drinks in minutes fifth (p=0.312).

At the sixtieth-minute of immersion, the mean difference of the calcium solubility in carbonated beverages was 3.06±0.81 mg/L and in fruit-based soft drinks was 2.26±1.35 mg/L; no significant difference was found between immersion in the two drinks at 60 minutes (p=0.242) (Table 3).

Table 3. Differences in mean calcium level between two types of beverages in the fifth and sixtieth minutes

Variable	Calcium Level (mg/L)			
	5 minute	p-value	60 minute	p-value
	Mean±SD		Mean±SD	
Carbonated drink	0.48±0.39	0.312	3.06±0.81	0.242
Fruit Juice drink	0.67±0.21		2.26±1.35	

***significant**

The GLM-RM test was carried out to determine the difference in the effect of calcium solubility in carbonated and fruit-based soft drinks in the fifth and sixtieth minutes. Table 4 presents no significant difference in the calcium solubility in carbonated drinks and juice drinks with a p-value=0.135. After sixty-minute immersion, the mean difference of the calcium solubility carbonated drinks was

Table 4.GLM-RM Analysis of the solubility effects of calcium in two types of drinks at the fifth and sixtieth minutes

Variable		Calcium Level (mg/L) (Mean ±SD)	p-value
5 minute		0.57±0.32	0.0001*
60 minute		2.66±1.14	
Calcium level of immersion in carbonated and fruit juice drink	Carbonated	2.58±0.80	0.135
	Fruit juice	1.59±1.26	

*significant

3.06±0.81 mg/L and in juice drinks was 2.26±1.35 mg/L. The statistical tests showed no significant difference between immersion in the two drinks at sixty minutes (p=0.242) (Table 3).

DISCUSSION

Table 1 shows an increase in the calcium solubility levels in the immersion of the teeth from the initial minute, the fifth minute and the sixtieth minute in carbonated drinks and fruit-based soft drinks and shows a significant relationship (p=0.001) (Table 2). This result aligned with the previous research conducted in 2015[14], which suggested increasing calcium solubility in tooth enamel immersed with carbonated drinks at 30, 45 and 60 minutes. The increase in calcium ion concentration was due to the presence of calcium ions in the tooth enamel, which dissolved so that the concentration of calcium ions in soft drinks increased.

The solubility of calcium ions in tooth enamel occurs due to the demineralisation process. Hydroxyapatite is balanced with a salivary environment filled with calcium and phosphate ions in the normal environment. As a result, hydroxyapatite will be reactive to hydrogen ions at pH≤5.5, which is the critical pH for hydroxyapatite. Hydrogen ion will bond with the phosphate group, or there is a conversion from PO_4^{3-} to HPO_4^{2-}. At the same time, the HPO_4^{2-} cannot survive in the normal hydroxyapatite environment dominated by PO_4^{3-} so the hydroxyapatite crystals will dissolve.

The present study results indicate that the amount of calcium solubility in tooth enamel depends on the length of time or immersion duration. The longer the teeth are exposed to carbonated drinks and juices, the more dental calcium will dissolve. The solubility of calcium and other minerals from the hard tissues of the teeth will cause demineralisation, which, if it occurs continuously without the remineralisation process, will cause damage to the tooth structure.[6,12]

Based on the results of statistical analysis, there was no significant difference in the amount of calcium solubility of tooth enamel immersed in carbonated drinks and fruit-based soft drinks for 5 minutes (p=0.312) and 60 minutes (p=0.242). The mean difference in enamel calcium solubility in the 5th minute of immersion in carbonated drinks was 0.48±0.39, while the mean difference in enamel calcium solubility in the immersion of juice drinks was 0.67±0.21 (Table 3). The mean difference in enamel calcium solubility in the sixtieth-minute immersion in carbonated drinks was 3.06±0.81, while the mean amount of enamel calcium solubility in the immersion of juice drinks was 2.26±1.35.

No significant difference was found between the immersion in the two soft drinks due to the acid content in carbonated drinks and juice drinks. Fruit-based soft drinks caused a pH below 5.5 which is a critical pH for dental hydroxyapatite, so the two drinks will cause the solubility of tooth enamel calcium ions.[16] Low pH will increase the hydrogen ion concentration and can damage dental hydroxyapatite.[16] Demineralisation of enamel occurs through diffusion, namely the movement of molecules or ions soluble in water or from enamel to saliva due to differences in the concentration of acids on the surface and in the enamel.[17]

In addition, sugar content such as sucrose in both caffeinated and juice drinks can be metabolised to form polysaccharides which allow bacteria to adhere to the tooth surface, that will cause tooth hydroxyapatite decay which dissolves dental calcium. Continuous process of metabolism will eliminate the enamel prism, which will cause tooth structure damage such as erosion.[4,15]

CONCLUSIONS

In the fifth minutes of immersion, fruit-based soft drinks caused higher calcium release level compared to carbonated soft drinks. In contrast, in the sixtieth-minutes of immersion, the calcium release is found to be higher in the carbonated soft drinks group.

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