Assessing the impact of immersing teeth in fresh orange juice and commercial orange juice on enamel hardness: an in vitro study

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Abstract. The pH of orange juice is below the critical pH of the enamel. Lately, consumption of orange juice has considerably increased because of its commercial availability. This study aims to investigate the impact of immersion time and juice types, both fresh and packaged, on enamel hardness. 60 premolars were immersed in fresh orange juice (n = 30) and commercial juice (n = 30) for 30 and 60 min and assessed the obtained data using repeated analysis of variance, Friedman test, Mann–Whitney U-test, and independent t-test. Enamel hardness decreased (P < 0.05) at every assessed time, and the commercial juice immersion significantly decreased the enamel hardness (P < 0.05). This study infers that enamel hardness is adversely affected by the pH, citric acid concentration, and immersion time of the orange juice.

1. Introduction

Hydroxyapatite is a naturally occurring mineral component in the enamel, dentine, and cementum. Apparently, minerals in the tooth structure are affected by the stability of the oral environment, especially the pH of the mouth. Hydroxyapatite has a critical pH, and any decrease in the pH below 5.5 increases the dissolution rate of hydroxyapatite. In addition, the pH of the mouth below 5.5 results in a progressive interaction between the acid ions and the phosphate group of hydroxyapatite, causing the crystal on the tooth surface to dissolve partially or completely. Demineralization is the loss of tooth mineral, which can be caused by acids that are either affected or not affected by bacteria [1].

Acids that are affected by bacteria are released by easily fermented materials such as monosaccharides and disaccharides. When easily fermented ingredients accumulate and interact with bacteria, such as Streptococcus mutans, in the oral cavity, a fermentation process occurs that produces an organic acid. This organic acid fermentation production could cause demineralization of teeth. In contrast, acids that are not affected by bacteria come from acidic food or drinks such as carbonated beverages and fruit juices. In the case, while bacteria play no direct role in demineralization, the environment on the tooth surface remains acidic because of the presence or absence of cariogenic bacteria on tooth surfaces. Thus, high consumption of acidic beverages or food increases the concentration and strength of acid ions on tooth surfaces, which could accelerate the demineralization process. Besides, food and beverages, acidic substances that are not affected by bacteria, can come from stomach acid in case of digestive disorders that cause vomiting [1]. Reportedly, demineralization adversely affects tooth enamel hardness; in fact, enamel hardness decreases as the demineralization
process increases [2]. Lately, consumption of commercial or packaged fruit juices in Indonesia has reached 30% [3] implying equally higher production of packaged fruit juices. Notably, the label of packaged fruit juices states the citric acid composition of a juice. Exposure of hydroxyapatite to citric acid causes an ion exchange between hydroxyapatite and citric acid, releasing calcium and phosphate ions from hydroxyapatite, thus accounting for demineralization [4].

This study aims to investigate the impact of immersion time and juice types, both fresh and packaged, on enamel hardness. We believe the result of this experiment would be crucial in raising the awareness of the public regarding the consumption of both packaged and fresh fruit juices and their impact on the oral health.

2. Methods

This study was conducted at the Dental Material Laboratory Faculty of Dentistry Universitas Indonesia and Metal Laboratory of Bandung Technological Institute, from October 2013 to November 2013. The study materials and tools included packaged orange juice (Buavita brand), fresh orange juice squeezed from Pontianak oranges (Citrus nobilis var. microcarpa), aquadest, 60 adult premolar teeth, decorative resin, hardener, Vaseline, Carborundum Disc, mikromotor, low-speed handpiece, mold, grinding and polishing machine, sandpaper number 1500, velvet, alumina 1 μ, Knoop hardness tester, preparation glass, plastisin, press tool, pH digital meter, medicine pot, and cotton.

The specimens for this study were prepared using 60 adult premolar teeth, marked by the formation of completed roots in the cervical portion to facilitate the separation of the crown and root with low-speed micromotor and Carborundum Disc. Crowns were placed at the base and at the center of the mold from a pralon pipe (height, 20 mm; diameter, 15 mm) that was polished with Vaseline, and the bottom was covered with a sticker with the buccal portion facing the base of the mold and attached to the sticker. Then, the mixture of the decorative resin and hardener were poured into the mold, which took approximately 30 min to solidify. After hardening, the specimen was removed from the mold, and the part of the specimen that contained the tooth was ground in the grinding and polishing machine with sandpaper number 1500 until the tooth was exposed. Of note, grinding did not exceed 1 mm. Then, the ground specimens were polished in the same machine with velvet and 1-μ alumina polish material for 30 min. All specimens were observed under the Knoop hardness tester lens; specimens were re-polished in case of scratches. Then, non-scratched specimens were inserted into the medicinal pot, and the polished specimen parts were covered with cotton so that it did not clash with the medicine pot wall when the pot was shaken. Finally, specimens were randomly divided into two equal large groups.

The hardness of all specimens was measured before treatment to obtain initial hardness values. The Knoop hardness tester was set to load for indenting 100 g for 10 s. All specimens were glued onto a preparatory glass with plastic and then pressed on the press tool. Next, specimens were placed under the lens on the Knoop hardness tester, and the focus was adjusted until the surface of specimens was clearly visible. In addition, the indent button was pressed so that the specimen could be indentated with the diamond penetrator for 10 s. After observing specimens for 10 s with the lens, lesions were visible in the form of a rhombus. Figure 1 shows the black line visible on the slide. By pressing a button on the Knoop hardness tester, we obtained the hardness number in Knoop hardness number. Of note, we performed indentation three times for each measurement, and the average of the three values was considered for its recording.

A digital pH meter was used to measure the pH and temperature of both types of orange juices used in this study. All specimens were immersed in each orange juice for 30 min, following which specimens were removed from the juice and rinsed with running water and dried with tissue paper. Then, the hardness value was measured. After that, each specimen was again immersed in orange juice for 30 min, and its hardness was measured until it reached the total time of immersion of 60 min.

The data were tested for normality using the Kolmogorov–Smirnov method. In addition, the difference in normality of enamel hardness data was tested. The difference data were searched to assess the magnitude of enamel degradation at each measurement time. After determining the data
distribution, independent \( t \)-test was performed to assess the significance of the difference in the early enamel hardness in both treatment groups. When the decline in enamel hardness after immersing in packaged or fresh juice was significant, the enamel hardness was tested with repeated analysis of variance (ANOVA) and Friedman test. Then, the significance of enamel hardness immersed in packaged orange juice and fresh orange juice was compared using the independent \( t \)-test and Mann–Whitney \( U \)-test. Finally, the significance of enamel hardness immersed in packaged orange juice and fresh orange juice was compared using the independent \( t \)-test. The p-value was set at 0.05 and with 95% confidence interval.

3. Results
Table 1 summarizes the mean values obtained before, after 30 min, and 60 min of immersion, standard deviations, and confidence intervals (Figure 1). The data of all groups were normally distributed, except for the 60-min immersion in fresh orange juice. We subtracted the results of the 30-min immersion from the hardness before immersion and from that of the 60-min immersion to obtain the magnitude of the decline. After observing decline in data, all groups tested for normality exhibited the normal distribution.

Table 1. The average value of enamel hardness and standard deviation before and after immersion in packaged and fresh orange juice

| Variable                    | \( N \) | Average ± SD   | 95% CI       |
|-----------------------------|--------|----------------|--------------|
| Packaged orange juice       |        |                |              |
| Before immersion            | 30     | 388.75 ± 21.55 | 380.70–396.80|
| After 30-min immersion      | 30     | 309.37 ± 29.15 | 298.49–320.26|
| After 60-min immersion      | 30     | 248.08 ± 27.03 | 237.98–258.17|
| Fresh orange juice          |        |                |              |
| Before immersion            | 30     | 392.80 ± 24.91 | 383.50–402.10|
| After 30-min immersion      | 30     | 341.47 ± 20.19 | 333.93–349.01|
| After 60-min immersion      | 30     | 312.79 ± 18.09 | 306.03–319.55|

SD, standard deviation; CI, confidence interval.
0
50
100
150
200
250
300
350
400
450
before
30 minutes
60 minutes

The hardness value of the enamel at any time of measurement.

Figure 1. The hardness value of the enamel at any time of measurement.

The first analysis of assessing the significance of the initial hardness of the freshly squeezed orange juice group and the packaged orange juice group using the independent t-test revealed no statistical significance ($P = 0.503$), implying that the initial hardness of both groups was the same. Next, the significance of enamel hardness discrepancies was assessed before, 30 min, and 60 min after immersion using repeated ANOVA for packaged orange juice group and the Friedman test for the fresh orange juice group. The results revealed a statistical difference ($P < 0.05$) in all data groups, suggesting that the more prolonged immersion in orange juice significantly decreased the enamel hardness.

In addition, the independent t-test was performed to assess the enamel hardness 30 min after immersion and the Mann–Whitney U-test 60 min after immersion in both groups. The results revealed a significant difference between both groups and at all times of measurement, suggesting a significant decline in the enamel hardness of those immersed in packed orange juice ($P < 0.05$), compared with those immersed in fresh orange juice. Furthermore, the independent t-test was performed to assess the significance of the difference in decreasing the enamel hardness of teeth immersed in packaged orange juice and fresh juice, which revealed a significant difference ($P < 0.05$) in both groups. Thus, it could be concluded that a decline in enamel hardness is higher with packaged orange juice than with fresh orange juice.

4. Discussion

This study investigated the impact of packaged and fresh orange juice on the decline in enamel hardness. In this study, all specimens were sharpened, and the outer surface of the tooth was not exposed; this is acceptable because demineralization can occur beneath the tooth surface in clinical conditions [5]. The 60-min immersion time was chosen because it can release mineral that can be assessed with a profilometer [6]. Previously, as some studies have also selected the immersion time of 15–60 min, the 60-min immersion is acceptable [5,7]. In addition, the 60-min immersion time simulates 1 month of juice consumption with a 2-min drinking assumption, per Stephan [8], who asked his participants to rinse their oral cavity with glucose solution for 2 min.

Apparently, enamel hardness decreases with time. In this study, the first treatment group, which was immersed in fresh orange juice, exhibited a significant difference in enamel hardness and a significant decline in enamel hardness at each measurement time. The results were repeated in the
second treatment group as well, implying that the longer the teeth are exposed to acids, the higher the decline in enamel hardness.

A study reported that consuming soft drinks might cause demineralization and decrease enamel hardness, also known as tooth erosion [9], which is the loss of minerals present in the tooth because of dissolution by acids [10]. In this study, the pH of fresh orange juice was 4.34 and that of packaged orange juice was 3.78. Thus, the pH value of juice in this study was below the critical pH of 5.5, rendering it capable of causing demineralization [1]. Besides the pH of acidic substances that affect demineralization, several factors such as buffer capacity, exposure frequency, duration of exposure, amount, and acidity of the substance affect demineralization [11]. In contrast, remineralization process in the oral cavity can be induced by two factors: increase in the pH of the oral cavity and the availability of a Ca$^{2+}$ and PO$_4^{3-}$ ions. Apparently, increasing pH in the mouth is affected by the salivary buffer capacity; after the pH increases, salivary ions can improve soluble acidic minerals [1]. Acids can be classified into extrinsic and intrinsic acids. While stomach acid due to vomiting, bulimia, and anorexia are intrinsic acids, acidic food and drinks constitute extrinsic acids [1]. Reportedly, acidic drinks might stimulate demineralization, acting as a factor that causes dental caries when demineralization is more dominant than remineralization [12]. Benjakul et al. reported the highest decline in enamel hardness in the longest enamel immersed in an acidic soup from Thailand (Kangsom) [13]. Thus, it could be inferred that the consumption of orange juice in large quantities and for an extended time could cause demineralization and increase the risk of caries.

Both fresh and packaged orange juice contain citric acid at different amounts. While fresh orange juice comprises 9.6 g/L, packaged orange juice comprises 16.8 g/L [14]. Typically, orange juice often represents drinks containing citric acid, and some studies have reported the potential of demineralization in beverages containing citric acid. Scaramucci et al. [15] reported that the enamel immersed in 1% citric acid solution with pH 3.8 exhibited the highest decline in hardness compared with that immersed in artificial juice and some other brands of juice [15]. In addition, Barbour et al. reported that teeth immersed in a solution containing citric acid had a significant decrease in enamel hardness compared with teeth immersed in a solution without citric acid, proving that citric acid plays an essential role in decreasing tooth enamel [16].

The concentration of citric acid in a solution affects the magnitude of mineral loss from the tooth structure of the enamel (enamel, dentine, and cementum). Shellis et al. [17] reported significant differences in the solubility rate of enamel in specimens immersed in 1% and 0.3% citric acid solution at pH 2.45 and 3.2, respectively, where the solubility of enamel specimens immersed in 1% citric acid solution was higher than that of those immersed in 0.3% citric acid solution. In addition, they observed an insignificant difference in the solubility of enamel specimens immersed in 1% citric acid solution with specimens immersed in 0.3% citric acid solution at pH 3.9, but suggested a higher enamel solubility in specimens immersed in 1% citric acid solution [17]. Furthermore, Misra [4] reported ion exchange when hydroxyapatite mineral is immersed in a citric acid solution as follows:

$$\text{HCl}^{2+} + 2\text{H}^2\text{O} \quad \text{(from solution to surface)}$$

$$\text{H}_2\text{PO}_4^{-} + \text{HPO}_4^{2-} + 1.5\text{Ca}^{2+} \quad \text{(from surface to solution)}$$

These ion changes demonstrated that the tooth surface loss of phosphate and calcium ions occurred because of nitric acid interaction. In addition, the loss exhibits the presence of demineralization on teeth. Thus, enamel hardness decreases as the demineralization process increases [2], suggesting that citric acid can cause a decline in enamel hardness.

As mentioned earlier, higher exposure of the teeth enamel to acidic substances results in higher loss of minerals and, thus, lesser hardness. Thus, teeth immersed in packaged orange juice exhibited a high decline in enamel hardness compared with those immersed in fresh orange juice because packaged orange juice is more acidic and has a higher citric acid content than fresh orange juice.
5. Conclusion
The decline in tooth enamel hardness increases with the duration of immersion in orange juice. The highest decrease in enamel hardness occurs in tooth specimens immersed in packaged orange juice because it has a lower pH and higher citric acid content. For further research, artificial saliva could be used to approach the clinical state of the oral cavity.

6. References
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