The effect of CPP-ACP paste on the surface hardness of glass ionomer cement when immersed in orange juice

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Abstract. This study aims to identify the effect of CPP-ACP paste on the surface hardness of glass ionomer cement (GIC) when immersed in orange juice. Eighteen specimens of Fuji IX GIC were divided into three groups: no CPP-ACP added (group A); CPP-ACP applied for three minutes (group B); and CPP-ACP applied for 30 minutes (group C). Specimens were immersed in orange juice and tested for surface hardness using a Vickers hardness tester. Data were analyzed using the one-way ANOVA ($p$ = <0.05). Group A resulted in a 31.77 ± 0.77 Vickers Hardness Number (VHN), group B in a 42.97 ± 1.08 VHN, and group C in a 51.92 ± 0.27 VHN. It was concluded that application of CPP-ACP paste for 30 minutes is effective in preventing a decrease in the surface hardness of GIC due to orange juice consumption.

1. Introduction
Glass ionomer cement (GIC), a restorative material that was introduced in the 1970s, consists of polycarboxylic acid and fluoroaluminosilicate glass. This material is available in powder and liquid form [1]. In Indonesia, GIC is still used for class II and class V restoration, not only for permanent but also for deciduous dentition. GIC has several advantages: it is biocompatible, bonds with enamel and dentine, and is anticariogenic. However, its physical and mechanical properties, such as compressive strength, tensile bond strength, and hardness, are generally lower than composite resin [1-2]. Hardness is one of the most important properties of GIC because it determines material resistance to plastic deformation [3]. The surface hardness of restorative materials can be tested with hardness tests, one of which is the Vickers hardness test (VHT) [4]. The VHT is performed by indenting tested material using a square-based-pyramid-shaped diamond indenter which will be given a certain weight. Any indentation created on the surface of the material will then be observed with a microscope and measured [5].

There are several factors that can influence the hardness value of GIC in the oral cavity, one of which is material dissolution caused by intrinsic and extrinsic factors [6, 7]. Intrinsic factors include anorexia nervosa, bulimia, or any other situation where stomach fluid enters the oral cavity. Extrinsic factors include acid from medication, food, or drinks [8]. Acidic drinks with a low pH can cause GIC particles to dissolve, which leads to a decrease in the surface hardness of GIC. As acidic drink consumption increases, more glass particles will dissolve. In Indonesia, one acidic drink that many people like is packaged fruit juice. Packaged fruit juice is fruit juice that is sold in a box and ready to consume [9]. A survey on packaged fruit juice consumption habits in Indonesia showed that more than 33% of respondents consumed packaged fruit juice two to three times per week, with orange as the favorite flavour [10]. Generally, the pH of orange juice ranges between 3.30 and 4.20 [11].
Many dental products have been developed to protect teeth from acid. One of them is casein phosphopeptide-amorphous calcium phosphate (CPP-ACP). CPP-ACP is a milk product that is used as a remineralization agent [12]. The principle of CPP-ACP is that the longer it is applied in the oral cavity, the more effective it will be [13]. Currently, various preventive materials include CPP-ACP in order to prevent dental caries. CPP-ACP can be found in various products such as tooth mousse, chewing gum, mouthwash, and toothpaste [14]. Several studies have examined the effect of CPP-ACP on GIC restorative material. Those studies reported that when CPP-ACP was applied to GIC, the latter had more compressive strength and higher microtensile bond strength, and initiated more calcium, phosphate, and fluoride ion release when exposed to neutral and low pH. The studies suggested that the increased compressive strength and microtensile bond strength of GIC occurred because cross-bonding occurred between CPP-ACP nanoparticles and the GIC matrix [15-16]. However, there have been no further studies on the effect of CPP-ACP on GIC hardness. Therefore, this study aims to identify the effect of CPP-ACP paste on the surface hardness of glass ionomer cement (GIC) when immersed in orange juice.

2. Materials and Methods

This study is a laboratory experiment in which 18 specimens were divided into three groups. Group A consisted of specimens to which CPP-ACP was not applied. CPP-ACP was applied to Group B’s specimens for three minutes, and CPP-ACP was applied to group C’s specimens for 30 minutes. The GIC specimens were made by making a cylindrical mold of 6 mm in diameter and 3 mm in height. It was then coated with silicone oil as a separator medium. GC Fuji IX GIC was manipulated according to manufacturer guidelines and placed into the mold. Eighteen specimens were made. A Mylar strip and an object glass were placed above the GIC to achieve a smooth surface. A 500 g weight was placed on it to achieve an even surface. The specimen-filled mold was placed into an incubator at 37 °C for 60 minutes. The mold was removed from the incubator after 60 minutes. Each specimen group was placed in a distilled water filled plastic pot and kept in the incubator at 37 °C for 24 hours [17].

Next, the specimens were removed from the incubator after 24 hours and their initial surface hardness was taken. The surface hardness value of each specimen was measured using the Vickers hardness tester with a 200 g weight for 15 seconds on three points [18]. The initial surface hardness value of each specimen was recorded.

The next step was to prepare CPP-ACP GC Tooth Mousse paste and packaged orange juice. The duration of the CPP-ACP paste application for group B (three minutes) matched the minimum application time indicated in the GC Tooth Mousse manufacturer instructions. Conversely, the duration of the CPP-ACP paste application for group C (30 minutes) corresponded to GC Tooth Mousse’s recommendation not to consume any food or drink for 30 minutes post-application. The specimens were then immersed in packaged orange juice whose pH had been measured with a pH meter. For the purposes of this study, the time spent consuming packaged orange juice was assumed at three times a week, for five minutes each time. In this study, the specimens were immersed for 180 minutes, which is equivalent to approximately three months of packaged orange juice consumption (5 × 3 × 12 = 180 minutes). Packaged orange juice was poured into a plastic container filled with the group A specimens and left for 180 minutes. CPP-ACP GC Tooth Mousse paste was spread onto the surface of the group B specimens and left there for 3 minutes. Packaged orange juice was then poured into a plastic pot filled with the group B specimens and left for 180 minutes. CPP-ACP GC Tooth Mousse paste was spread onto the surface of the group C specimens and left there for 30 minutes. Packaged orange juice was then poured into a plastic pot filled with the group C specimens and left for 180 minutes. All the specimens were kept in the incubator at 37 °C. After 180 minutes of immersion, the specimens were removed from the incubator and dried.

The experiment continued by re-measuring the surface hardness of the specimens after immersion. The surface hardness value was measured using a Vickers hardness tester with a 200 g weight for 15 seconds on three points [18]. Statistical analysis of the results was then performed using the SPSS 16.0 statistics application. The normality test for the data was done using the Shapiro-Wilk Test, and the
one-way ANOVA ($p$-value = <0.05) was used to find out if there were any significant statistical differences.

3. Results and Discussion

3.1 Results
In this study, the surface hardness of GIC was tested after being immersed in packaged orange juice. Specimens were divided into three groups and were treated differently before immersion: no CPP-ACP application (group A); a three-minute CPP-ACP application (group B); and a 30-minute CPP-ACP application (group C). The packaged orange juice was measured with a pH meter and a pH of 4.51 was obtained. A surface hardness test was performed using the Vickers hardness test method on three different points with a 200 g weight for 15 seconds.

The test results in Table 1 show that there were changes in the surface hardness value of GIC in each specimen group. There was a decrease in the surface hardness value of GIC for all specimens, with the greatest difference found in group A.

| Group  | Surface Hardness/VHN ± SD Before | Surface Hardness/VHN ± SD After |
|--------|----------------------------------|---------------------------------|
| Group A| 52.00 ± 0.93                     | 31.77 ± 0.77                    |
| Group B| 52.00 ± 0.93                     | 42.97 ± 1.08                    |
| Group C| 52.00 ± 0.93                     | 51.92 ± 0.27                    |

After being tested using the one-way ANOVA, the surface hardness value of GIC group A showed a significant change after immersion in orange juice ($p$ = <0.05). The original surface hardness value of specimen A had a Vickers Hardness Number (VHN) of 52.00 and it decreased to 31.77 VHN after immersion. The surface hardness value of GIC in group B also showed a significant change ($p$ = <0.05) after immersion in orange juice. The original surface hardness value of specimen B was 52.00 VHN and it decreased to 42.97 VHN after immersion. The surface hardness value of GIC in group C also showed a significant change ($p$ = <0.05) after immersion in orange juice. The original surface hardness value of specimen B was at 52.00 VHN and it decreased to 51.92 VHN after immersion. The decrease in surface hardness value in this group was the lowest of all.

3.2 Discussion
This study was conducted to learn the effects of CPP-ACP paste on the surface hardness of glass ionomer cement after immersion in orange juice. The specimens were 6 mm × 3 mm cylindrical Fuji IX GIC and the orange juice was Buavita brand packaged orange juice. The original surface hardness of the specimens was tested with a Vickers hardness tester and the specimens were then divided into three groups: immersion in orange juice with no CPP-ACP application (group A); CPP-ACP applied to the specimens for three minutes followed by orange juice immersion (group B); and CPP-ACP applied to the specimens for 30 minutes followed by orange juice immersion (group C). All the specimens were immersed for 180 minutes, or the equivalent of approximately three months of orange juice consumption. The surface hardness value of the specimens was then measured.

The results of the study showed that a significant decrease in surface hardness value after immersion occurred in the group of specimens immersed in orange juice without prior CPP-ACP application (group A). The surface hardness value in group A experienced a decrease of as much as 20.23 VHN. The declining value could be caused by the acidic orange juice (pH = 4.51). H⁺ ions from the orange juice would diffuse into GIC components and replace metal cations, cross-bonding with polycarboxylic acid molecules in the cement matrix. According to the concentration gradient, metal
cations would diffuse outward and release from the surface [8, 19]. As the amount of metal cations decreases, the amount of oxygen increases on the glass tissue at the surface, leading to damage of the glass tissue. The simultaneous reaction of H\(^+\) and F\(^-\) ions further dissolves the glass tissue. It could be said that immersion in acidic drinks can cause the glass particles to dissolve and decrease the surface hardness of GIC. As the immersion time increases, the more glass particles dissolve and the lower the surface hardness value will be [8,20].

The results of group A correspond to the results of a previous study on the effect of orange juice on GIC hardness. In that study, GIC specimens were immersed for one day, one week, one month, or six months. The results showed that the longer the GIC specimens were immersed, the lower their surface hardness value became [7]. This result suggested that there would be significant changes in surface hardness value after immersion in orange juice.

Group B also showed a significant difference in GIC hardness after immersion. The decrease in value was as much as 9.03 VHN. This occurred due to H\(^+\) ion diffusion into GIC particles, replacing metal cations in the matrix. However, unlike group A, the decrease in surface hardness value was smaller [8,19]. This was because of the CPP-ACP that was spread on the specimens before their immersion in orange juice. In acidic conditions, CPP will bind to ACP and play the role of an acid buffer by producing calcium phosphate (CaHPO\(_4\)) ions, which can balance the decreasing pH level [1,13]. When the declining pH is balanced, dissolution of GIC decreases [16]. CPP-ACP on GIC also plays a role as a calcium ion reservoir and slows the diffusion process of free calcium ions in the matrix, which then leads to a decrease in the dissolution of GIC material [15]. Calcium and phosphate ions from CPP-ACP were deposited to fill the porous GIC surface; therefore, the surface hardness of GIC should increase [15-16]. However, the deposit of CPP-ACP calcium and phosphate ions onto group B GIC was not satisfactory because of the short application period. Thus, the specimens in this group experienced a significant decrease in their surface hardness value.

The process that occurred with this GIC is similar to what happens to teeth. In the demineralization process, H\(^+\) ions diffuse into enamel and replace calcium and phosphate ions [8]. When CPP-ACP is applied to teeth, CPP-ACP deposits calcium and phosphate ions, especially in the form of CaHPO\(_4\), which balance a pH decrease. CaHPO\(_4\) diffuses into enamel as Ca\(^{2+}\) and PO\(_4^{3-}\) ions, thus reforming hydroxyapatite [1, 13]. CPP-ACP also plays a role as a calcium ion reservoir that slows down free calcium diffusion. This event limits mineral loss when a demineralization process occurs and provides a calcium source for remineralization. For instance, CPP-ACP slows down the process of dental caries [21]. Based on these results, it can be said that the second hypothesis, which suggested that there would be a significant change in the surface hardness value of group B GIC, can be validated.

Group C specimens, which were spread with CPP-ACP for 30 minutes before being immersed in orange juice, also experienced a decline in surface hardness value. This decline was the lowest among all the groups and it did not result in a significant difference. The value decrease was as much as 0.08 VHN. The small decrease is due to the 30 minutes of CPP-ACP application before orange juice immersion. CPP-ACP products buffer the increasing acidity by producing CaHPO\(_4\) [16]. A similar process occurred in group B, but the deposit of calcium and phosphate ions occurred for a longer time in group C because of the longer duration of CPP-ACP application. Therefore, there was no significant decrease in surface hardness even though the specimens were exposed to acid [13].

The test results of group C were consistent with previous study on the effect of CPP-ACP on enamel lesions after immersion in an acidic solution. In that study, specimens were divided into two groups: one given a 30-minute CPP-ACP application and the other with no CPP-ACP application. Specimens were then immersed in an acidic solution, observed by polarized microscope, and measured for their mineral loss by microradiograph. The study showed that CPP-ACP application for 30 minutes could avoid enamel demineralization. On the other hand, the specimen group that did not receive CPP-ACP application experienced demineralization [22]. Based on these results, the third hypothesis, which suggested that there would be no significant difference in the surface hardness value of group C specimens after immersion, can be validated.
In addition to testing the surface hardness value of GIC before and after immersion, a comparison of the surface hardness value among all three groups was performed with a statistical test. The results showed that there was a significant difference across all groups. The difference in surface hardness value between groups A and B and between groups A and C occurred because of CPP-ACP application to one of the groups. Moreover, there was also a significant difference between the surface hardness values of groups B and C, which occurred because of the different duration of CPP-ACP application to the GIC specimens. These results correspond to the CPP-ACP application principle, in that as the duration of CPP-ACP application increases, the more effective it will be in the oral cavity [23].

4. Conclusion
The study showed that CPP-ACP paste application can influence the value of glass ionomer cement surface value CPP-ACP paste application for 30 minutes was effective to avoid the decreasing surface hardness value of glass ionomer cement which was cause by orang juice consumption.

Another test are needed to determine glass ionomer cement mass that dissolved in orange juice. Furthermore, it is important to inform people about the decrease of glass ionomer cement surface hardness value probability after consuming packaged orange juice and recommend them to not eat or drink for 30 minutes after CPP-ACP application.

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