



Effect of Fresh Orange Juice and Industrial Orange Juice on Enamel Microhardness of Primary Teeth: An In-Vitro Study

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Article Info	ABSTRACT
<p>Article type: Original Article</p>	<p>Objectives: Increased consumption of non-alcoholic carbonated soft drinks, diet sodas, and fruit juices greatly contributes to dental erosion. This study aimed to compare the microhardness of primary tooth enamel before and after exposure to fresh and industrial (commercial) orange juices.</p>
<p>Article History: Received: 10 Feb 2025 Accepted: 17 May 2025 Published: 22 Feb 2026</p>	<p>Materials and Methods: In this in vitro experimental study, 30 enamel blocks were obtained from primary teeth, and randomly divided into three groups (n=10). The pH levels of fresh and commercial orange juices were measured. The enamel blocks were immersed in fresh and industrial orange juices for 10 minutes, 4 times a day, over a period of 15 days. Specimens in the third group were stored in artificial saliva to serve as the control group. Enamel microhardness was assessed using the Vickers hardness test both before and after immersion in orange juices. Data were analyzed using paired t-test and ANOVA (alpha=0.05).</p>
<p>*Corresponding author: Department of Pediatric Dentistry, Faculty of Dentistry, Isf.C., Islamic Azad University, Isfahan, Iran Email: h.attarzadeh@iau.ac.ir</p>	<p>Results: There was a significant decrease in enamel microhardness in both the fresh and commercial orange juice groups (P<0.001), with no significant difference in this regard between the two orange juice groups (P=0.209).</p> <p>Conclusion: The microhardness of primary enamel can decrease following exposure to both fresh and commercial orange juices, but no significant difference was found between the two types regarding the enamel microhardness reduction.</p> <p>Keywords: Tooth, Deciduous; Dental Enamel; Hardness; Citrus Sinensis</p>
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INTRODUCTION

Dental erosion is defined as the chemical loss of tooth structure caused by acid, rather than the bacteria [1]. This process typically begins with the enamel, and can progress to the underlying dentin, which, due to its composition, erodes at an accelerated rate. In severe cases, the erosion may even reach the dental pulp [1]. The primary cause is an acidic environment, originating from either external sources—such as acidic foods, beverages, and medications—or internal sources, like stomach acid introduced through vomiting [2,3]. Modern dietary habits have significantly

increased the frequency of acidic challenges on teeth, thereby elevating the risk of dental erosion [4-6]. This condition is now recognized as a common problem among children and adolescents worldwide, with studies reporting a prevalence ranging from 10% to 80% [7, 8]. A key etiological factor is the rising consumption of carbonated soft drinks, diet beverages, and fruit juices [9-11]. Importantly, the duration of acid contact with the tooth surface is a more critical factor than the quantity consumed. Carbonated drinks are often held in the mouth longer, and the practice of consuming bottled drinks at bedtime in children prolongs this

contact time, significantly increasing the risk for both erosion and caries [11,12]. Deciduous teeth are particularly susceptible due to their thinner enamel and lower mineral content compared to permanent teeth [8]. Dental erosion can lead to several clinical issues, including tooth hypersensitivity, a reduction in tooth height, and impaired esthetic appearance, all of which are common sources of patient anxiety [8]. The potential of many commonly consumed beverages to cause such erosion has been well established. For example, studies have confirmed that diet sodas [13] and certain grape juices [14] can damage the enamel surface and increase its roughness.

Although the erosive nature of many soft drinks and processed juices has been extensively studied, there remains a notable lack of research focusing specifically on both fresh and commercial orange juices. This is a significant oversight, given that orange juice is extremely popular among children [7, 10, 13]. Since orange juice is highly acidic and a known contributor to enamel wear, this study was designed to fill this knowledge gap. Its primary objective was to measure and compare how exposure to fresh versus commercial orange juice affects the microhardness of primary tooth enamel. This specific investigation is novel because it directly contrasts the effects of these two common juice types on primary teeth, which are known to be more susceptible to damage. By providing a clear comparison of their erosive potential, this research aimed to deliver crucial insights into the relative risks these popular beverages pose to children's dental health.

MATERIALS AND METHODS

In this *in vitro* experimental study (ethical code: IR.IAU.KHUISF.REC.1399.145), primary first and second molars that had been extracted due to therapeutic needs were collected. The collected teeth were required to be completely free from corrosion, cracks, caries, and hypocalcification; teeth that did not possess intact enamel were excluded from the study. The teeth were stored in water for a maximum duration of one month. Following collection, the extracted teeth underwent a gentle cleaning process with a scaler, and were

subsequently treated with sodium hypochlorite (5.25%) to eliminate any residual stains or bacteria on their surfaces, after which they were thoroughly rinsed under running water. All teeth were then sectioned along their sagittal axis using a diamond disc and a cutting machine to create samples measuring 2×2 mm. Next, the enamel specimens were embedded in transparent self-cure acrylic resin (Acropars Co., Tehran, Iran), and to dissipate the heat generated during the polymerization of acrylic resin, the samples were maintained in cold water. The samples then underwent a polishing procedure using 1200- and 1400-grit silicon carbide abrasive papers (Matador®, Rüggeberg GmbH & Co. KG, Germany), and the baseline microhardness of enamel blocks was recorded after drying the enamel surface. For this purpose, the enamel blocks were positioned in a Vickers microhardness tester (Buehler, Germany), and each enamel surface underwent three indentations at three distinct points for testing. An applied force of 10 g was maintained for 15 seconds, and the mean of the three readings was calculated to establish the baseline microhardness value [15]. Following the baseline microhardness assessment, the enamel samples were randomly divided into three test groups, each containing 10 samples. The first group was immersed in a commercial orange juice (Sunich Co., Iran), the second group in fresh, pulp-containing orange juice, and the third group, which served as the control group, was stored in artificial saliva. The pH values were recorded at 3.7 for the commercial juice, and 3.2 for the fresh juice.

A controlled immersion cycle was followed for 15 consecutive days. Each sample was exposed to 40mL of the assigned juice 4 times per day at 3-hour intervals, with each session lasting 10 minutes. This protocol was designed to mimic a frequent consumption pattern [16]. After every exposure, the samples were rinsed with distilled water for 10 seconds before being returned to the artificial saliva storage medium. The artificial saliva, with a pH of 7, contained 150mmol/L KCL, 1.5mmol/L CaCl₂, and 0.9mmol/L

KH_2PO_4 in 100 mL of distilled water, and was refreshed every 24 hours. Upon completion of the 15-day cycle, all samples were washed, dried, and their microhardness was re-evaluated. Normality of the data was assessed using the Kolmogorov–Smirnov test in SPSS version 22. All variables were normally distributed ($P > 0.05$), allowing the use of parametric tests (paired t-test and one-way ANOVA) The resulting data were analyzed using paired t-test to compare pre- and post-treatment microhardness values within the groups, and ANOVA to assess differences among the groups using SPSS software version 22, with a P-value below 0.05 denoting statistical significance.

RESULTS

The results clearly showed that tooth enamel became significantly softer after exposure to both fresh ($P < 0.001$) and commercial ($P < 0.004$) orange juices. On the other hand, enamel samples in the artificial saliva control group maintained their hardness, showing no significant change by the end of the study period ($P = 0.172$), as shown in Figure 1.

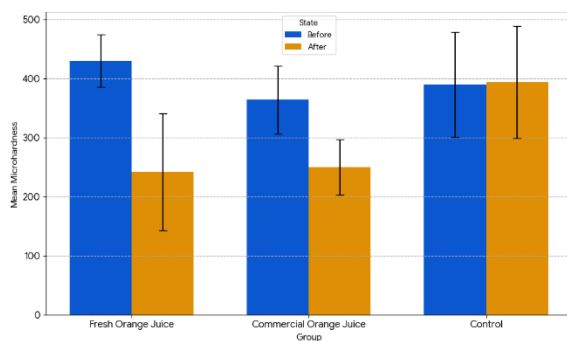


Fig 1. Microhardness of the three groups before and after the intervention

Pairwise comparisons showed that exposure to fresh orange juice caused a significant reduction in enamel microhardness compared with the artificial saliva control group ($P < 0.001$). Similarly, commercial orange juice also resulted in a significant decrease in hardness relative to the control ($P < 0.004$). When the two juice groups were compared directly, fresh orange juice produced a numerically greater decrease in enamel

hardness; however, this difference was not statistically significant ($P = 0.209$).

Crucially, even though fresh juice had a larger effect on enamel, the difference in erosive potential between the fresh and commercial juices was not statistically significant ($P = 0.209$).

DISCUSSION

This study demonstrated that both fresh and commercial orange juices significantly softened tooth enamel. Interestingly, the degree of softening was comparable between the two juice types. The underlying mechanism for this softening is rooted in the acidic nature of the juices. Enamel demineralization begins when the environmental pH falls below a critical level of 5.5. In this acidic state, a process called demineralization is triggered, causing essential minerals, primarily calcium and phosphate, to leach out from the tooth's hard structure. As enamel strength is directly dependent on its mineral content, this leaching action inevitably leads to a softer surface [17]. In the present study, the fresh orange juice recorded a pH of 3.2 and the commercial juice had a pH of 3.7, placing both far beneath this critical threshold and directly explaining the observed erosion. The principal component responsible for this effect is citric acid, a natural constituent of orange juice. Independent research validates that citric acid by itself can cause a significant reduction in enamel hardness [18], and that the degree of mineral loss is influenced by its concentration [19]. The existing literature, such as a study by Meira et al. [20] acknowledges that fruit juices vary in their potential to erode enamel, partly due to differences in their inherent mineral composition. It is important to recognize that while a low pH is the initial driver of demineralization, the final erosive outcome is modulated by other elements. These include the drink's buffering capacity (its ability to sustain an acidic environment), duration of tooth contact, and presence of safeguarding minerals like calcium and fluoride [17, 21]. In fact, some commercially produced juices are supplemented with calcium and phosphate for the specific purpose of decreasing their

damaging effect [17, 22]. The present results indicated a trend where fresh juice caused slightly more softening than commercial juice, a finding that aligns with studies by Lee and Kim [23] and de Melo et al [24]. A comparable pattern was noted by Tocolini et al, [14] in a study on grape juice. However, contrasting results exist, such as those from Gumilang and Djauharie [17] who found commercial juice to be more erosive. This inconsistency could be attributed to our methodological approach, which employed brief, repeated exposure cycles interspersed with periods in artificial saliva, thereby more closely simulating actual consumption of juice. The established scientific view firmly connects acidic drinks to dental erosion, with individuals experiencing frequent drops in oral pH being at higher risk [15]. Larsen and Richards [25] further specified that beverages with a pH lower than 4.2 are particularly damaging, a classification that encompasses the juices used in the present study. An additional factor is temperature, as warmer liquids are known to increase the rate of enamel dissolution [13]; since the tests were conducted at room temperature in the present study, the recorded erosive effects might actually be an underestimate. The central finding of the present study that orange juice erodes enamel is consistent with a wide body of literature, regardless of the assessment method used [20, 23, 24, 26-29]. When compared to other drinks, beverages like Coca-Cola are often more erosive than orange juice [26, 27]. This is because cola typically contains phosphoric acid, which is stronger than the citric acid in juice and can cause more permanent surface etching. The duration of time the acid is in contact with teeth is also a critical factor; prolonged exposure leads to more severe damage as the tooth's protective shell is worn away [27, 28]. Supporting this, Torres et al. [28] found that while both cola and a calcium-fortified soy-based juice caused softening, cola was more destructive due to its higher titratable acidity. The added calcium to the soy juice helped reduce its erosive effect. The type of tooth structure also influences the rate of erosion. Dentin, being less mineralized,

often erodes faster than enamel [20]. However, this is not always the case, as some studies like Zimmer et al. [26] reported greater enamel loss, highlighting how different research methodologies can yield varying results. Special consideration is needed for children's primary teeth, which are more vulnerable to erosion than permanent teeth due to their thinner enamel and lower mineral content [16, 21, 30]. Erosion in primary teeth is a known risk factor for future erosion in permanent teeth [31, 32]. The rough surfaces created by erosion also enhance more bacterial plaque accumulation, increasing the risk of cavities [33]. Therefore, early signs of erosion should be a warning, prompting healthcare providers to educate families about dietary risks [34]. Behavioral habits are equally critical. Practices like holding juice in the mouth before swallowing or drinking it at bedtime significantly prolong acid exposure. Therefore, educating patients about changing these habits is a powerful preventive strategy. Ultimately, erosion will progress continuously if the acidic challenge is not prevented [20].

Studies by Ahmed and Saleem [35] and Zedan and Jafar [36] shed light on how different materials behave in dental practice. Ahmed and Saleem [35] looked at how natural and industrial orange juices can change the surface roughness of orthodontic bonding composites, which is important for maintaining oral health. In conclusion, while the present in vitro study could not replicate every aspect of the oral cavity, it provided clear evidence that orange juice softens enamel. Since dental erosion is a complex issue shaped by many risk and protective factors, continued research into these interactions is essential.

CONCLUSION

The microhardness of primary tooth enamel decreased after immersion in fresh and commercial orange juices. There was no difference in erosive effect of fresh and commercial orange juices on primary tooth enamel. Given the widespread consumption of these beverages by children, it is crucial for dental practitioners and parents to be aware

of these results. Implementing strategies to mitigate the effects of acidic drinks, such as promoting good oral hygiene and limiting exposure duration, could help protect primary teeth from erosion. Further research is needed to explore long-term effects and preventive measures.

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CONFLICT OF INTEREST STATEMENT

None declared.

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