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The Effect of Exposure to Dried Fruits on the Surface Micro-Hardness of Dental Enamel

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ABSTRACT

Background: This study investigated the effect of exposure of enamel surfaces to dried fruit suspension including dates, raisins, and dried apricot on their micro-hardness changes. **Methods:** In this in vitro study, fifty enamel sections of bovine incisor teeth were mounted inside the acrylic resin so that the enamel surface would not be exposed to the acrylic. After surface polishing, the initial micro-hardness was measured by a Vickers hardness-testing machine. The samples were randomly placed into five different solutions including apricot, raisin and date suspension, citric acid (positive control) or sorbitol (negative control), 5 times a day, each time for 5 minutes, and then in artificial saliva for 60 minutes. This process was repeated for 20 days. Eventually, the final micro-hardness of the samples was measured. Micro-hardness changes between groups were compared through ANOVA and TUKEY test using SPSS 23 software with a significance level of $P<0.05$. **Results:** After exposure, the micro-hardness of the teeth was significantly reduced in all three suspensions prepared from dried fruit ($P<0.05$). Apricot and date had the highest and lowest effects on reducing the micro-hardness of teeth, respectively. Sorbitol solution did not have a significant effect on changing the micro-hardness of teeth ($P=0.13$). **Conclusion:** The suspension of studied fruits (apricot, raisin, date) causes a significant reduction in micro-hardness of the dental enamel surface, indicating the negative effect of frequent consumption of dried fruits over long periods of time on dental health.

Keywords: *Apricot; Enamel surface; Date; Dried fruits; Micro-hardness; Raisin*

Introduction

Diet is influenced by the environment and people's health. Furthermore, increasing income and urbanization have replaced the traditional diet with treated sugars, fats, high oil and meat. These changes can threaten human health, environmental sustainability and biodiversity (Tilman and Clark, 2014).

One of the solutions to this problem is increasing the consumption of fruits and vegetables because it has significant benefits for health and reduces global greenhouse gas emissions and deforestation. It also prevents many chronic diseases linked to diets such as obesity, type II diabetes, coronary heart disease and some cancers

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(Westhoek *et al.*, 2014). Fruits are usually produced seasonally, and therefore, fresh fruits may not be available throughout the year. Therefore, they are dried in different ways to increase their life span. Dried fruits are considered important and healthy snacks around the world. They have the advantage of being available during the year and can be a healthy alternative to salty and sweet snacks (Chang *et al.*, 2016). Drying reduces the weight and volume of fruits extensively; thus, reducing the packaging costs, storage, and transportation (Kamiloglu *et al.*, 2016). Fruits and vegetables are excellent sources of antioxidant compounds such as vitamins (especially vitamins C and E), flavonoids, and carotenoids which protect against cardiovascular disease, cancer and age-related degenerative changes (Schieber *et al.*, 2001). The US National Health and Nutrition Examination Survey (NHANES) in 1999-2004 reported the relation between dried fruit consumption and the reduction of the risk of non-communicable diseases (Keast *et al.*, 2011).

Dried fruits can have a protective effect on teeth in several ways. If they are chewed and have a pleasant taste, the flow of saliva will be stimulated; this flow of saliva has a protective effect on teeth against decay. Dried fruits also contain polyphenols which cause antimicrobial effects (Sadler, 2016). Some dried fruits have a high rate of sorbitol, which is not metabolized by oral bacteria, and therefore, has no cariogenic effect (Sadler *et al.*, 2019). The Dietary Guidelines for Americans (2015-2020) emphasize the role of consuming dried fruits in getting the required dietary fiber. Although it has been suggested that excessive consumption of dried fruits can lead to extra calories, there was no mention of their link with caries (Rahavi *et al.*, 2019). In order for dried fruits to contribute to dental caries, the sugars present in the food matrix need to be solubilized and diffused into dental plaque. The rate of solubilizationdepends on the location of the sugars in the matrix (inside or outside the cellular structure), the fruit texture, and the force and frequency of chewing. Other influential factors include plaque thickness, the length of time dried fruits remain in the mouth, allowing the sugars to dissolve, and the buffering capacity of saliva (Sadler *et al.*, 2019). In vivo plaque acid measurements showed inconsistent evidence for the demineralization of raisins (Utreja *et al.*, 2009). In an in vitro study regarding plaque acidogenicity, raisins and dates were compared with 52 other snack foods, such as hard and soft candies, baked goods and beverages; no significant difference was found between dried fruits and other snack foods concerning teeth (Edgar *et al.*, 1975).

In addition to emphasizing the importance of nutrition for the general health of the child, the pediatric dentist is obliged to use every opportunity to eliminate any undesirable eating habits and replace more appropriate foods in terms of dental health. Dried fruits can be commercialized as snacks between meals instead of sweet junk foods containing carbohydrates. Before making such a recommendation, it is necessary to study the dental effects of various dried fruits, such as dental demineralization and erosion. Since that dietary recommendations about dried fruits are different, in this study, a number of common dried fruits in Iran, such as date, raisins and dried apricot, were examined.

Furthermore, the potential of dental demineralization due to the consumption of dried fruits in comparison with sorbitol as negative control and citric acid as a positive control was investigated. This was done through the evaluation of changes in the surface micro-hardness of enamel blocks in an in vitro study.

Materials and Methods

Preparation of enamel sections: Thirty bovine incisor teeth were collected and disinfected in 1% thymol solution for one week. The inclusion criterion was sound incisor teeth without obvious crack, and the exclusion criterion was any fracture or cracks determined after microscopic evaluation. Periodontal tissue and its extra tissues were removed by curette (Gracey curette 1/2; Hu-Friedy, Chicago, IL., USA), and the surface of the teeth was cleaned with a brush and prophylaxis

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paste (Kemdent, Swindon, Wiltshire, UK). Then, the roots of the teeth were cut from their crown at the cemento enamel junction (CEJ) of the tooth by a micro-motor. On the buccal surface, the approximate sites of the incisions were drawn in the form of 4×4 mm squares with a sharp black pencil and gauge. Afterwards, the teeth were mounted on the plates of the cutting machine with wax and placed inside the three-axis Computerized numerical control (CNC) cutting machine (Nano-pars. Mashhad, Iran), with a rotation speed of 2000 rpm, and a disk plate thickness of 0.2 mm, and separated 4×4 mm sections from the surface of the teeth.

Mounting samples: Enamel sections were mounted on the surface of a cylindrical acrylic resin (Acropars self-cure, Acropars, Marlik, Medical Co. Iran) with a 5 mm thickness. The enamel sections were placed in such a way that they were not exposed to acrylic. After this stage, the enamel surface was examined under a microscope to be free of any cracks or fractures, and fifty suitable samples were selected. Surface polishing was performed on the surface of mounted samples with 1000, 1500, and 2000 grit sandpaper. A code was assigned to each sample, which was recorded on its acrylic. Fifty samples were divided into five groups (N=10) using *Research Randomizer* software. They included a date, apricot, raisin, sorbitol (negative control) and citric acid (positive control).

Initial hardness test: Micro-hardness was measured using Vickers hardness and a universal testing machine (STM20, SANTAM, Tehran, Iran). The hardness number of Vickers with pyramidal diamond indenter was calculated through the following formula (Pahk *et al.*, 2000):

HV=F/A=1.8544F/d2

HV: kg /m^2, F:kg, d:mm

First, the samples were placed in the desired position under a microscope. Their surface was examined with a magnification of ×40, so that the place of the application of surface force would be free from any defects. A force of 50 g for 15 seconds was recorded on the sample by the indenter as a positive sign. The load was applied three times, and their mean was recorded as the final hardness number of each sample.

Preparation of dried fruit suspensions and control solutions: Dried fruits, in which no sulfur or additive was used in their drying process, were utilized in this study. To prepare the suspension, 50 g of dried date, raisin and apricot were weighed by digital scales (Libror AEU-210, Shimadzu Corporation, Kyoto, Japan), and each was mixed in 50ml of water with a mixer (Kenwood Coporation, Tokyo, Japan) for 5 minutes.

Citric acid solution (3%), as a positive control, and sorbitol solution (50%), as a negative control, were prepared by dissolving 0.3 g citric acid crystals and 50 g sorbitol in 100 ml of water respectively. The pH for suspensions and control solutions was measured by the pH meter device (WTW, Weilheim, Germany) at room temperature. The device was calibrated with standard pH levels of 4 and 7 before use.

Preparation of artificial saliva for the pH cycling process: In this phase, 4.1 mM potassium dihydrogen phosphate (KH_2PO_4), 4.0 mM sodium hydrogen phosphate (Na_2HPO_4), 24.8 mM potassium hydrogen carbonate ($KHCO_3$), 16.5 mM sodium chloride (NaCl), 0.25 mM magnesium chloride (MgCl2), 4/1 mM citric acid, and 2.5 mM calcium chloride ($CaCl_2$) were mixed. The pH of this solution was 6.7.

Sample size: The sample size was calculated according to Pollard (Pollard, 1995), with a 95% confidence level and 80% power level. At least 8 samples were needed; however, they were increased to ten. The formula for determining the sample size was as follows:

$$n = \frac{(Z_{1-\frac{\propto}{2}} + Z_{1-\frac{\beta}{2}})^2 (s_1{}^2 + s_2{}^2)}{(\tilde{\mu}_1 - \tilde{\mu}_2)^2}$$

$$= \frac{(1.96 + 0.84)^2 (1.22^2 + 0.96^2)}{(3.51 - 1.94)^2} \cong 8$$

$\propto$: The first type of error; $s_1{}^2$: The variance of the first sample; $s_2{}^2$: The variance of the second sample; $\tilde{\mu}_1$: Estimation of the mean of the first

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sample; $\tilde{\mu}_2$: Estimation of the mean of the second sample; Z: Area under the normal chart

PH cycling process: For each group, pH cycling was performed in the following steps: First, the samples were immersed in 50 ml of the selected dried fruit suspension for 5 minutes. Then, they were rinsed with a syringe containing distilled water to the extent that they did not have any obvious contamination with the immersed material, which was tried to be limited to 50 ml. Finally, they were immersed in 50 ml of artificial saliva solution for 60 minutes.

This cycle was performed 5 times a day for twenty days, in general 100 times for each group. Samples were kept in distilled water at pH=7 between these steps when the cycling was not performed. All these steps were performed at room temperature. Moreover, fruit suspensions were prepared freshly every day. The Final hardness test was performed after the end of the pH cycling. The percentage of tooth surface hardness loss was calculated with the following formula:

$$SHL\% = \frac{\text{Final microhardness-Initial microhardness}}{} \times 100$$

Ethical considerations: This research has been approved by the Ethics Committee of the School of Dentistry, Mashhad University of Medical Sciences, under code No 960962, and IR.MUMS.DENTISTRY.REC.1394.325.

Data analysis: Multivariate analysis of variance (MANOVA) was used to compare micro-hardness in different groups due to the existence of correlation. If the data were not normal, the Friedman test would be used, and the comparisons were in pairs with the Wilcoxon test. The applied software was SPSS23, and the significance level was 0.05.

Results

In the present study, the effect of dried fruits on bovine enamel micro-hardness was investigated. Before starting the experiments and placing the teeth in the fruits suspension, all teeth had the same level of hardness, but after applying the pH cycling process, the hardness level of the teeth changed significantly. All suspensions of different fruits significantly reduced the hardness level of teeth (P<0.05, **Table 1**). Among the selected dried fruits, apricot and date went through a change of 227.76 HV and 108.88 HV regarding hardness levels. This indicated the highest and lowest effect on reducing the hardness of teeth. Furthermore, the Sorbitol solution did not show a significant effect regarding the micro-hardness of teeth (P=0.13). Compared to the positive control group, apricot did not differ from citric acid significantly in the level of micro-hardness reduction; but, date and raisin had a significantly less adverse effect on micro-hardness compared with citric acid (**Table 2 and Figure 1, 2**).

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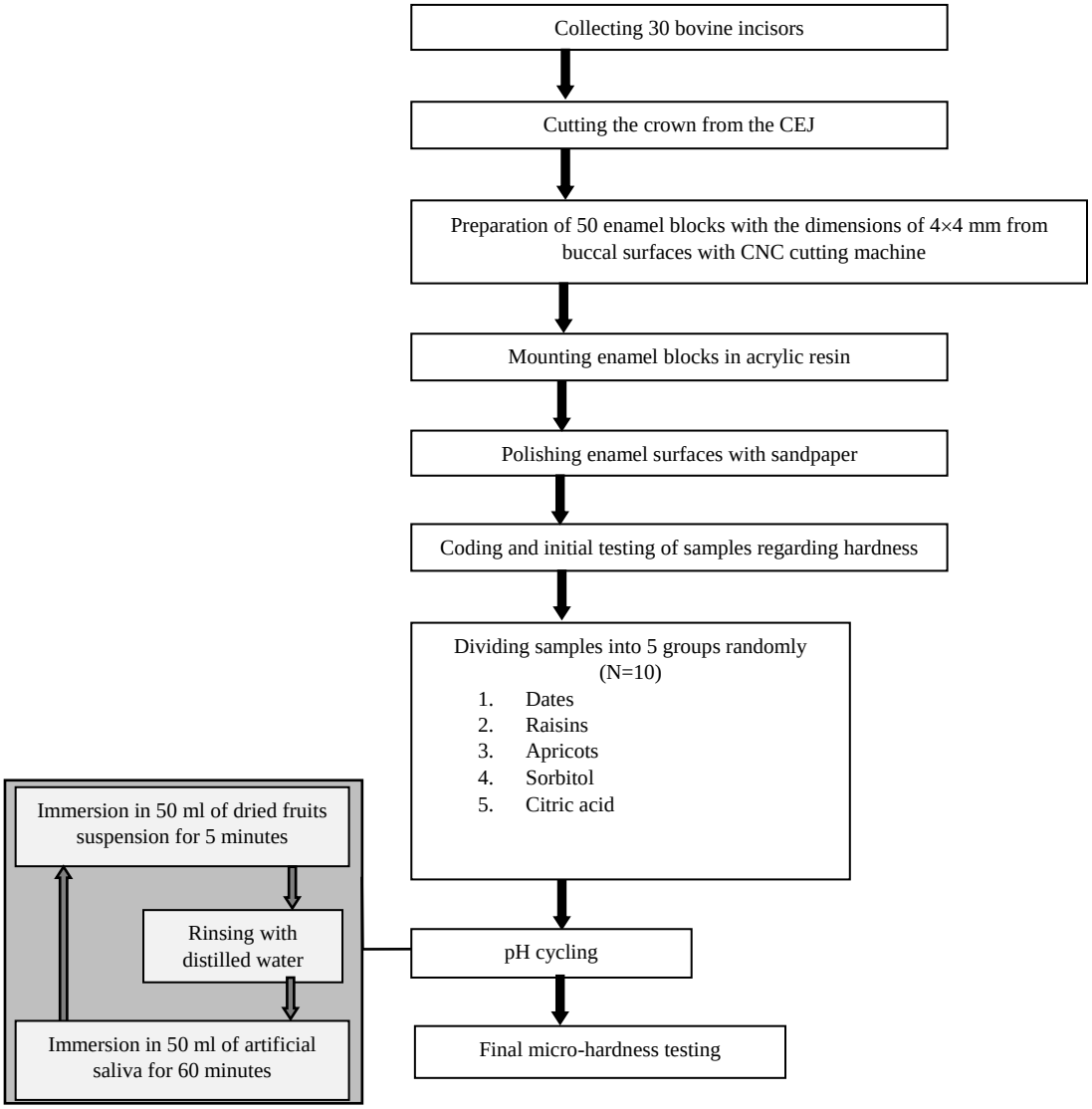

Figure 1. Laboratory stages of the study

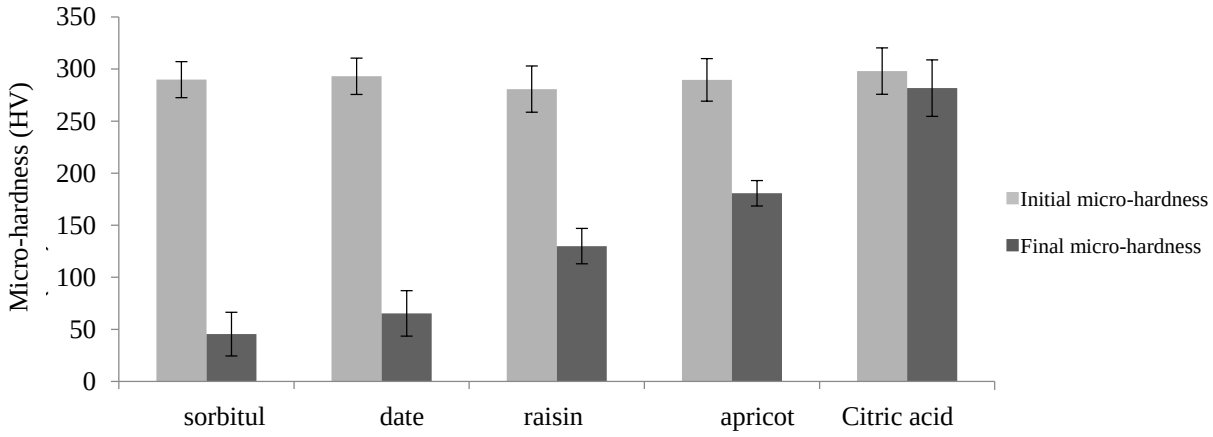

Figure 2. Enamel micro-hardness changes before and after the pH cycling process. Data were presented as mean±SD

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Table 1. Initial and final micro-hardness (HV) in the studied groups.

Groups	pH suspension	Number	Initial micro-hardness	Final micro-hardness	P-value
Apricot	4.1	10	293.06±17.31	65.30±20.96	<0.0001
Raisin	4.2	10	280.74±17.44	129.90±21.81	<0.0001
Date	6.2	10	289.56±22.16	161.68±19.24	<0.0001
Citric acid	3.1	10	289.89±20.40	45.42±12.17	<0.0001
Sorbitol		10	298.10±22.30	281.77±27.10	0.137

The final micro-hardness in the apricot and citric acid groupswas not statistically different (P= 0.54). But, the comparison of the final microhardness between groups was statistically and significantly different in other cases (P<0.0001 for all cases). Data were presented as mean ± SD.

Table 2. Changes in the micro-hardness level and the reduction in the percentage of surface hardness level (SHL%) in each group.

Groups	Hardness Change	SHL%
Apricot	-227.76	77.71
Raisin	-150.84	53.72
Date	-108.88	37.60
Citric acid	-244.43	84.32
Sorbitol	-16.40	5.50

The rate of hardness change in dates, raisins, and apricots was statistically and significantly different from the sorbitol group (P<0.0001). The rate of hardness change in date and raisin was statistically and significantly different from the citric acid group (P<0.0001). The rate of hardness change in the apricot group compared with the citric acid group was not statistically significant. (P= 0.75).

Discussion

All suspensions of dried fruits cause a significant reduction in the hardness level of the teeth during the pH cycling process.

The dried fruits to which sugar is not added during the drying process are called traditional dried fruits. They are rich in dietary fiber, low in fat, and contain useful amounts of micronutrients such as potassium, manganese, iron, and vitamin K, depending on the particular type of dried fruit. However, the possible adverse effects of dried fruits on teeth is a limiting factor for their consumption as a snack. Dried fruits contain sugar, and although the process of drying water destroys them, their amount of sugar is the same as a fresh fruits. Since dried fruits are sticky and have a high concentration of sugar, it is better to consume them with meals to reduce their impact on dental health (Moynihan, 2003). Dried apricot showed the highest level of change in hardness following the citric acid control solution, and its effect on the reduction of hardness was significantly higher than date and raisin and sorbitol groups (P<0.0001). Moreover, its effect was not significantly different from the citric acid group (P=0.75). This can be attributed to the lower pH level and higher acidity of this solution, which is related to citric and malic acids (Grobler *et al.*, 1989). In the present study, the measured pH of dried apricot suspension was lower than date and raisin (pH=4.1). Examination of sources revealed that there were few studies regarding the effect of apricot on dental erosion. Grobler *et.al* examined enamel erosion rate by five different fruits including apricot, apple, grape, orange and guava. In the first six minutes of their study, the apricot erosion rate proved to be higher than other fruits, although its pH level was moderate. This can be due to the low carbohydrate content and high rate of acid (citric and malic acid), which could cause erosion faster than others. The erosion in a period of forty minutes was mostly reported in grapes, whose pH level was higher than apricot (Grobler *et al.*, 1989). In the present study, raisin was tested at pH=4.6, which

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significantly reduced tooth micro-hardness. Raisin is made up of about 60% sugar, mainly fructose and glucose (Camire and Dougherty, 2003), which might be considered unhealthy. However, it is rich in dietary fiber (4.3–5.4 g/100 g) (Camire and Dougherty, 2003, Li and Komarek, 2017), which contributes to its probiotic effect, because it is selectively used by host microorganisms (Gibson *et al.*, 2017, O'Grady *et al.*, 2019) . Wong reported that when raisin is consumed alone, it does not reduce the pH level of the mouth to below the threshold of 5.5, causing demineralization of the enamel. In addition, it does not remain on the teeth for a long time (Wong *et al.*, 2013). Raisin contains photo-chemical antimicrobials which inhibit the growth of oral bacteria linked to dental caries (Rivero-Cruz *et al.*, 2008). In previous studies, however, the effect of raisin on hardness has been directly investigated in only one case, and often on grape juice. For example, Gonçalves *et.al* investigated the erogenous potential of five different types of grape juice on incisions from bovine teeth placed in tested solutions 4 times a day for 10 minutes for 15 days. All solutions had a significant effect on the loss of hardness, and the potential for erosion regardless of their type and pH level (Gonçalves *et al.*, 2012).

Another study directly examined the effect of raisins on erosion. Issa et.al studied the effect of whole fruit, juice and vegetable on demineralizing enamel in situ, and asked participants to consume the studied materials 7 times a day for 10 days. The tested materials were fresh apples, oranges, grapes, carrots, tomatoes, fruit juice and raisin. The results showed that the rate of demineralization of all these materials was significant, and similar to the sucrose solution. There was no difference between juice, fresh fruit, and dried form (raisin) in terms of change in demineralization (Issa *et al.*, 2011). A different method was used to assess the degree of demineralization, making its quantitative and accurate comparison with the present study difficult. Raisin contains high sugar (about 64%), which causes severe demineralization of dental incisions. This issue should be considered in schools, because raisin is one of the five recommended fruits to be eaten in a day, and is often considered a healthy snack for school children. Therefore, long-term and frequent consumption of raisin as a snack by children can increase demineralization and the risk of dental caries (Issa *et al.*, 2011), which is consistent with the results of this study.

In the present study, the effect of the date on the micro-hardness of teeth enamel revealed that the hardness of enamel decreases significantly after exposure to date suspension (P<0.0001). The date with pH=6.2 has changed the hardness of tooth enamel to 108.88 HV. This can be attributed to the high viscosity of this solution and the high amount of carbohydrates (70%) in its structure. This carbohydrate is in the form of sugar (including fructose, glucose, and sucrose) quickly absorbed by the body. Most of the sugar in date is fructose and glucose, which are almost equal in the amount in date. The amount of sucrose, however, is much lower (Al-Farsi and Lee, 2008). There have been no studies regarding the effect of the date on enamel micro-hardness. A study pointed to the role of the date in the inhibition of the growth of *Streptococcus mutans*, being the main cause of tooth decay. Therefore, the use of date was introduced as a factor in preventing tooth decay (Sayyedi *et al.*, 2007). The pH-cycling used in this study was based on a specific order; an environment with a neutral pH level was replaced with an acidic environment in order to periodically simulate changes in the pH level occurred during the metabolism of sugar in vivo. This is one of the advances in the pH cycling model which can simulate demineralization and re-mineralization in the decay process (Buzalaf *et al.*, 2010). Vickers micro-hardness measures the resistance of enamel surfaces against indentor penetration. It is generally an easy, reliable, non-destructive, and highly sensitive method to examine mineral changes and dissolution of hard dental tissue (Feagin *et al.*, 1969). Citric acid is a common acid used in most studies to determine the effect of food erosion because it is the predominant natural hydroxy acid found in fruits and juices; therefore, it is used as a positive control in this study. In

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addition, citric acid can be easily prepared for both in vitro and in situ studies (West *et al.*, 2001).

A contact time of 5 minutes was chosen. This was because one study showed the pH level of saliva and its calcium phosphate saturation returned to its original level after 5 minutes of rinsing with citric acid. The adopted technique in current research is suitable for in vitro simulations, and can investigate the effect of different parameters on dental erosion (Bashir and Lagerlöf, 1996). In addition, the average chewing time of dried fruits in the mouth is 5 minutes.

Dried fruits are an important and useful snack around the world. Carbohydrates, mainly sugars, are their predominant component (USDA, 2018). Due to the high amount of sugar, dried fruits are expected to have a high glycemic index (70 and higher), which may increase insulin reactions. However, recent studies have shown that dried fruits have low to moderate glycemic index, and glycemic and insulin responses are comparable to fresh fruits (Kim *et al.*, 2008). This may be due to the presence of fiber and phenolic which can modify the blood sugar response (Widanagamage *et al.*, 2009). Dried fruits per serving (40 g or about a quarter cup) are good sources of dietary fiber, water-soluble vitamins and minerals, and contain a wide range of bioactive compounds such as phenolic acids, flavonoids and carotenoids. The positive health effects of bioactive compounds in dried fruits are probably related to their strong antioxidant activity (Alasalvar *et al.*, 2020).

Dried fruits can increase the amount of fruit consumed in our diet regardless of the season (Garcia-Viguera *et al.*, 1994). Several benefits have been identified regarding the effects of dried fruits on dental health. Therefore, both the positive and negative properties of dried fruits on the teeth should be considered in further scientific studies.

Conclusion

The suspension of the fruits studied (apricot, raisin, and date) have a significant effect on reducing the hardness of teeth. Among the selected dried fruits, apricot had the highest, and date, the lowest effect on reducing tooth hardness. The consumption of dried fruits frequently and over long time periods of time can have adverse effects on dental health.

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Conflict of interest

The authors declared no conflict of interest.

Author contribution

Sardar F involved in drafting the manuscript or revising it critically for important intellectual content. Ajami B involved in preliminary idea, supervision of research implementation, final review of the manuscript. Hasani F participated in performing laboratory steps and writing the manuscript. Boskabady M involved in the conception and design of data, analysis and interpretation of data.

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