Original Article

Mineralization potential of Fresh Milk and Mineral Water Treatment on Enamel Surface after Demineralization by Citric Acid

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Abstract

Introduction: Evidence shows that people from different regions have different teeth characteristics based on their daily water consumption. This exploratory study aimed to measure the mineralization potential of mineral water and fresh milk on the demineralized enamel surface. **Methods:** The enamel surface was softened for 10 min in 100 ml of 1% citric acid under 150 rpm agitation in all specimens. Afterward, the specimens were mineralized with 100 ml of mineral water and milk under 150 rpm agitation. The mineral content of the enamel surface before and after the treatment in nanoscale was measured using an energy-dispersive X-ray spectrometer (EDXS). Before and after treatment, the results of EDXS were analyzed using a one-way ANOVA test. **Results:** The results showed that initial treatment with citric acid significantly increased the surface roughness of the enamel surface. Moreover, in treatment with fresh milk, the potassium and sodium content of the enamel surface increased. It was also reported that mineral water treatment increased the sodium content of the enamel surface. **Conclusion:** The findings suggested that milk and mineral water might slightly recover the damage induced by citric acid on the enamel surface.

Keywords: Demineralization, Enamel Surface, Milk, Mineralization, Mineral Water

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Introduction

Dental erosion by acidic beverages happens by chronic exposure to erosive acids at low pH and is defined by leaving a small demineralized zone behind (1–3). Conventionally, the demineralization process is characterized by changes in the mineral content of the tooth enamel after acid softening. Remineralization is also defined as calcium and phosphate uptake through the surface enamel (4,5). Therefore, mineralization is the chemical process of enamel formation through the combination of calcium, phosphate, and hydroxyl ions. Evidence confirmed that cavities develop when the rate of demineralization exceeds the rate of remineralization, typically in a process that requires a long time (6).

The demineralization of enamel occurs for several reasons; however, it most importantly happens due to fermentable carbohydrates and the acid by-product of oral microbes. The results of studies suggest that treatment with minerals may protect sound enamel from acid surface softening and enhance the remineralization of erosive lesions (7). The detection of enamel loss is based on the surface hardness measurement by calculating the depth, baseline, and post-treatment roughness (8). The surface hardness values reflect average hardness for the given mineral content of the enamel; nevertheless, they do not clarify the crystalline structure of redeposited minerals (9). The surface roughness and mineralization of tooth enamel can be measured with various techniques. Micro EDX fluorescence is a powerful technique for mapping the surface roughness and mineral content of enamel and other dental materials after chemical erosion (10–12).

Water is one of the most common drinking liquids in human life, and each type of mineral water has a corresponding effect on teeth. Moreover, tooth enamel contains a high mineral concentration in the human body, and the primary mineral compound in enamel is hydroxyapatite, a crystalline calcium phosphate (13–15). Calcium phosphate is highly soluble in saliva and serves as a source of calcium and phosphate required for decalcified teeth (16). Therefore, this study mainly aimed to determine the effect of mineral water and milk on tooth enamel. Furthermore, the findings of this study may help people understand the impact of milk and water rinsing on surface rehardening of acid softened enamel, which happens daily by consuming acidic drinks.

Materials and Methods

Tooth preparation

A total of 30 extracted human teeth were collected. It is noteworthy that teeth with pathological or gingival problems were excluded from the study. The teeth were prepared according to the technique provided by Pahlavan et al. (17) and Azer et al. (18). For this purpose, the extracted incisor teeth were collected and stored in refrigeration until testing. Teeth structures were investigated with a 4x magnifier and transillumination (AdDent, USA) to ensure they were free of cracks, restorations, or caries. The labial surface of the teeth was sectioned to form specimens with the same size of square enamel block (7 mm×7 mm×2 mm) using a diamond disc (NTI, UK) and cooled with profuse water. The enamel surface was flatted using abrasive paper. The specimens were stored in artificial saliva for 48 h at 37°C before testing to keep 100% relative humidity. The initial mineral contents of the enamel surface (Ca, Mg, K, and Na) were measured at the baseline using an energy-dispersive X-ray spectrometer (EDXS; Skyray Instrument, USA).

Treatment

According to the technique developed by Esteves-Oliveira et al. (5), the enamel surface of specimens was softened for 3 min in 100 ml of 1% citric acid (pH=4) under 150 rpm agitation. The samples were rinsed with distilled water for 30 seconds and hydrated in artificial saliva (Thermo Fisher, Malaysia) before being subjected to the remineralization regime. Subsequently, specimens were treated with 100 ml of mineral water (300 ppm), distilled water, and milk under 150 rpm agitation for 24 h. The distilled water was used as a control. Specimens were rinsed with distilled water for 30 sec and hydrated in artificial saliva to maintain 100% relative humidity. Finally, the mineral contents of the enamel surface (Ca, Mg, K, and Na) were measured at the end of the hardening regime using an EDX spectrometer (Skyray Instrument, USA). The enamel surface roughness in treated citric acid was evaluated by an enamel surface scanning electron micrograph (400x, 500x, and 1,000x). Table I presents the mineral composition of mineral water (Evian™) and Fresh milk (Nestle™). The EDX measurement extended for six hours for all samples.

Table I: Mineral composition of the treatments

	Mineral water	Fresh milk
Calcium	80 $mg.L^{-1}$	5.0 $mg.L^{-1}$
Magnesium	26 $mg.L^{-1}$	0.1 $mg.L^{-1}$
Potassium	1 $mg.L^{-1}$	1.3 $mg.L^{-1}$
Sodium	6.5 $mg.L^{-1}$	1.7 $mg.L^{-1}$

Statistical Analysis

The results of EDXS before and after the treatment and those of surface roughness through scanning electron micrograph were statistically evaluated using a one-way ANOVA test. P-values of < 0.05 were considered statistically significant. The graphs were prepared using MS Excel 2010.

Results

The result showed that after treatment with citric acid, the surface roughness of enamel significantly increased ($F (2, 6) = 2.57$, $P<0.05$). In contrast, in treatment with fresh milk, the surface roughness of milk decreased significantly, indicating milk's protective properties on dental enamel (figures 1, 2, and 7). Moreover, based on the results, fresh milk decreased the surface roughness of the enamel surface, suggesting the healing properties of milk on citric acid-treated enamel ($F (2, 6) = 1.98$, $P>0.05$). It was also found that mineral water increased the surface roughness due to mineral aggregation on the enamel surface.

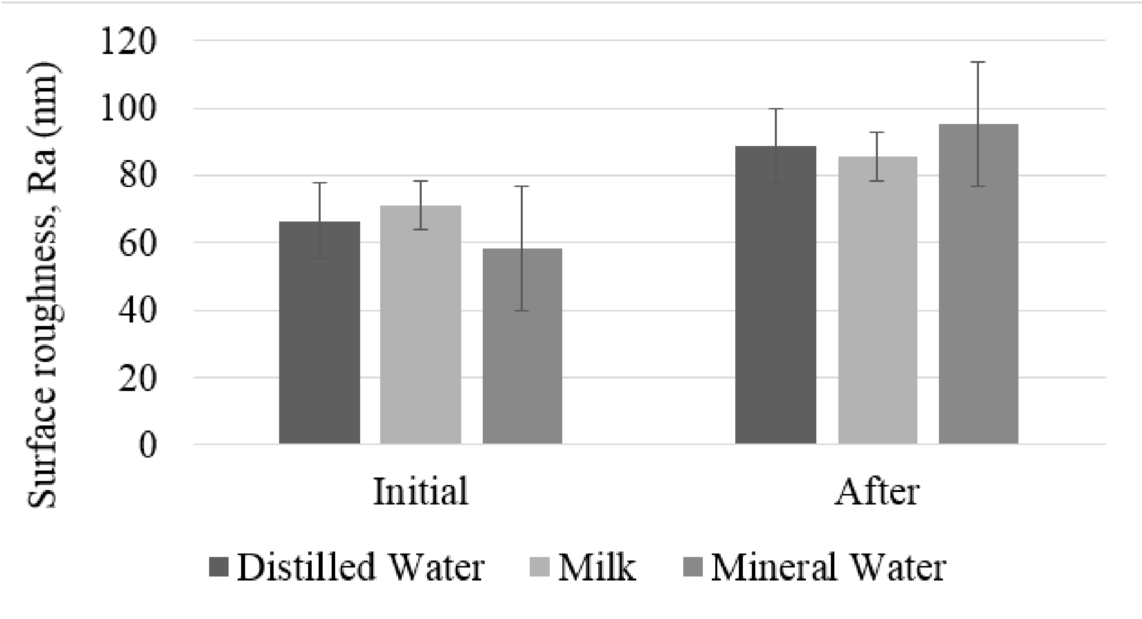

Figure 1: Surface roughness of enamel surface treated with citric acid

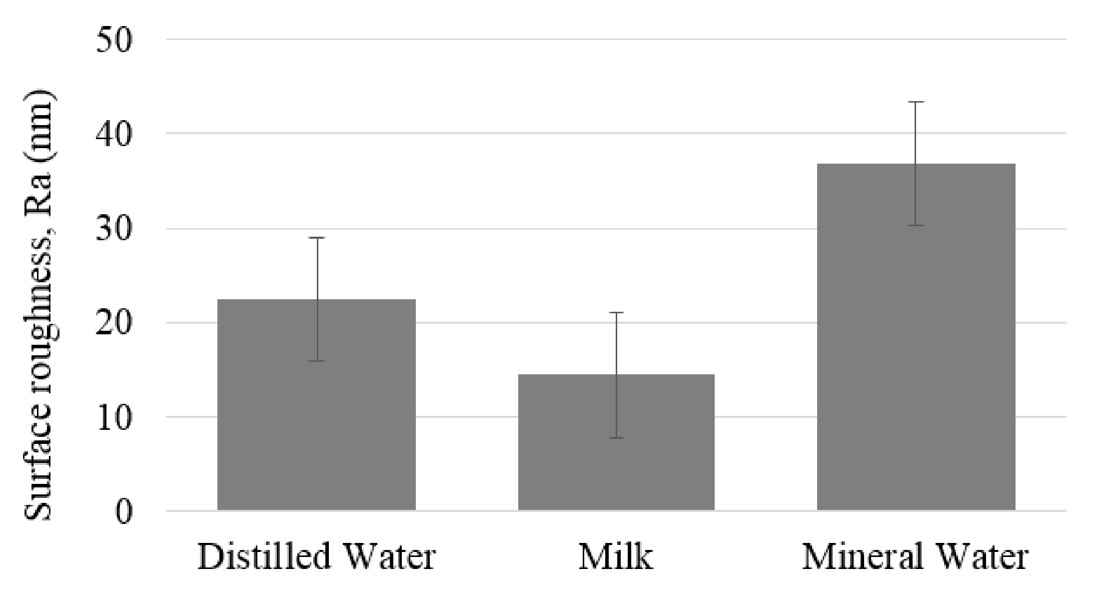

Figure 2: Quantity of induced surface roughness of enamel treated with different materials

The result of EDXS showed that after treatment with citric acid, the calcium content of enamel slightly changed (Figure 3). However, statistical analysis confirmed no significant difference in calcium content of enamel surface treated with different drinking materials (F (2, 6) =1.88, P>0.05). It was also reported that the calcium content of enamel was slightly washed by mineral water and milk due to morphological changes (Figure 3).

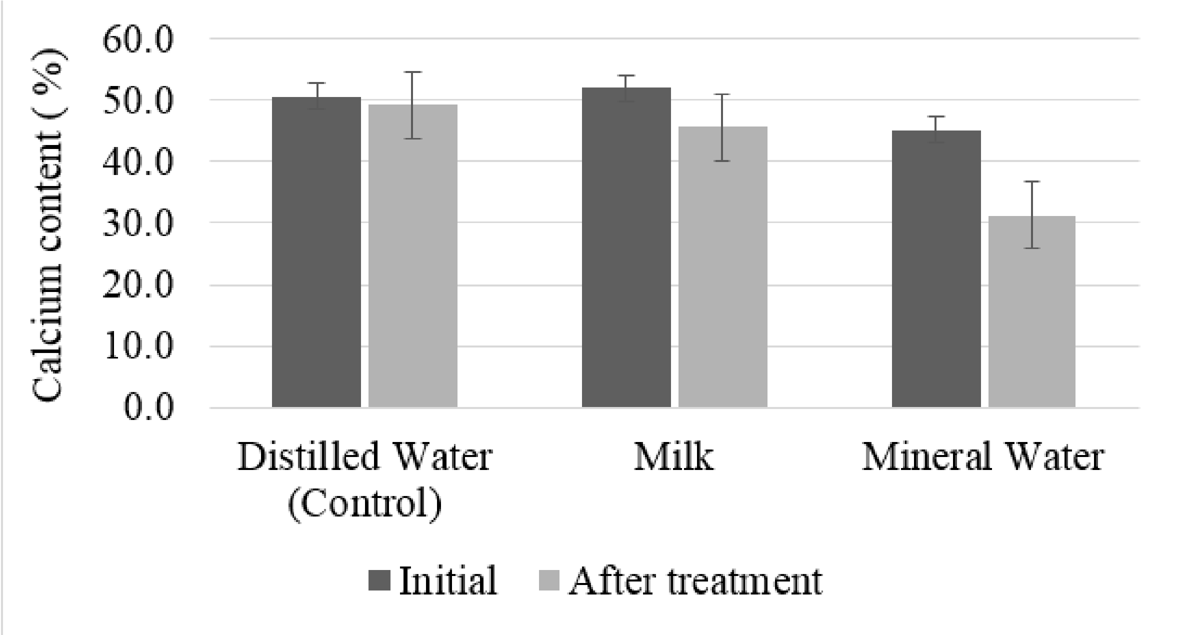

Figure 3: Calcium content of enamel surface initially and after treatment with different materials

Based on the result of EDXS, the treatment of the enamel surface with fresh milk slightly increased the magnesium content of the enamel surface. It hardened the softened enamel after citric acid treatment. On the other hand, the results showed that in the control group, the magnesium content of the enamel decreased after treatment with citric acid (Figure 4). Nonetheless, statistical analysis showed that the difference in magnesium concentration of enamel surface treated with different materials was not significant (F (2, 6) =2.44, P>0.05).

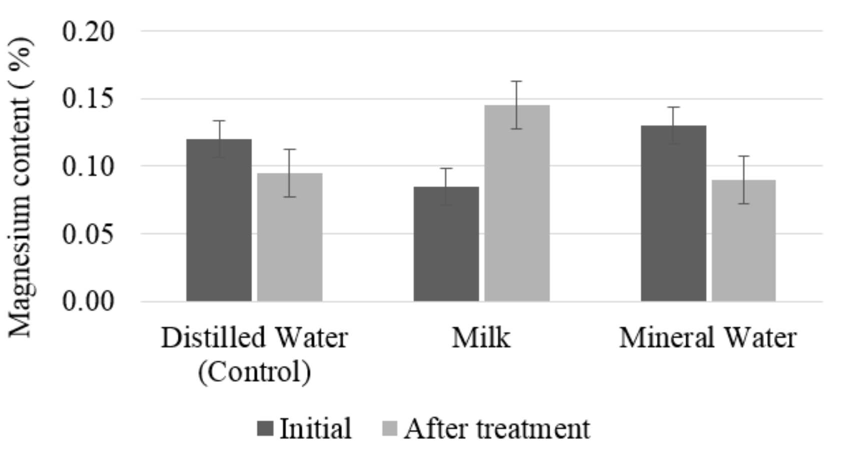

Figure 4: Magnesium content of enamel surface initially and after treatment with different materials

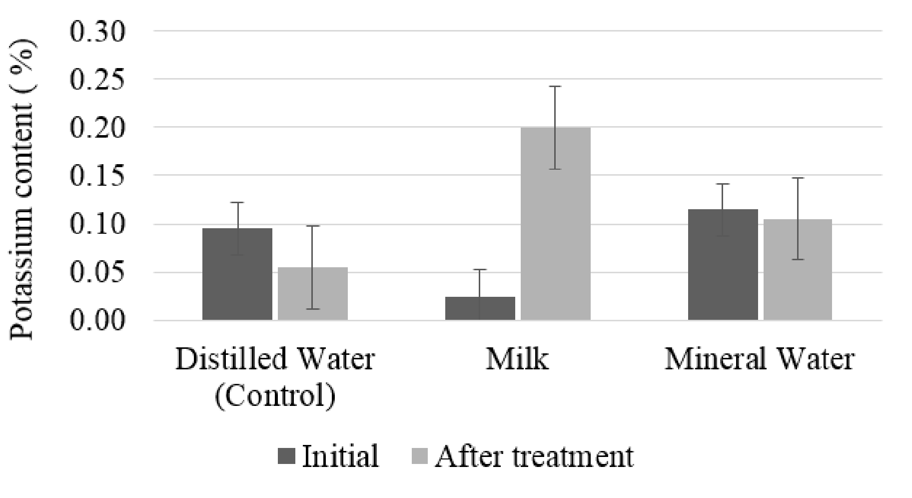

Figure 5: Potassium content of enamel surface before and after treatment with different materials

The results of statistical analysis revealed that the potassium content of enamel surface treated with different materials was significantly different (F (2, 6) =10.59, P<0.01). Moreover, EDXS confirmed that the enamel surface treatment with fresh milk increased the potassium content of the enamel surface and healed the damage of citric acid. In addition, mineral water partially recovered the damages of citric acid. On the other hand, the results demonstrated that citric acid reduced the potassium content of the enamel surface (Figure 5). According to the statistical analysis, the sodium content of the enamel surface treated with different materials was significantly different (F (2, 6) =35.71, P<0.01). Therefore, the results of EDXS confirmed that the treatment of the enamel surface with fresh milk and mineral water recovered the damage of citric acid to a higher level than the initial concentration. Furthermore, the results showed that the citric acid slightly decreased the sodium content of the enamel surface (Figure 6).

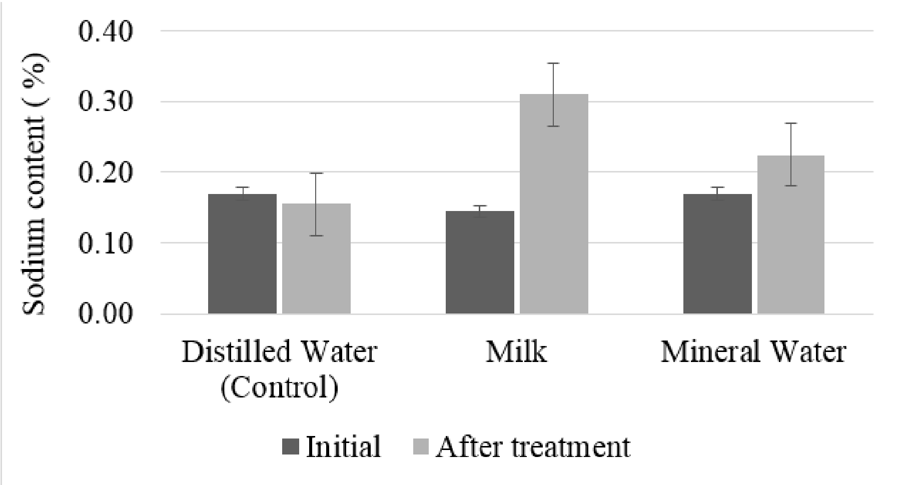

Figure 6: Sodium content of enamel surface before and after treatment with different materials

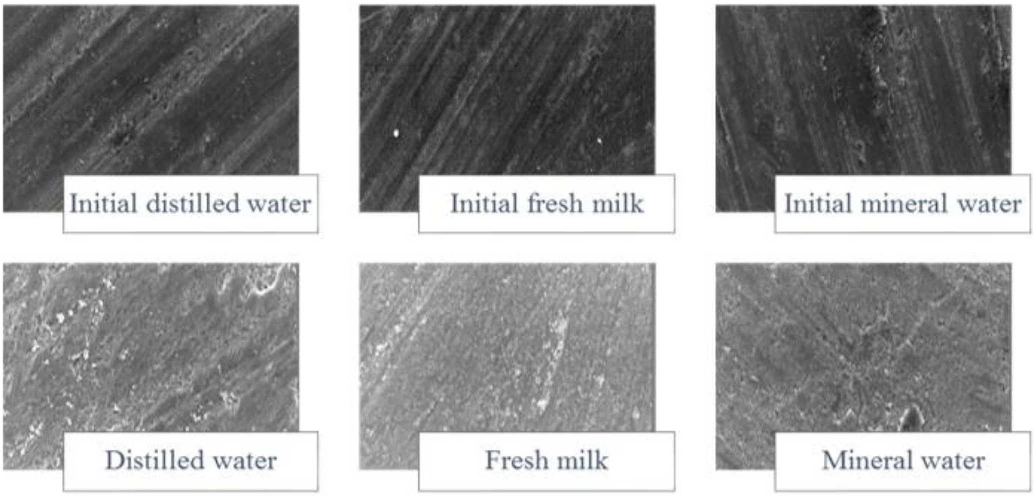

Figure 7: Electron micrograph of surface roughness of enamel treated with different materials (500x)

Discussion

In the current study, the effects of mineral water and fresh milk on the nano-hardness of tooth enamel surface were measured. Mineral water and dairy products contain inorganic Na^{+}, K^{+}, Mg^{2+}, and Ca^{2+}, similar to the mineral content of enamel, including Al^{-}, Fe^{2+}, Mg^{2+}, Ca^{2+}, Na^{+}, and K^{+} (19). It is noteworthy that tooth enamel is almost entirely composed of calcium phosphate with specific mineral content (20). Additionally, citric acid leads to enamel surface roughness through anatomical and morphological change (21–23). Therefore, the results of the present study suggested that fresh milk might heal the enamel surface after treating citric acid.

The potassium and sodium content of the enamel surface increased in treatment with milk to a higher level than the initial concentration. Furthermore, mineral water increased the sodium content of the enamel surface and slightly covered the damage induced by citric acid on the enamel surface. Evidence revealed that the enamel mineral loss of uptake is correlated with the change of its nano-hardness (5). Assessment techniques, such as microhardness and profilometry, were used to evaluate the loss of dental hard tissue by erosive challenges (12). The nature of remineralization of eroded enamel depends on initial demineralization conditions and the ratio of calcium to phosphate content of saliva (6, 24). Remineralization on the enamel surface happens by the uptake of calcium and phosphate. However, the evidence revealed that distilled water has no minerals content and if distilled water contacts the tooth, it sucks out the minerals from the enamel and accelerates enamel destruction (25–27).

Highly supersaturated calcifying solutions can be used to enhance mineral appositions (28). It is noteworthy that remineralization of the enamel depends on the pH and the amount of calcium and phosphate available in the water (16). Evidence shows that demineralization begins at the nanoscale level at the crystal surface inside the enamel and can continue unless be halted (6). Moreover, EDXS is a pioneering technique to evaluate the content of specific substances, such as the enamel surface (12). The results of a study on eroded dentin using transverse microradiography showed that phosphate-rich solution could provide better remineralization than the calcium-rich one. The surface nano-hardness and EDXS results on eroded enamel were reported in this study and expanded the previous research conducted on eroded enamel (24). The findings suggested that milk and mineral water might slightly recover the damage induced by citric acid on the enamel surface at the nanoscale level.

Conclusion

Tooth remineralization with the consumption of mineral water or fresh milk is an area of potential interest for oral health. The results demonstrated that the citric acid led to the anatomical change of the enamel surface, and the mineral water led to increased sodium content compared to the initial concentration on the enamel surface. Furthermore, mineral water slightly covered the damage induced on the enamel surface. Additionally, an increase was observed in the potassium and sodium content of the enamel surface treated with fresh milk.

Conflict of interest

Authors declare no conflict of interest.

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